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Review article

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THE INFLUENCE OF POLYPHENOL-RICH EXTRACTS ON THE PRODUCTION OF PRO-INFLAMMATORY MEDIATORS IN MACROPHAGES

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Recent decades have seen a rise in chronic inflammatory diseases such as diabetes, cardiovascular diseases, asthma, rheumatoid arthritis, neurodegenerative diseases. Importantly, such chronic inflammatory diseases also increase the risk of cancer development and there is a pressing need to identify new anti-inflammatory drugs. One promising source of new medication are natural polyphenolic compounds and polyphenol-rich preparations, extracts and foods, which have strong antioxidant properties. This paper reviews the anti-inflammatory role of polyphenolic-rich natural extracts, and their ability to modulate crucial pro-inflammatory mediators, such as cyclooxygenase-2, prostaglandin E_2, inducible nitric oxide synthase, and nitric oxide, in macrophage cells. Our research confirms that natural compounds have health potential, and could be used in the treatment or prevention of inflammatory diseases.

Key words: *inflammation, macrophages, polyphenol- phenol-rich extracts, nitric oxide, cyclooxygenase, prostaglandin, reactive oxygen species*

INTRODUCTION

Many years of scientific research have shown that a properly-balanced diet has a crucial impact on human health, and can prevent many diseases (1). The World Health Organization (WHO) emphasizes the need to include fruits, vegetables, and whole grains in the diet, especially considering the growing consumption of foods rich in sugar, salt/sodium and fat. Moreover, according to WHO recommendations, consuming at least 400 g of vegetables and fruit per day may reduce the risk of the development of numerous chronic diseases, such as cardiovascular diseases, stroke, diabetes, autoimmune diseases and most cancers (2-4). This beneficial effect has been attributed to the high levels of multiple nutraceuticals found in plants.

A large proportion of these nutraceuticals are polyphenols, and research into these bioactive natural compounds has become increasingly popular in recent years. The group comprises about 10,000 compounds characterized by an aromatic ring structure and which are classified into four main groups, *viz.* phenolic acids, flavonoids, stilbenes and lignans (5, 6). So far, a number of properties of single polyphenols such as resveratrol, epigallocacechin gallate (EGCG), curcumin, or polyphenol-rich foods, such as green tea, coffee, olive oil and berries have been well described (7-9); however, a large number of the compounds and their effects remain relatively poorly understood, and further research is necessary. Nevertheless, a growing body of research indicates that polyphenol and polyphenol-rich preparations consumption may play a vital role in health through the regulation of many biological processes, importantly at the cellular level, where the compounds have been found to modulate the activity of a series of growth factors and enzymes, or even influence gene transcription. While the most widely reported aspect of polyphenols is their antioxidant activity, various *in vitro* and *in vivo* studies and clinical trials have shown a number of other beneficial properties, such as anti-microbial, anti-inflammatory, and anti-cancer effects (10-14). Moreover, understanding the mechanism of inflammation and the crucial role played by macrophages in this process has allowed researchers to focus on the design of therapeutics based on natural compounds that target these immune cells. Macrophages play a key role in the maintenance of tissue homeostasis and influence pathogenesis of a variety of human diseases associated with chronic inflammation, such as diabetes, atherosclerosis, rheumatoid arthritis, colitis, endometriosis and obesity. Importantly, macrophages participate both in tumor initiation and progression, and high levels of infiltration of these cells in the tumor environment is often a predictor of poor prognosis for cancer patients (15, 16). Hence, by modulating the effect of macrophages, bioactive compounds can play an important role in the protection of human health and treatment of chronic diseases at many levels. The purpose of this article is to review the current data presenting the anti-inflammatory activity of selected polyphenolic-rich extracts acting by inhibiting pro-inflammatory mediators such as cyclooxygenase-2 (COX-2), prostaglandin E_2 (PGE_2), inducible nitric oxide synthase (iNOS) and nitric oxide (NO).

INFLAMMATION

Inflammation is a protective response of the immune system to noxious stimuli and conditions, ranging from irritation and tissue injury to infection. It has a crucial role in regeneration,

tissue repair, remodeling and homeostasis and the response involves a wide range of immune cells and molecular mediators (17, 18). Its initiation is rapid and usually lasts a few minutes. Firstly, specific receptors, named pattern-recognition receptors (PRRs) are activated, transmitting signals to the nucleus, where a selective set of genes are induced *via* both transcriptional and posttranscriptional mechanisms. One crucial transcriptional factor in the selective induction of inflammatory genes is nuclear factor-κB (NF-κB). The response also involves the action of a range of factors that control the expression of genes governing inflammatory factors, such as tumor necrosis factor alpha (TNF-α), inteleukin-1 (IL-1), IL-6 and chemokines. These factors include activator protein-1 (AP-1), cyclic-adenosine monophosphate (cAMP) response element binding protein (CREB), a cAMP-induced factor; E2F, a transcription factor activated by the adenovirus E1A protein in adenovirus-infected cells; serum responses factor (SRF) and the associated ternary complex factors (TCFs), which is responsible for the serum induction of Fos transcription factors. The process also recruits a number of immune cells, such as macrophages, neutrophils, dendritic cells, mast cells and lymphocytes, as well as non-immune cells, inter alia endothelial cells, epithelial cells, and fibroblasts.

Additionally, two types of inflammation can be distinguished, acute and chronic. Acute inflammation is considered as a part of innate immunity and the first line of the host defense against foreign stimuli (19). It is mainly related to maintaining proper homeostasis of the organism. The duration of inflammatory responses varies depending on the level of damage caused by the stimuli, and the longer this response persists in the organism, the greater chance of more adverse consequences arising (17, 19). If inflammation is not resolved, typically by anti-inflammatory mediators such as transforming growth factor-β, IL-10 and glucocorticoids, the condition can develop into a chronic form. From this moment, the inflammatory response is itself responsible for the pathogenesis, not the foreign stimulus. Importantly, this state, characterized by unbalanced and uncontrolled levels of pro- and anti-inflammatory mediators, may lead to numerous civilization disorders. Another damaging process specific to inflammation is oxidative stress. In normal conditions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an essential role in mitochondrial processes and provide a defense against microbial pathogens (20). However, during inflammation, overproduction of ROS and RNS can damage or inhibit the normal function of lipids and proteins, and can lead to DNA mutation and damage, which can be a predisposing factor for cancer or other serious diseases. Immune cells, and especially macrophages are involved in the production of ROS and RNS. Throughout chronic inflammation, macrophages are constantly activated, resulting in increased oxygen uptake and the production of a variety of ROS and RNS, including NO or hydrogen peroxide. When the host antioxidant activity/capacity is not sufficient, endogenous compounds such as polyphenols might be used as ROS scavengers (20, 21).

Also, dysregulated apoptosis or the persistence of apoptotic cells that escape clearance may lead to serious consequences, including chronic inflammation, autoimmune disease, or cancer. In this case, natural compounds, including polyphenols, such as EGCG or resveratrol, can modulate cellular pathways related to apoptosis. Treatment with polyphenols can induce apoptosis through the activation of proteins related to the programmed-cell death pathways such as caspase-3, -9 and -8, as well as through the inhibition of other proteins, such as the inhibitor of apoptosis protein-2 (c-IAP2), X-linked IAP (XIAP), Bcl-2, Bcl-xL and Bid (22, 23).

Some of the most common diseases related to chronic inflammation are type 2 diabetes, rheumatoid arthritis, atherosclerosis, asthma, inflammatory bowel disease (IBD), glomerulonephritis and cardiovascular diseases; in addition, various neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and multiple sclerosis have been associated with an inflammatory state (24). Furthermore, chronic inflammation is believed to be a preceding state in around 15 – 20% of all cancer cases (17). For instance, the risk of colorectal cancer is elevated during inflammatory bowel disease, liver cancer during chronic hepatitis, stomach cancer during *Helicobacter*-induced gastritis and bladder cancer during *Schistostoma*-induced bladder. In addition, obesity, hyperglycemia, or excessive lipid accumulation can lead to low-grade inflammation that also promotes carcinogenesis long before tumor formation (17).

Fortunately, knowledge about intracellular molecules in inflammatory signaling cascades has expanded, allowing these molecules to be targeted to develop anti-inflammatory therapies. Nowadays, depending on the cause and severity of inflammation, non-steroidal anti-inflammatory drugs (NSAIDs), steroids or monoclonal antibodies can be used in treatment (25). Obviously, while antibiotics or antifungal agents should be prescribed for bacterial or fungal infections, the most frequently chosen drugs are NSAIDs such as naproxen, ibuprofen and aspirin due to their pain-reducing properties. At the same time NSAIDs are not addictive, unlike opiate/opioid analgesics. Nevertheless, all available NSAIDs are associated with potential adverse effects, increasing the risk of various cardiovascular and gastrointestinal problems, kidney or liver diseases (26). The next most commonly-used group of anti-inflammatory medications are corticosteroids, which are used to treat allergies, dermatological diseases and gastrointestinal problems as well as neurological diseases, such as multiple sclerosis; however, they can also cause various side-effects, including severe ones, such as insulin resistance or peptic ulceration (27). These pharmaceuticals may also demonstrate unfavorable drug interactions, and result in impaired quality of life and high costs, especially for biological drugs (28). Therefore, the development of new anti-inflammatory therapeutics is essential.

POLYPHENOL-RICH EXTRACTS MODULATING PROSTAGLANDINS, CYCLOOXYGENASE AND NITRIC OXIDE

Prostaglandins

Among the complicated cascades of mediators involved in the generation of the inflammatory response, prostaglandins (PGs) play a crucial role; PGs contribute to the development of the cardinal signs of inflammation and their biosynthesis is significantly increased in inflamed tissue. This group of molecules is characteristic for most tissues and organs. They are produced from arachidonic acid, which is released from the plasma membrane by phospholipases and metabolized by PGG/H synthase or by cyclooxygenase (COX) and their respective synthases (29). The principal bioactive PGs are prostaglandin E_2 (PGE_2), prostacyclin (PGI_2), prostaglandin D_2 (PGD_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and of these, PGE_2 is synthesized in the greatest amounts in the body (30). Under physiological conditions, PGE_2 also plays an important role in regulating blood pressure, gastrointestinal integrity and the immune response (31). During inflammation, PGE_2 synthesis rapidly increases, and its presence at high levels is related to classical symptoms, such as redness, swelling and pain. It also

immediately recruits leukocytes and elicits infiltration by immune cells. Macrophages are the main cells involved in the production of PGE_2, and its level dramatically increases after lipopolysaccharide (LPS) stimulation (30).

Cyclooxygenase (COX)

At the molecular level, the biosynthesis of PGs depends on the activity of the cyclooxygenase (COX) enzyme, known as prostaglandin-endoperoxide synthase, which exists as distinct COX-1 and COX-2 isoforms. COX-1 is constitutively expressed, while COX-2 is induced by inflammatory stimuli, hormones, or growth factors (29). Alternatively, the lipoxygenase enzyme is active both in leukocytes and in macrophages synthesizing leukotrienes. PG biosynthesis can be blocked by several drugs, including NSAIDs (cyclooxygenase antagonists) and COX-2 selective inhibitors, or corticosteroids. Importantly, single phenols, as well as food, plant preparations or extracts rich in these compounds, also effectively regulate the production of PGE_2 and the expression of COX-2 (30).

Nitric oxide (NO)

NO is another crucial molecule secreted during the immune response. It is produced by the conversion of L-arginine to L-citrulline by a class of enzymes known as nitric oxide synthases (NOS) (32). The third isoform of NOS is known as inducible NOS (iNOS or NOS_2) and is expressed only in response to certain inflammatory stimuli such as cytokines or bacterial products. Various cell types express iNOS during host defense against microbial and viral pathogens, leading to the secretion of NO radicals in the host cell or in the microbe itself (33). Macrophages are the main cells of the immune system that are involved in the regulation of iNOS and NO activity. They are known to act through the stimulation of cellular receptor molecules such as Toll-like receptors and CD14. After activation with LPS, the CD14 receptor plays an important role in the inflammatory response by activating the NF-κB pathway, which has an essential function in the modulation of iNOS and NO (32). Under pathological conditions, the expression of iNOS can be unbalanced and over-activated in macrophages. This state exacerbates inflammation and often participates in the pathology of inflammatory diseases including atherosclerosis, diabetes, rheumatoid arthritis, transplant rejection, septic shock and multiple sclerosis (34).

Polyphenol-rich extracts

Many synthetic inhibitors of iNOS have been developed; this group encompasses various arginine and non-arginine analogs, including amidinic compounds, five-membered and six-membrane heterocyclic compounds, steroidal compounds and chalcone derivatives. Natural inhibitors are also well-known therapeutics, and numerous studies have shown that phenols elicit anti-inflammatory activity by inhibiting NO production and reducing the expression of iNOS (35). Hu and co-workers found that edible mushrooms are also rich sources of bioactive components; a study of *Pleurotus eryngii* polyphenol-rich extract (PPEP), which contained gallic acid monohydrate, 3-(3,4-dihydroxyphenyl)-propionic acid, methyl gallate, ellagic acid, syringic acid and catechin found that PPEP suppression of NO overproduction (reduction by 98.7% at 200 μg/mL) resulted in inhibition of iNOS protein expression in LPS-stimulated RAW264.7 cells: the extract diminished iNOS protein expression by 95.4% (200 μg/mL) (36).

In another study, RAW264.7 cells were stimulated with LPS or Pam3CSK4 (inflammatory inducer, toll-like receptor 1/2 agonists) and then treated with methanol extract from *Piper cubeba* L. (Pc-ME; 25, 50 and 100 μg/mL). The extract was rich in flavonoids, such as quercetin, kaempferol and luteolin. After 24-hour treatment with Pc-ME, NO production was significantly reduced in a concentration-dependent manner to the level of unstimulated macrophages at 100 μg/mL. Prednisolone, a common anti-inflammatory drug used as a control, reduced NO secretion only by 40%. Also, mRNA expression of iNOS in LPS-stimulated macrophages was found to be effectively down-regulated by Pc-ME after six hours of treatment (37).

Another study examined the properties of aqueous extract derived from the petals of safflower (*Carthamus tinctorius* L.; SFA), whose main constituents are safflomin A and safflomin B, both being major compounds of carthamus yellow (CY). This study was carried out on RAW264.7 cells, firstly treated for two hours with SFA (250, 500, and 1000 μg/mL) or CY (250, 500, 1000, and 2000 μg/mL), and then stimulated with LPS, for a further 24 hours. Both SFA and CY treatment significantly reduced NO and PGE_2 production in a concentration-dependent manner. Although the results were comparable, SFA inhibited PGE_2 production much more strongly. Similarly, while both SFA and CY markedly suppressed COX-2 and iNOS expression in a concentration-dependent manner, SFA appeared to be more effective (38).

Cheng and co-workers examined the influence of insoluble (i.e. non-extractable) phenols, which are the components of cell walls, and soluble (i.e. extractable) phenols, which are compartmentalized within the plant cell vacuoles (39, 40). The authors tested the biological activity of two crude blueberry preparations containing both extractable polyphenols (EPP) and non-extractable polyphenols (NEPP). The EPP fraction was found to be rich in anthocyanins, such as malvidin-3-glucoside, malvidin-3-galactoside, and malvidin-3- arabinoside, while NEPP was rich in acylated anthocyanidins. A higher concentration of phenolic compounds was identified in the EPP fraction (40). LPS-stimulated RAW264.7 macrophages were first treated with EPP and NEPP for 48 h (10, 100, 200, 400 μg/mL) and then incubated for 6, 12, 24, 48, and 72 hours in the presence of 100 μg/mL EPP or NEPP. In both cases, the extracts reduced NO production in a concentration- and time-dependent manner, respectively. Both EPP and NEPP were found to have a stronger inhibitory effect on NO secretion than the positive control, in this case tea polyphenols. Moreover, EPP had a slightly greater influence on NO production than NEPP, the reason may be that most active ingredients were present in significantly higher contents in EPP than in NEPP. During the time-dependent experiments, both extracts caused a similar effect. In both cases, incubation with extract for 48 hours resulted in over 50% inhibition of iNOS mRNA expression. Additionally, both extracts reduced the mRNA expression of iNOS after 6, 12, 24, 48, and 72 hours, but the greatest down-regulation (to ~20%) was observed after six hours. In addition, COX-2 mRNA expression was markedly inhibited in a concentration-dependent manner in LPS-induced macrophages: EPP down-regulated expression by about 60%, while NEPP by about 40% (40).

Elsewhere, pre-treatment with *Syzygium cumini* seed fractions was found to significantly reduce NO production; again, 70% methanol caused the strongest effect (inhibition by 48.77%). The following solvents were used during extraction: hexane, ethyl acetate, methanol (ME), 70% ME and water (41). Additionally, down-regulation of iNOS (2-fold reduction) and COX-2 (1.6-fold reduction) mRNA expression was observed. The authors attribute the anti-inflammatory properties of *Syzygium cumini* seed fractions to their polyphenol content (41); their previous research indicated a high content of

polyphenols, such as gallic acid, ellagic acid, myricetin, ferulic acid, phenolic acids (42).

Another study examined effect of methanol extract derived from the leaves and twigs of *Gouanialeptostachya* (Gl-ME) on RAW264.7 cells and peritoneal macrophages obtained from male C57BL/6 mice. The extract was primarily composed of resveratrol, followed by quercetin, luteolin, kaempferol, anthraquinone-2-carboxylic acid, 2-methylanthraquinone, and curcumin (43). It was found that after 24-hour treatment, 200 and 300 μg/mL GI-ME significantly decreased the production of NO in both LPS-treated RAW264.7 cells and peritoneal macrophages in a concentration-dependent manner. NO secretion was also suppressed in RAW264.7 cells treated with the extract and active with pam3CSK, a TLR2 ligand, and poly(I:C), a TLR3 ligand. In addition, PGE_2 production was markedly reduced in LPS-activated RAW246.7 after treatment with GI-ME (300 μg/mL), and two standard compounds, L-NAME and indomethacin, dose-dependently blocked the production of NO and PGE_2 under the same conditions. Moreover, GI-ME down-regulated mRNA expression of COX-2 and iNOS, suggesting that the inhibition of inflammatory mediator release occurred at the level of transcriptional regulation (43) (*Table 1*).

In another study, LPS-stimulated macrophages were treated for 24 hours with 40% ethanol extract from *Trifolium pretense* L. leaves (40% PeTP; 0.5, 1, and 2 mg/mL). A significant and concentration-dependent reduction of NO and PGE_2 was observed, as well as of iNOS and COX-2 protein expression. In the case of iNOS, 2 mg/mL of PeTP decreased its expression to the level of unstimulated RAW264.7 cells (44).

The level of PGE_2 in LPS-activated RAW264.7 was also found to be markedly reduced after treatment with litchi (*Litchi chinensis* Sonn.) flower ethanolic extract (LFEE1), which was rich in flavanoids, phenolic acids and proanthocyanidin A2. These findings correlated with a concentration-dependent reduction in COX-2 protein expression, with 0.3 mg/mL suppressing protein expression by 70%. Moreover, LFEE treatment significantly reduced NO production and iNOS protein expression in a concentration-dependent manner: treatment down-regulated iNOS by 67% in LPS-mediated RAW264.7 cells (45) (*Table 1*).

Elsewhere, macrophages were pretreated with different concentrations of pomegranate (*Punica granatum* L.) peel polyphenols (PPPs;100 μg/mL), and its main components, *viz*. punicalagin (PC; 50 μg/mL), and ellagic acid (EA; 50 μg/mL) for one hour and then stimulated with LPS (1 μg/mL) for 20 min or 24 hours. The NO and PGE_2 measurements indicated that NO was much more strongly inhibited than PGE_2, both after one hour and after 24 hours. EA caused the most significant reduction of NO production, while PGE_2 was the most inhibited by EA after one hour, and by PC after 24 hours. Nevertheless, the extract and its major constituents caused a similar inhibitory effect (46). Also, treatment with *Ternstroemia gymnanthera* stem bark extract (by 88.99% at 200 μg/mL) was found to markedly inhibit NO production, which correlated with down-regulation of iNOS expression at the mRNA and protein level (47).

Crude *Ecklonia cava* flake extracts (CEF) appear to have beneficial effects in LPS-induced RAW264.7. In this study, five extracts were tested, differing from each other with regard to the solvent (water or HCl) and temperature conditions, *viz*. 25°C, 50°C, 80°C, or 95°C (48). Unfortunately, the chemical profile was not characterized in this study, although it has previously been noted that *Ecklonia cava is* rich in polyphenols (49). CEF appeared to have an anti-inflammatory effect. Two extracts (CEF-W, 95°C and CEF-1 N HCl, RT) significantly reduced the production of NO in a concentration-dependent manner (3.13 and 6.25 μg/mL). In addition, CEF-W, 95°C and CEF-1 N HCl, RT inhibited iNOS expression at the protein level. Although, the production of NO was slightly reduced by CEF-1 N HCl, 50°C, it was not sufficient to attenuate the expression of the iNOS protein (48).

Crude polyphenols extracted from the blossoms of *Citrus aurantium* L. var. *amara* Engl. (CAVAP-W) significantly suppressed the expression of iNOS at the mRNA level. Unfortunately, the production of NO was not determined. CAVAP-W also exerted a significant, concentration-dependent decrease in the expression of COX-2 at the mRNA and protein levels in LPS-induced RAW264.7. The extract was rich in phenols, such as neohespeidoside, neoeriocitrin, rhoifolin, hesperidin, naringin, rutin, veronicastroside, neohesperidin, and hesperetin (50).

Spent hops (*Humulus lupulus* L.) extract (SHE) was also found to significantly decrease COX-2 expression at the mRNA (by 47%) and protein level (by 32%) after 24 hours of treatment in mice macrophages. Similarly, SHE reduced iNOS protein expression to 2%, and NO production by almost a half for all tested concentrations; this effect was similar to that of the steroidal drug budesonide, used as a positive control. No significant changes were observed for iNOS mRNA expression. SHE was characterized by high flavonol, proanthocyanidin, hydroxycinnamic acid and flavanol monomer content (51).

Another phenolic-rich extract is grape seed extract (GSE), derived from *Vitis vinifera* L. seeds. In this study, three varieties of GSE were used: Syrah, Merlot and Carignan. All of them were rich in flavan-3-ols, flavanols, and gallic acid. GSE treatment resulted in the downregulation of iNOS mRNA expression and NO production, with Syrah causing the strongest effect (52).

Japanese quince (*Chaenomeles japonica*) leaf polyphenol-rich extract (JQLPE) has also been found to have anti-inflammatory activity (53). The extract was rich in 33 phenolic compounds, with chlorogenic acid and naringenin hexoside being its main constituents (54). JQLPE (10, 25, and 50 μg/mL) markedly reduced Ptgs2 mRNA expression in LPS-activated murine macrophages, with the highest concentration reducing expression by 47.95%. it also reduced COX-2 protein expression by 49.93% after 24 hours. Similarly, iNOS expression was effectively inhibited, by about 50% at both the mRNA and protein levels, as was the production of NO. In this study, the extract exerted anti-inflammatory activity, which was similar to the positive control: budesonide (53).

These findings confirm the anti-inflammatory potential of phenol-rich plant extracts, through their ability to suppress PGE_2 and NO production, as well as down-regulating COX-2 and iNOS expression. Interestingly, the above findings indicate that natural extracts have similar inhibitory effects on the pro-inflammatory mediators as steroid medications. Furthermore, the extract of *Piper cubeba* L. was found to act more strongly than the corticosteroid prednisolone, and crude blueberry extract caused better anti-inflammatory effects than tea polyphenols, which were used as a positive control (37, 40, 51). These results clearly suggest that phenolic-rich extracts may find application in the treatment of inflammatory diseases or as dietary supplements.

ANIMAL MODELS OF INFLAMMATION

While most studies on the anti-inflammatory role of polyphenols have been performed on macrophages *in vitro,* some researchers also have used animal models. For instance, in 2020, Zhang with co-workers examined the anti-inflammatory properties of polyphenolic extract from *Moringa oleifera* leaves (MOPE) on a mouse model of colitis. C57BL/6 mice were

Table 1. Selected polyphenol-rich extracts with inhibitory activity on pro-inflammatory mediators in macrophages.

Extract name	Phenols content	Model	Treatment	Anti-inflammatory effect	Ref.
*Pleurotuseryngii*polyphenol-rich extract (PPEP)	Total phenolic content was 0.11 ± 0.02 µmol GAE/mg of PPEP. The main compounds were: gallic acid monohydrate, 3-(3,4-dihydroxyphenyl)- propionic acid, methyl gallate, ellagic acid, syringic acid, and catechin.	LPS-stimulated RAW264.7	25, 50, 100, 200 µg/mL for 24 h	↓ iNOS protein expression ↓ NO production	36
Methanol extract from *Piper cubeba* L. (Pc-ME)	The main compounds were: flavonoids, such as quercetin, kaempferol, and luteolin.	LPS- or Pam3CSK4-stimulated RAW264.7	50 and 100 µg/mL for 6 or 24 h	↓ iNOS mRNA expression ↓ NO production	37
Dried safflower *Carthamus tinctorius* L. petals aqueous extracts (SFA) and the main constituent – carthamus yellow (CY)	Carthamus yellow (CY) is the main constituent of safflower and is composed of safflomin A and safflomin B	LPS-stimulated RAW264.7	SFA: 250, 500, and 1000 µg/mL CY: 250, 500, 1000, and 2000 µg/mL 2 h pre-treatment, followed by LPS-stimulation for 24 h	↓ iNOS protein expression ↓ COX-2 protein expression ↓ NO production ↓ PGE_2 production	38
Crude blueberry extracts: extractable polyphenols (EPP) non-extractable polyphenols (NEPP)	Higher concentration of phenols in EPP than in NEPP. The main compounds in EPP were: anthocyanins, such as malvidin-3-glucoside, malvidin-3-galactoside, and malvidin-3- arabinoside The main compounds in NEPP were: acylated anthocyandins	LPS-stimulated RAW264.7	10, 100, 200, 400 µg/mL pre-treatment for 48 h, followed by LPS-stimulation for 6, 12, 24, 48, and 72 h	↓ iNOS mRNA expression ↓ COX-2 mRNA expression ↓ NO production	40
Five *Syzygiumcumini* seeds fractions During extraction following solvents were used: hexane, ethyl acetate (EA), methanol (ME), 70% methanol (70% ME) and water (WE).	The chemical composition was not evaluated.	LPS-stimulated RAW264.7	100 µg of all fractions for 24 h	↓ iNOS mRNA expression ↓ COX-2 mRNA expression ↓ NO production	41
Gouanialeptostachya leaves and twigs methanol extract (Gl-ME)	The main compounds were: resveratrol, followed by quercetin, luteolin, kaempferol, anthraquinone-2-carboxylic acid, 2-methylanthraquinone (2-MA), and curcumin	LPS- or pam3CSK-stimulated RAW264.7 peritoneal macrophages obtained from male C57BL/6 mice	50, 100, 200, 300 µg/mL pre-treatment for 18 h, followed by LPS-stimulation for 24 h	↓ iNOS mRNA expression ↓ COX-2 mRNA expression ↓ NO production ↓ PGE_2 production	43
40% prethanol extract of *Trifolium pretense* L. leaves (40% PeTP)	The chemical composition was not evaluated.	LPS-stimulated RAW264.7	0.5, 1, and 2 mg/mL for 24 h	↓ iNOS protein expression ↓ COX-2 protein expression ↓ NO production ↓ PGE_2 production	44
Litchi (*Litchi chinensis*Sonn.) flower ethanolic extract (LFEE)	The main compounds were: flavanoids (102.73 mg/g), phenolic acids (60.31 mg/g), and proanthocyanidin A2 (79.31 mg/g)	LPS-stimulated RAW264.7	0.1, 0.2 and 0.3 mg/mL 1 h pre-treatment, followed by LPS-stimulation for 24 h	↓ iNOS protein expression ↓ COX-2 protein expression ↓ NO production ↓ PGE_2 production	45
Pomegranate *Punica granatum* L. peel polyphenols (PPPs) and main components punicalagin (PC) and ellagic acid (EA)	The polyphenol content of PPPs was 57.09%. The main compounds were: gallic acid, PC (punicalagin-α and punicalagin-β), catechin, chlorogenic acid, epicatechin, and EA. PC content was 464.48 mg/g, and EA was 71.50 mg/g.	LPS-stimulated RAW264.7	PPPs: 100 µg/mL PC: 50 µg/mL EA: 50 µg/mL 1 h pre-treatment, followed by LPS-stimulation for 20 min or 24 h	↓ NO production ↓ PGE_2 production	46
Ternstroemiagymnanthera stem bark aqueous extract (TGSBE)	Total phenolic content was 240.9 mg GAE/g. The detailed chemical composition was not evaluated.	LPS-stimulated RAW264.7	100 and 200 µg/mL for 12 or 24 h	↓ iNOS mRNA expression ↓ iNOS protein expression ↓ NO production	47

Crude *Ecklonia cava* flake extracts (CEF) During extraction following solvents were used: water and HCl, and different temperature conditions: room temperature (RT), 25°C, 50°C, 80°C, or 95°C.	The chemical composition was not evaluated.	LPS-stimulated RAW264.7	3.13 and 6.25 µg/mL 24 h pre-treatment, followed by 6 or 24 h LPS-stimulation	↓ iNOS protein expression ↓ NO production	48
Crude polyphenols extracted from blossoms of *Citrus aurantium* L. var. *amara* Engl. (CAVAP-W)	The main compounds were: neohespeidoside, neoeriocitrin,rhoifolin, hesperidin, naringin, rutin, veronicastroside, neohesperidin, and hesperetin.	LPS-stimulated RAW264.7	15.625, 31.25, 62.5, 125, 250, and 500 µg/mL for 12 or 24 h	↓ iNOS mRNA expression ↓ COX-2 mRNA expression ↓ COX-2 protein expression	50
Spent hops (*Humulus lupulus* L.) extract (SHE)	The content of total phenolic compounds was 62.29 g/100 g of the extract. The main compounds were: flavonols, proanthocyanidins, hydroxycinnamic acids, and flavanol monomers. Flavonols and proanthocyanidins were the dominant phenolic compounds in extract and constituted 39.24 and 37.42% of the total phenolics, respectively.	LPS-stimulated RAW264.7	5, 10, 25 µg/mL for 6 h or 24 h	↓ COX-2 mRNA expression ↓ COX-2 protein expression ↓ iNOS protein expression ↓ NO production	51
Grape (*Vitis vinifera* L.) seed extract (GSE). Three varieties of GSE were used: Syrah, Merlot, and Carignan.	Phenol-content of Syrah (161.66 mgGAE/g), Merlot (142.33 mgGAE/g), and Carignan (112.66 mgGAE/g). The main compounds were: flavan-3-ols (epigallocatechin gallate, epicatechin gallate, epigallocatechin, catechin, epicatechin, procyanidin B1, procyanidin B4 and procyanidin B2), flavonols (kaempferol, myricitrin and quercetin) and gallic acid.	LPS-stimulated RAW264.7	0.5, 1, 5 µg/mL for 6 or 24 h	↓ iNOS mRNA expression ↓ NO production	52
Japanese quince (*Chaenomeles japonica*) leaf polyphenol-rich extract (JQLPE)	The main compounds were: chlorogenic acid and naringenin hexoside	LPS-stimulated RAW264.7	10, 25, 50 µg/mL for 6 h or 24 h	↓ COX-2 mRNA expression ↓ COX-2 protein expression ↓ iNOS mRNA expression↓ iNOS protein expression ↓ NO production	53
Moringa oleifera leaves (MOPE)	The main compounds were: astragalin, chlorogenic acid, isoquercitrin, kaempferitrin, luteolin, quercetin, and rutin	induced colitis to C57BL/6 mice with 3% DSS water	50 and 200 mg/kg for 14 days	↓ CD3+ Tcells, CD177+ neutrophils, and F4/80+ macrophages infiltration	55
Polyphenol-rich plant extract (PRPE) from Asteraceae, *Lactuca*; Liliaceae, *Allium cepa*; Lamiaceae, *Ajuga*; and Verbenaceae and *Lippia*	The main compounds were: chlorogenic acid, chicoric acid, and phenylpropanoid caffeic acid glycosides, quercetin and glycosylated quercetin	obesity model on C57BL/6 mice induced with high-fat/high-sucrose diet	supplementation containing 25% of polyphenols *ad libitum* for 180 days	↓ F4/80+ macrophages infiltration	57
Resveratrol (RSV)		ISO-induced myocardial remodeling model on BALB/c mice	100 mg/kg for 14 days	↓ CD45 + /F4/80 + /Ly6C macrophages infiltration ↓ ICAM-1 and VCAM-1mRNA expression	58
Resveratrol		acute lung injury/acute respiratory distress syndrome in C57BL/6 mice induced with LPS	30 mg/kg treatment after 3 and 24 h after LPS injection	↓ M1 macrophages infiltration ↑ M2 macrophages infiltration	59

treated with MOPE (50 mg/kg and 200 mg/kg, respectively) or 5-aminosalicylic acid (5-ASA; a positive control) individually for 14 days, and from the eighth day, the animals were also given water containing 3% dextran sulfate sodium to induce colitis. It was found that MOPE extract treatment reduced the infiltration of $CD3^+$ T cells, CD177+ neutrophils, and F4/80+ macrophages, and caused a similar effect to 5-ASA. These findings demonstrate the anti-inflammatory effects and mechanisms of MOPE in the colon, indicating its potential use in preventing inflammation-driven diseases. The main polyphenols detected in MOPE astragalin, chlorogenic acid, isoquercitrin, kaempferitrin, luteolin, quercetin, and rutin, as determined by UPLC-QTOF-MS/MS analysis (55).

The anti-inflammatory properties of another polyphenol-rich plant extract (PRPE) derived from different food plants, such as Asteraceae, *Lactuca*; Liliaceae, *Allium cepa*; Lamiaceae, *Ajuga*; and Verbenaceae and *Lippia* were also in an *in vivo* model of obesity, which may be consistently associated with metabolic disorders, cardiovascular complications or cancer (56). The extract mainly contained hydroxycinnamic acids (chlorogenic acid, chicoric acid, and phenylpropanoid caffeic acid glycosides) and flavonoids (quercetin and glycosylated quercetin). Male C57BL/6Rj mice received a chow diet or high-fat/high-sucrose diet for 180 days; after that time, some animals were also supplemented with PRPE containing 25% of polyphenols *ad libitum* for another 180 days. The mice that had consumed a high-fat/high-sucrose diet demonstrated hyperglycemia and hypercholesterolemia, increased oxidative stress and endotoxemia, as well as expanded adipose tissue with enlarged adipocytes, enhanced macrophage infiltration and accumulation of cholesterol and oxysterols. Their median lifespan was also reduced. When the animals were supplemented with PRPE, these parameters were lower; supplementation also caused a significant reduction in the levels of the oxidative stress marker malondialdehyde and a significant increase in blood

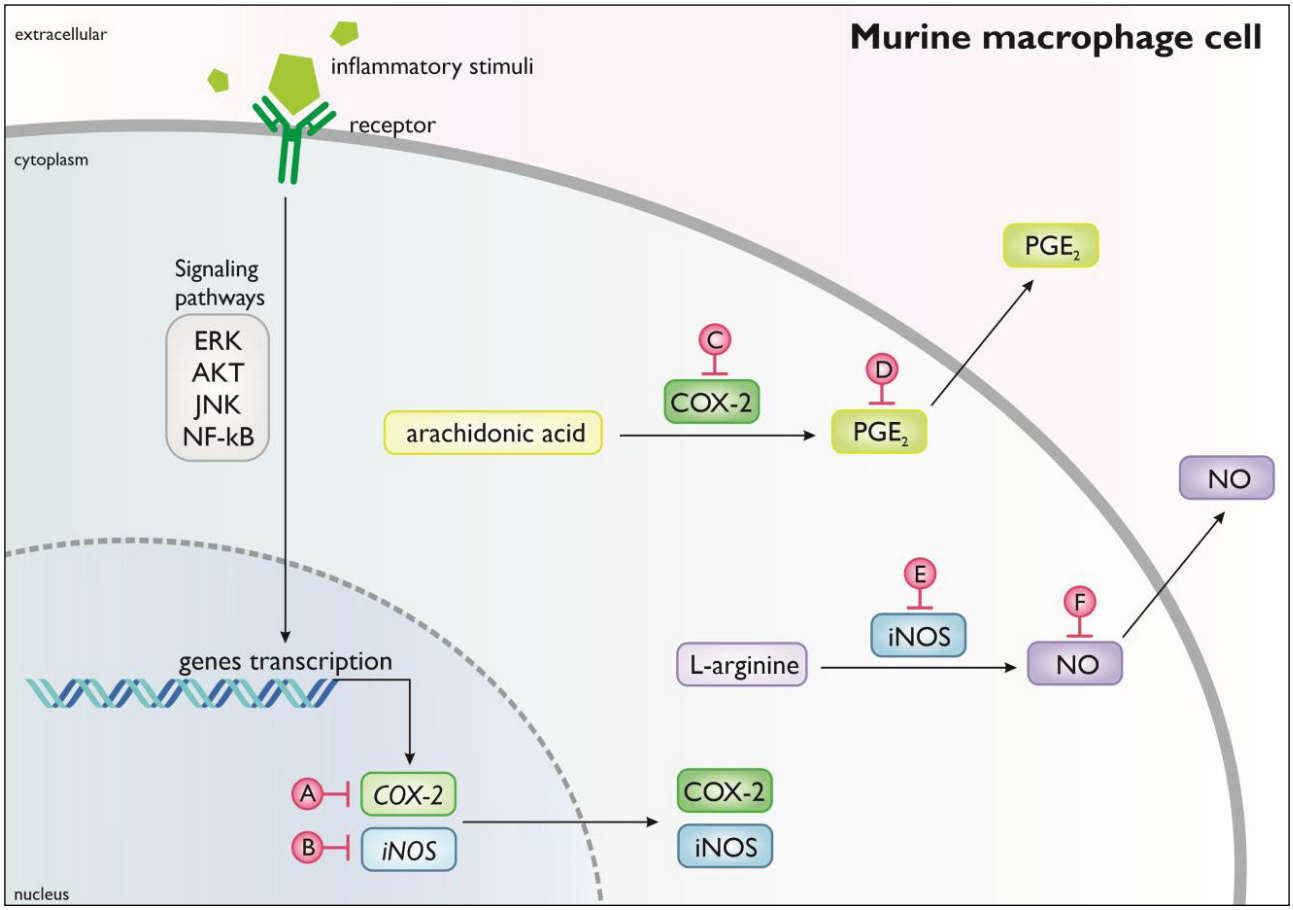

Fig. 1. The scheme presenting the inhibitory impact of polyphenol-rich extracts on pro-inflammatory mediators in murine macrophage cells. (A) EPP, NEPP; 70% ME; Gl-ME; CAVAP-W; SHE; JQLPE; (B) Pc-ME; EPP, NEPP; 70% ME; Gl-ME; TGSBE; CAVAP-W; GSE; JQLPE; (C) SFA, CY; 40% PeTP; LFEE; CAVAP-W; SHE; JQLPE; (D) Gl-ME; SFA, CY; 40% PeTP; LFEE; PPPs, PC, EA; (E) PPEP; SFA, CY; 40% PeTP; LFEE; TGSBE; CEF; SHE; JQLPE; (F) Pc-ME; PEPP; EPP, NEPP; SFA, CY; 70% ME; Gl-ME; 40% PeTP; LFEE; PPPs, PC, EA; TGSBE; CEF; SHE; GSE; JQLPE.

Abbreviations: 40% PeTP, 40% prethanol extract of *Trifolium pretense* L. leaves; 70% ME, 70% methanol *Syzygium cumini* seeds fraction; AKT, protein kinase B; CAVAP-W, crude polyphenols extracted from blossoms of *Citrus aurantium* L. var. *amara* Engl.; CEF, crude *Ecklonia cava* flake extracts; COX-2, cyclooxygenase-2; CY, carthamus yellow; EA, ellagic acid; EPP, NEPP, crude blueberry extracts: extractable polyphenols, non-extractable polyphenols; ERK, extracellular signal-regulated kinase; Gl-ME, *Gouanialeptostachya* leaves and twigs methanol extract; GSE, grape (*Vitis vinifera* L.) seed extract; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; JQLPE, Japanese quince (*Chaenomeles japonica*) leaf polyphenol-rich extract; LFEE, litchi (*Litchi chinensis* Sonn.) flower ethanolic extract; NF-kB, nuclear factor kappaB; NO, nitric oxide; PC, punicalagin; Pc-ME, methanol extract from *Piper cubeba* L.; PGE_2, prostaglandins E_2; PPEP, *Pleurotus eryngii* polyphenol-rich extract; PPPs, PC, EA, pomegranate *Punica granatum* L. peel polyphenols; SFA, dried safflower *Carthamus tinctorius* L. petals aqueous extracts; SHE, Spent hops (*Humulus lupulus* L.) extract; TGSBE, *Ternstroemia gymnanthera* stem bark aqueous extract.

antioxidant defenses. In addition, the number of infiltrating F4/80+ macrophages was reduced. However, PRPE treatment did not affect adipose tissue hypertrophy or adipocyte size, it only markedly reduced its number. Hence, this polyphenolic extract appears to have significant beneficial roles in preventing adipose oxidation and inflammation (57).

In 2020, the anti-inflammatory role of resveratrol (RSV) was assessed on a myocardial remodeling model in mice. Male BALB/c mice were administered with RSV (100 mg/kg of body weight daily) for 14 days; in addition, from day 8, they were also administered isoproterenol to induce pro-inflammatory cytokine expression and macrophage infiltration. It was found that the expression of ICAM-1 and VCAM-1, adhesion molecules necessary for macrophage recruitment into inflammation sites, was significantly up-regulated by isoproterenol. The RSV treatment decreased these levels, as confirmed by flow cytometry and RT-PCR (58). Resveratrol was also found to have an influence on inflammation in a model of acute lung injury/acute respiratory distress syndrome in male C57BL/6 mice. The mice were first injected with LPS for 48 hours to induce inflammation, and then treated with 30 mg/kg resveratrol after three hours and 24 hours. Flow cytometry analysis revealed that LPS significantly increased the level of infiltrating pro-inflammatory M1 macrophages, while resveratrol treatment largely decreased it; in addition, anti-inflammatory M2 macrophage levels were up-regulated (59). The findings indicate that resveratrol, thanks to its beneficial properties, may be used also in the treatment of diseases related to lung inflammation. These few *in vivo* studies investigating the anti-inflammatory activity of the polyphenol-rich extracts and their polyphenol constituents clearly demonstrate that supplementation with natural compounds can bring many health benefits. The inhibitory effects of polyphenol-rich extracts on iNOS, NO, COX-2 and PGE_2 expression and activity are summarized in *Table 1*.

Among numerous natural compounds, polyphenols are recognized as bioactive molecules with a variety of beneficial properties. This review describes the anti-inflammatory activity of several polyphenol-rich extracts, and presents their great potential in the treatment of inflammatory-related diseases. The described extracts have been found to have a significant impact on crucial pro-inflammatory mediators, reducing the gene and protein expression of COX-2 and iNOS, as well as decreasing the production of PGE_2 and NO (*Fig. 1*). Importantly, in most presented cases, polyphenol-rich extracts were also able to modulate cellular signaling, inhibit NF-κB translocation from the cytoplasm to the nucleus, and effectively suppress the phosphorylation of extracellular signal-regulated kinase (ERK), protein kinase B (AKT) and c-Jun N-terminal kinase (JNK). However, most of the reported studies were conducted *in vitro* using RAW264.7 macrophages, and further well-designed animal and clinical studies are necessary to confirm their anti-inflammatory activity. Studies on the bioavailability of polyphenols may be complicated by the interactions with digestive enzymes, proteins or other molecules; in addition, gut microbiota can transform and influence the bioavailability and effects of polyphenols. In contrast, polyphenols and their metabolic products may also inhibit the activity pathogenic bacteria, while stimulating the growth of beneficial cells exerting prebiotic-like effects.

Polyphenols and polyphenol-rich extracts possess noteworthy anti-inflammatory properties. Hopefully, in the future, they may become parts of the treatment of inflammatory diseases.

Abbreviations: 5-ASA, 5-aminosalicylic acid; AKT, protein kinase B; AP-1, activator protein-1; cAMP, cyclic-adenosine monophosphate; c-IAP2, inhibitor of apoptosis protein-2; CAVAP-W, crude polyphenols extracted from the blossoms of *Citrus aurantium* L. var. *amara* Engl.; CEF, crude *Ecklonia cava* flake extracts; COX-2, cyclooxygenase 2; CREB, cyclic-adenosine monophosphate response element binding protein; CY, carthamus yellow; EGCG, epigallocacechin gallate; EPP, extractable polyphenols; ERK, extracellular signal-regulated kinase; G1-ME, methanol extract derived from the leaves and twigs of *Gouanialeptostachya*; GSE, grape seed extract; ICAM-1, intracellular-adhesion molecule-1; IL-6, interleukin-6; iNOS, nitric oxide synthases; JQLPE, Japanese quince (*Chaenomeles japonica*) leaf polyphenol-rich extract; JNK, c-Jun N-terminal kinase; LFEE, litchi (*Litchi chinensis* Sonn.) flower ethanolic extract; LPS, lipopolysaccharides; MOPE, polyphenol extract from *Moringa oleifera* leaves; NEPP, non-extractable polyphenols; NF-κB, nuclear factor-κB; NO, nitric oxide; NOS, nitric oxide synthase; NSAIDs, non-steroidal anti-inflammatory drugs; PeTP, ethanol extract from *Trifolium pretense* L. leaves; PGs, prostaglandins; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGI_2, prostaglandin I_2; PPEP, *Pleurotus eryngii* polyphenol-rich extract; PPPs, pomegranate (*Punica granatum* L.) peel polyphenols; PRPE, polyphenol-rich plant extract; RNS, reactive nitrogen species; ROS, recactive oxygen species; RSV, resveratrol; SFA, aqueous extract derived from the petals of safflower (*Carthamus tinctorius* L.); SHE, spent hops (*Humulus lupulus* L.) extract; SRF, serum responses factor; TCFs, ternary complex factors; TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion protein-1; XIAP, X-linked IAP.

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