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**Permalink**

**Journal**

**ISSN**

**Authors**

Felgus-Lavefve, Laura
Howard, Luke
Adams, Sean H
et al.

**Publication Date**

**DOI**

Peer reviewed

# The Effects of Blueberry Phytochemicals on Cell Models of Inflammation and Oxidative Stress

Laura Felgus-Lavefve,[1] Luke Howard,[1] Sean H Adams,[2,3] and Jamie I Baum[1,4]

[1]Department of Food Science, University of Arkansas, Fayetteville, AR, USA; [2]Department of Surgery, School of Medicine, University of California Davis, Sacramento, CA, USA; [3]Center for Alimentary and Metabolic Science, School of Medicine, University of California Davis, Sacramento, CA, USA; and [4]Center for Human Nutrition, University of Arkansas System Division of Agriculture, Fayetteville, AR, USA

**ABSTRACT**

Blueberries have been extensively studied for the health benefits associated with their high phenolic content. The positive impact of blueberry consumption on human health is associated in part with modulation of proinflammatory molecular pathways and oxidative stress. Here, we review in vitro studies examining the anti-inflammatory and antioxidant effects of blueberry phytochemicals, discuss the results in terms of relevance to disease and health, and consider how different blueberry components modulate cellular mechanisms. The dampening effects of blueberry-derived molecules on inflammation and oxidative stress in cell models have been demonstrated through downregulation of the NF-$\kappa$B pathway and reduction of reactive oxygen species (ROS) and lipid peroxidation. The modulatory effects of blueberry phytochemicals on the mitogen-activated protein kinase (MAPK) pathway and antioxidant system are not as well described, with inconsistent observations reported on immune cells and between models of endothelial, dermal, and ocular inflammation. Although anthocyanins are often reported as being the main bioactive compound in blueberries, no individual phytochemical has emerged as the primary compound when different fractions are compared; rather, an effect of whole blueberry extracts or synergy between different phenolic and nonphenolic extracts seems apparent. The major molecular mechanisms of blueberry phytochemicals are increasingly defined in cell models, but their relevance in more complex human systems needs further investigation using well-controlled clinical trials, in which systemic exposures to blueberry-associated molecules are measured concurrently with physiologic indices of inflammation and oxidative stress. *Adv Nutr* 2022;13:1279–1309.

**Statement of Significance:** Blueberries have been extensively studied for their health benefits using in vitro models of inflammation and oxidative stress, however a comprehensive review of the in vitro literature is lacking. This review provides the first in depth overview of in vitro models of inflammation and oxidative stress using blueberry polyphenols.

## Introduction

Blueberries (*Vaccinium* genus) have been widely studied for their high phytonutrient content, particularly phenolic compounds. Dietary polyphenols found in blueberries consist of flavonoids (anthocyanins, flavonols, proanthocyanidins) and phenolic acids (**Figure 1**). Anthocyanins are the pigments responsible for the color of berries and blueberries have one of the highest anthocyanin contents among foods (1). The individual anthocyanin profile of blueberries is complex and contains 5 of the 6 anthocyanidins commonly present in food: malvidin, cyanidin, delphinidin, petunidin, and peonidin (2, 3). The glycoside moieties attached to the anthocyanidin are predominantly galactose, arabinose, and glucose (2, 4), with all combinations of the 5 anthocyanidins and 3 sugars found across blueberry cultivars. Blueberries are also rich in flavonols, with a predominance of quercetin derivatives (5, 6), and proanthocyanidins, formed by polymerization of catechin and/or epicatechin units (7). Nonflavonoid phenolic acids are mainly represented by chlorogenic acid, which results from the esterification of caffeic acid with a quinic acid molecule (2, 7). Blueberry phytochemicals used in the treatment of cells in vitro often consist of a whole extract, delivered as a reconstituted powder, juice, or pomace (residue after the juice extraction), concentrated or not. Specific classes of phytochemicals such as polyphenol-rich extracts and phenolic fractions, including anthocyanins, phenolic acids, and proanthocyanidins are prepared using solvent extraction and purified through solid phase extraction.
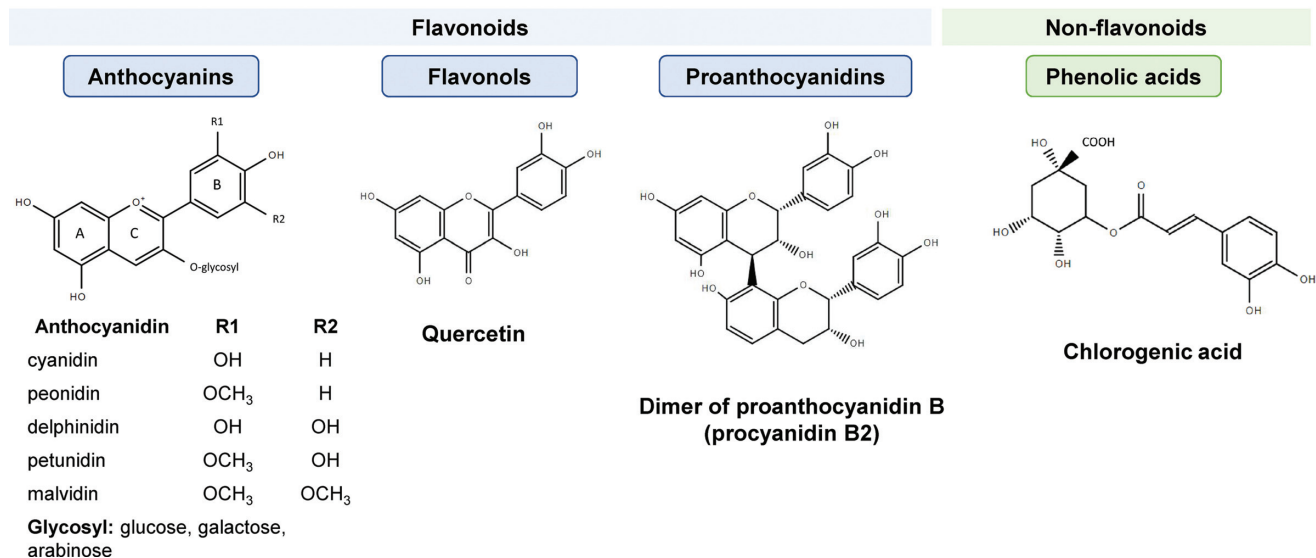
**FIGURE 1** Classes and structures of the main phenolic compounds found in blueberries.

Polyphenols found in blueberries have been shown to contribute to their health benefits (8). A number of reviews discuss the association between blueberry consumption and cardiovascular health (8, 9), inflammatory markers (10), type 2 diabetes, neuroprotection, and ocular health (8). These claims on the health benefits of blueberry consumption are supported by epidemiological studies (11, 12), animal studies (13, 14), and diverse cell culture models (15, 16). Randomized controlled trials have investigated antioxidant and anti-inflammatory effects of blueberry in the context of hypertension, cardiovascular diseases, arthritis, insulin resistance, and metabolic syndrome and supplemented with doses between 20 and 50 g of wild blueberry powder, equivalent to 1 to 2 cups of fresh blueberries daily for 6 to 16 wk (17–21). However, few reported direct modulation of molecular markers via blueberry supplementation, including circulating inflammatory cytokines and adhesion molecules.

To complete and extend the body of literature covering the in vivo physiological effects of blueberry feeding, the current review considers mechanisms of action by focusing on in vitro responses to blueberry components. Evaluation of the bioactive potential of berry phytochemicals or extracts often uses cell models (e.g., immortalized cell lines induced with LPS, proinflammatory cytokines, oxidant species, or physical stimulus), which serve as controlled, simplified systems (22, 23). Numerous limitations exist regarding cell culture conditions and the artificial environment in which the cells are maintained, because they are not completely representative of the body's physiology (24). In general, cells are treated with parent compounds, disregarding potential host and microbial metabolism between consumption and the moment compounds reach the target organ (25, 26). Cells are also not always treated with amounts representative of physiological concentrations in the body, which can be low due to the limited absorption of polyphenols (27, 28). They are relatively simple to access and maintain, and provide insights into the cellular mechanisms of the studied compounds (29). Although blueberry phytochemicals may impact a multitude of health-related mechanisms, we focused on 2 intrinsically related systems (inflammation and oxidative stress), the regulation of which are central to health, and when dysregulated, underlie many disease outcomes. The objective of this narrative review is to discuss observations related to the modulatory role of blueberry phytochemicals on key pathways implicated in systems, and to consider the results from a physiological perspective.

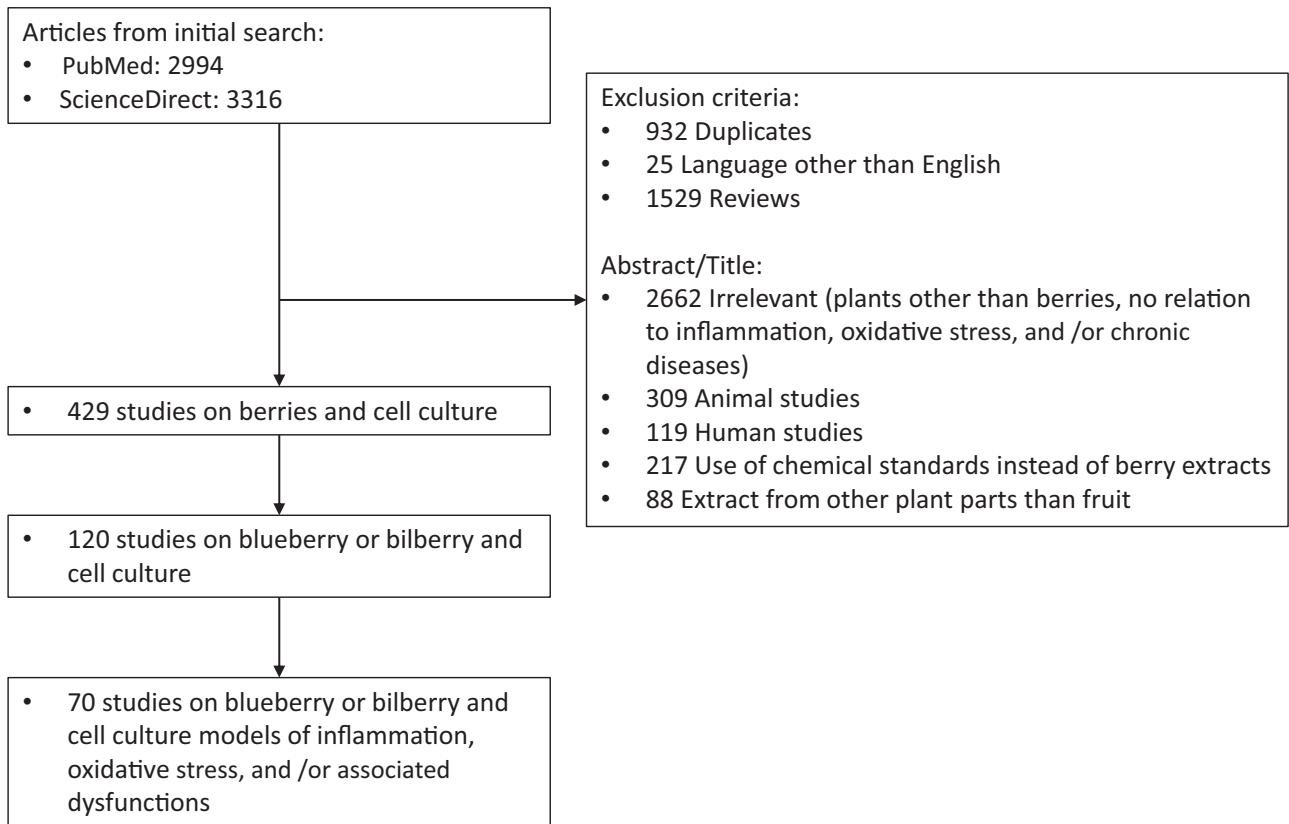### Search strategy

Search queries containing the keywords "inflammation", "anti-inflammatory", "oxidative stress", "cell culture", "in vitro", and "berry" (title/abstract/keywords) were conducted in the PubMed and Science Direct databases. The search was

**FIGURE 2** Flow diagram of included and excluded studies.

conducted for articles through to August 2021. Duplicates, reviews, articles written in languages other than English, and studies using animal models or human participants were excluded (**Figure 2**) with the caveat that studies focusing on animals and/or humans but containing complementary cell-based experiments were included and an evaluation of the cell model findings used in the current review. From all studies on berries and cell culture-based models retrieved, only studies using blueberries were included. In this article, the term "blueberry" encompasses fruits from the *Vaccinium* genus described as blueberries, including *V. angustifolium*, *V. corymbosum, V. ashei, V. uliginosum*, and the European blueberry also referred to as bilberry, *V. myrtillus*. In addition, relevant references from earlier reviews were manually entered. A total of 70 articles related to blueberry and cell culture models of inflammation, oxidative stress, and related conditions were included.

### Blueberry phytochemicals: in vitro models of inflammation and oxidative stress

*Immune system and inflammation models.*
Inflammation is the innate immune system reaction to a stimulus generated by pathogens, damaged cells, carcinogens, toxic compounds, changes in concentrations of reactive oxygen species (ROS), and some foods or metabolites (e.g., certain SFAs and fatty acylcarnitines). General

response mechanisms of inflammation have been extensively reviewed elsewhere (30–32). In brief, the NF-$\kappa$B and mitogen-activated protein kinase [MAPK, subdivided into extracellular-signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38] inflammatory pathways are activated following an external stimulus (e.g., pathogen) and/or by proinflammatory cytokines (e.g., TNF-$\alpha$). Their activation generates the production of proinflammatory cytokines, including TNF-$\alpha$, IL-1$\beta$, and IL-6, which upon release mobilize immune cells (33). Abnormal activation of inflammation-associated proteins, including NADPH oxidase (NOX) (34), inducible NO synthase (iNOS), and cyclo-oxygenase (COX)-2 (35), and failure to resolve the infection or injury can lead to chronic inflammation linked to diseases and cardiometabolic dysfunction.

Studies on murine cell lines include the extensively used monocyte/macrophage RAW 264.7 cells and primary bone marrow-derived macrophages (BMDMs) that can be readily activated by binding ligand to several toll-like receptors (TLRs). Numerous studies on blueberry treatment of murine cell lines use TLR4-activated models, through the addition of the bacterial cell wall component LPS at doses varying from 0.01 to 10 $\mu$g/mL (details in **Table 1**). This produces a strong inflammatory response through, but not limited to, the expression of IL-6, TNF-$\alpha$, or NO (36).

The capacity of blueberry components to reduce proinflammatory marker gene expression and secretion in

**TABLE 1**   Cell-culture-based studies on the effect of blueberry phytochemicals on immune system and inflammation models

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| Whole and/or polyphenolic extracts: | | | | | | | |
| RAW 264.7 | Water and ethanol extracts (5–625 $\mu$g/mL, 24 h[2]) | LPS (10 $\mu$g/mL, 24 h) | Vehicle, challenge | 3 | Viability (MTT), NO (Griess) | Dose-dependent ↓ NO production | [52] |
| RAW 264.7 | Polyphenol extract (75 $\mu$g/mL, 30 min) | LPS (40 ng/mL, 6 h) | Vehicle, challenge | 3 | Gene signals (microarray hybridization), COX-2, IL-1$\beta$, IL-6, PTGS2, TNC, TNF, CCL22, IFI1, IFI47 gene expression (qPCR) | ↓ TNF, IL-1$\beta$, IL-6, TNC, PTGS2, and COX-2 | [37] |
| RAW 264.7 | Polyphenol extract (dose study: 10–400 $\mu$g/mL, 48 h[2] time study: 100 $\mu$g/mL, 6–72 h[2]) | LPS (1 $\mu$g/mL, 24 h) | Vehicle, challenge, positive (tea polyphenols, 100 $\mu$g/mL) | 3 | IL-1$\beta$, IL-6, IL-12 gene expression (qPCR) | Better ↓ IL-1$\beta$ and IL-6 for doses 200 $\mu$g/mL and less. Dose- and time-dependent ↓ of IL-12 | [47] |
| RAW 264.7 | Polyphenol extract (100 $\mu$M, 1 h + 24 h[3]) | LPS (5 $\mu$g/mL, 24 h) or conditioned media from 3T3-L1 adipocytes (24 h) | Vehicle, challenge | 3 | NO (Griess), iNOS, TNF-a, IL-10, MCP-1 (qPCR) | ↓ of LPS- and conditioned media-induced NO secretion. ↓ of LPS-induced IL-10 gene expression, no effect on LPS-induced iNOS and TNF-$\alpha$ gene expression. ↓ of conditioned media-induced iNOS expression | [53] |
| RAW 264.7 | Polyphenol extract (2.7–8.8 mg fresh blueberry equivalent/mL, 24 h) | LPS (100 ng/mL, 18 h) | Not described | 3 | TNF-$\alpha$, IL-6 (ELISA) | ↓ of TNF-$\alpha$ and IL-6, with varying inhibitory concentrations depending on the blueberry cultivar. Negative correlation between cytokines IC50 and phenolic acid content of the extracts | [42] |
| RAW 264.7 | Whole and polyphenol extracts (50–150 $\mu$g/mL, 1 h + 4 h[3]) | LPS (1 $\mu$g/mL, 4 h) | Vehicle, challenge, positive (DEX, 10 $\mu$M) | 3 | Viability (MTT), COX-2, iNOS, IL-6, IL-1$\beta$ gene expression (qPCR) | ↓ of IL-1$\beta$ gene expression by polyphenol extract, but not COX-2, iNOS, and IL-6. No effect of whole extract | [45] |

(Continued)

TABLE 1 (Continued)

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| RAW 264.7 | Freeze-dried pomace extract (10 μg/mL, 1 h) | LPS (10 ng/mL, 4 h) | Vehicle, challenge, positive (DEX, 10 μM) | 3 | Viability (MTT), COX-2, iNOS, IL-6, IL-1β gene expression (qPCR) | ↓ of the 4 gene expressions | (40) |
| RAW 264.7 | Whole extract (250 μg/mL for hybrid cultivars, 50 μg/mL for lowbush, 1 h) | LPS (1 μg/mL, 18 h) | Vehicle, challenge, positive (DEX, 10 μM) | 2 | Viability (MTT), ROS (DCFDA), NO (Griess), IL-1β, COX-2, iNOS, IL-6 gene expression (qPCR) | ↓ proinflammatory cytokines transcription. Better effect for cultivars with higher anthocyanin content | (39) |
| RAW 264.7 | Whole extract (0.2–0.5 mg/mL, 3 h) | LPS (1 μg/mL, 24 h) | Vehicle, challenge | 3–4 | Cell viability (MTT), TNF-α, IL-6, IL-10 (ELISA), COX-2, iNOS (WB) | Slight ↓ IL-6 at 0.2 but not 0.5 mg/mL. Dose-dependent inhibition of TNF-α. ↑ IL-10, no effect on COX-2 and iNOS production | (41) |
| RAW 264.7 | Extractable (EPP) and nonextractable (NEPP) polyphenol extracts (10–400 μg/mL, 6–72 h[2]) | LPS (1 μg/mL, 24 h) | Vehicle, challenge, positive (tea polyphenols, 100 μg/mL) | 3 | NO (detection kit), iNOS, COX-2 gene expression (qPCR), NF-κB (WB) | ↓ NO production, iNOS gene expression and inhibition of NF-κB activation by both extracts. Higher inhibitory effect of EPP than NEPP on COX-2 gene expression | (50) |
| U-937, differentiated into macrophages | Phenolic extract (15.6–500 μg/mL, 30 min for luciferase assay, 2 h for ELISA) | F. nucleatum (MOI 100, 6 h for luciferase assay, 48 h for ELISA) | Vehicle, challenge, positive (BAY-11–7082, inhibitor of NF-κB, 25 μM) | 3 | Viability (MTT), NF-κB activity (luciferase activity), TNF-α, IL-1β, IL-6, CXCL8, MMP-8, MMP-9 (ELISA) | Dose-dependent ↓ of NF-κB activation, IL-1β, IL-6, and TNF-α production. ↓ in CXCL8 (doses ≥ 125 μg/mL). ↓ MMP-8 and MMP-9 secretion | (56) |
| THP-1 (monocytes) | Blueberry and bilberry pomace (0.04–1 mg/mL, 1.5 h) | LPS (1 μg/mL, 1 h) | Vehicle, challenge | 3 | Viability (flow cytometry), NF-κB (flow cytometry), IL-8 (ELISA), CCL22, IL-8, IL-10, IL-6, TNF-α, IP10, IL-23, MMP-9 (multiplex assay), IL-6, TGF-β, TNF-α, IL-10, IL-23 (qPCR), COX-2 (fluorometric kit) | ↓ of NF-κB translocation by bilberry extract (1 mg/mL) and blueberry extracts (0.2 and 1 mg/mL). ↓ of proinflammatory cytokines by both berries, except for CCL22 and IL-10. Greater ↓ of COX-2 by blueberry (dose-dependent) than bilberry pomace | (59) |

*(Continued)*

**TABLE 1** (*Continued*)

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| THP-1 (monocytes) | Whole extract (10 µg/mL, 20 min + 20 min[3] for WB or 24 h[3] for other assays) | TNF-α or IFN-γ (100 ng/mL, 30 min or 24 h) | Vehicle, challenge | 4 | STAT1, STAT3, ERK, JNK, p38, NF-κB phosphorylation (WB), TNF-α, MCP-1, IL-6 (ELISA), MCP-1, IL-6, ICAM-1, T-bet, IL-12, IL-1β, IL-10 (qPCR) | IFN-γ challenge: ↓ in STAT1 and STAT3 phosphorylation. No effect on MAPK pathway. Significant ↓ of TNF-α and MCP-1, complete inhibition of IL-6. ↑ in IL-10 gene expression TNF-α challenge: ↓ MCP-1 and TNF-α secretion. ↑ NF-κB phosphorylation and ↓ ERK. No significant changes for p38 and JNK. ↑ gene expression of IL-6, IL-8, MCP-1, IL-1β, IL10, and ↓ ICAM-1 | (61) |
| THP-1, differentiated into macrophages | Polyphenol extract (1.53 mg/mL, 2 h) | LPS (5 µg/mL, 48 h) | Vehicle, challenge | 5–6 | Viability (LDH), caspase-3 (fluorescence), NO (Griess), TNF-α, IL-6, and IL-10 gene expression (qPCR), IL-6 secretion (Luminex xMAP) | No effect of blueberry treatment on NO secretion. ↑ TNF-α and ↓ IL-10 gene expression compared to LPS alone. ↓ IL-6 secretion, but no significant effect on IL-6 gene expression | (60) |
| PBMCs | Anthocyanin (10 µg/mL, 30 min[3] for WB or 24 h[3] for other assays) | IFN-γ (100 ng/mL, 30 min or 24 h) | Vehicle, challenge | 4 | STAT1, STAT3, and p38 phosphorylation (WB), MCP-1, IL-6, ICAM-1, (qPCR) | ↓ STAT1 and STAT3 phosphorylation but ↑ p38 phosphorylation. ↓ ICAM-1, MCP-1, and IL-6 gene expression | (61) |
| Phenolic and polyphenolic fractions: | | | | | | | |
| RAW 264.7 | Whole and polyphenol extracts, anthocyanin, proanthocyanidin phenolic acid fractions (50 µg/mL, 1 h) | LPS (1 µg/mL, 6 h) | Vehicle, challenge, positive (DEX, 10 µM) | 3 | Viability (MTT), COX-2, iNOS, IL-6, IL-1β, MCP-1 gene expression (qPCR) | ↓ COX-2 by all fractions except phenolic acid. Better ↓ iNOS and IL-1β by proanthocyanidin. Better ↓ of MCP-1 expression by anthocyanin | (43) |

(*Continued*)

**TABLE 1** (*Continued*)

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| U-937, differentiated into macrophages | Proanthocyanidin fraction, viability tests (3.9–500 μg/mL, 1 h[3]), other tests (31.25–125 μg/mL, 2 h for ELISA, 30 min for NF-κB) | Leukotoxin (viability: 1 μg/mL, 1 h), A. actino-mycetemcomitans LPS (1 μg/mL, 6–24 h) | Vehicle, challenge, positive (BAY-11–7082, 25 μM) | 3 | Viability (MTT), IL-1β, IL-6, CXCL8, TNF-α, MMP-3, and MMP-9 (ELISA) | ↓ leukotoxin cytotoxic effect on cells by proanthocyanidin extract. Dose-dependent ↓ of proinflammatory cytokine and MMPs production | (58) |
| Anthocyanin fractions: | | | | | | | |
| RAW 264.7 | Anthocyanin (400–1200 μg/mL, 1 h) | LPS (1 μg/mL, 24 h) | Vehicle, challenge, positive (DEX, dose not described) | 3 | Viability (MTT), NO (Griess), PGE₂, INF-γ, IL-1β, and IL-6 (ELISA), MCP-1, IL-6, TNF-α, IL-1β, COX-2 gene expression (qPCR), COX-2, NF-κBp65 proteins (WB) | Dose- and time-dependent ↓ of NO production. Dose-dependent ↓ of cytokines. No significant effect on TNF-α gene expression. ↓ COX-2 protein expression and NF-κB p65 | (51) |
| RAW 264.7 and BMDM | Anthocyanin (RAW 264.7: 2.5–20 μg/mL, 12 h + 1 to 3 h[3], BMDM: 20 μg/mL, 12 h + 3–12 h[3]) | LPS (RAW 264.7: 100 ng/mL, 1 h for WB, 3 h for other assays; BMDM: 3–12 h[3]) | Vehicle, challenge | 3–9 | RAW 264.7: TNF-α, IL-1β gene expression (qPCR), TNF-α cytokines (ELISA), NF-κB translocation (WB)  BMDM: ROS (DCFDA), TNF-α, IL-1β, NOX-1 gene expression (qPCR) | RAW 264.7: ↓ IL-1β and TNF-α gene expressions and TNF-α secretion. ↓ NF-κB translocation  BMDM: no alteration in ROS production after 3 h but ↓ in cells expressing Nrf-2 after 12 h. ↓ IL-1β and NOX-1 in both cell phenotypes | (44) |
| U-937, differentiated into macrophages | Anthocyanin (1–25 μg/mL, 1 h or 3 h[2]) | LPS (1 μg/mL, 1 h[2] or 3 h) | Vehicle, challenge | 3 | TNF-α gene expression (qPCR) | ↓ TNF-α, significant when the treatment was administered before the LPS induction | (57) |
| THP-1 | Anthocyanin (10 μg/mL, 20 min + 30 min[3] for WB or 24 h[3] for qPCR) | IFN-γ (100 ng/mL, 30 min or 24 h) | Vehicle, challenge | 3 | STAT phosphorylation (WB), IFN-γ R1, IFN-γ R2, SOCS1, SOCS3 (qPCR) | ↓ IFN-γ R1 expression induced by challenge and ↑ in IFN-γ R2 expression decreased by challenge. ↓ SOCS1 and SOCS3 mRNA expression. ↓ STAT phosphorylation, but not through inhibition of protein tyrosine phosphatase activation | (62) |

*(Continued)*

**TABLE 1** (Continued)

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | N[1] | Controls | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| Others: | | | | | | | |
| RAW 264.7 | Polyphenol extract (19–78 $\mu$g/mL, 1 h), volatile extract (7–28 ng/mL, 1 h) | LPS (100 ng/mL, 24 h) | 3 | Vehicle, challenge | Viability (MTS), NO (Griess), IL-6, TNF-$\alpha$, PGE$_2$ (ELISA), COX-2, NF-$\kappa$B, I$\kappa$B$\alpha$ (WB) | ↓ proinflammatory markers by both extracts. Higher ↓ of IL-6 by volatile extracts | (38) |

[1]N, number of experimental replicates, as stated by the authors. BMDM, bone marrow-derived macrophages; CCL22, CC chemokine ligand 22; COX-2, cyclo-oxygenase-2; CXCL, chemokine (C-X-C motif) ligand; DCFDA, 2,7'-dichlorofluorescin diacetate; DEX, dexamethasone; ERK, extracellular-signal-regulated kinase; ICAM, intercellular adhesion molecule-1; IFI, interferon-inducible protein; iNOS, inducible nitric oxide synthase; I$\kappa$B$\alpha$, NF-$\kappa$B inhibitor $\alpha$; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide; Nrf-2, Nuclear factor erythroid 2-related factor 2; NOX-1, NADPH oxidase; PGE$_2$, prostaglandin E2; PTGS2, prostaglandin-endoperoxide synthase; ROS, reactive oxygen species; STAT, signal transducers and activators of transcription; TNC, Tenascin C; SOCS, suppressor of cytokine signaling; WB, Western blot.

[2]Blueberry treatment administered after challenge.

[3]Blueberry and challenge in coadministration. If no symbol is indicated, the cells were treated with the blueberry extracts first before (Table 2) challenge.

LPS-induced murine cell models (RAW 264.7 and BMDMs) has been shown in several studies, although the anti-inflammatory effect seems to be carried out by an array of compounds rather than an individual blueberry phenolic fraction. Diverse blueberry products, including blueberry pomace, whole blueberry extracts, polyphenol-rich extracts, or specific phenolic fractions lowered the production of cytokines by the cells in a dose-dependent manner. Further details on the treatments and doses used in the studies are reported in Table 1. For instance, blueberry phytochemicals reduced the gene expression and secretion of proinflammatory cytokines induced by LPS, particularly IL-6 (37–43), IL-1$\beta$ (37, 39, 40, 43–45), and TNF-$\alpha$ (37, 38, 41, 42, 44) in RAW 264.7 cells and BMDMs compared with the LPS-induced control. However, it is not clear which fraction or specific phenolic compound exerts a more potent effect, especially since their activities seem to be cytokine specific. For example, Esposito et al. (43) reported a better ability of a proanthocyanidin fraction to reduce IL-1$\beta$ compared with the polyphenol extract, anthocyanin, or phenolic acid fractions. However, IL-6 gene expression was not suppressed by that same proanthocyandin fraction, and monocyte chemoattractant protein (MCP)-1 expression (43), a chemokine that regulates monocyte infiltration (46), was inhibited by the blueberry anthocyanins but not by the other phenolic fractions. Although the large variation between the blueberry treatment doses used across different reports hinders direct comparisons between the studies, most have shown a dose-dependent regulation of cytokines (39, 41, 44). Cheng et al. (47), however, reported 40–60% inhibition of IL-1$\beta$ gene expression with ≤200 $\mu$g/mL blueberry extract, but no effect when the treatment dose was increased to 400 $\mu$g/mL (47). This observation was tentatively explained by the increased phagocytic activity of the macrophages in the presence of a high polyphenol concentration.

iNOS is an enzyme responsible for the synthesis of NO, a mediator secreted by neutrophils and macrophages to induce vasodilation, mediate the immune response, or regulate apoptosis (48, 49). After stimulation of RAW 264.7 cells by LPS, blueberry extracts inhibited iNOS gene expression (40, 50) and NO production (38, 39, 50–53), with extracts from anthocyanin-rich cultivars (39) and blueberry proanthocyanidin fractions (43) being particularly effective (Table 1). COX-2 also plays a central role in the induction of inflammation. It is involved in the formation of prostaglandins, including prostaglandin E2 (PGE-2) responsible for the induction of pain (54). Blueberry extracts have consistently reduced COX-2 gene expression in RAW 264.7 cells (37–40, 43, 50, 51) (Table 1), although Mueller et al. (41) and Grace et al. (45) did not report a significant reduction of iNOS and COX-2 gene expression by whole blueberry or polyphenol-rich extracts. Although most studies have focused on blueberry polyphenols, Gu et al. (38) reported the anti-inflammatory effect of the volatile extracts of several berries compared with their phenolic counterpart. Volatile and phenolic blueberry extracts showed

similar inhibitory effects on inflammatory cytokines, NO, and COX-2 production, even though the volatile fraction was tested at a lower concentration than the phenolic fraction. This provides initial evidence that the volatile fraction of blueberries also contains molecules with anti-inflammation properties, but this remains to be validated.

The anti-inflammatory activities of blueberry phytochemicals have also been demonstrated in cell lines derived from humans (23), including the U-937 and THP-1 monocyte-like cells, that can be differentiated into macrophages after stimulation with phorbol-12-myristate-13-acetate (PMA) (55), and human primary peripheral blood mononuclear cells (PBMCs). In the context of blueberry studies, a variety of compounds have been used to induce inflammation, including *Fusobacterium nucleatum* bacteria (56), LPS (57–60), or cytokines (61, 62) (Table 1). Blueberry extracts exerted an inhibitory effect toward cytokine secretion (56, 58–60) and matrix metalloproteinase (MMP)-8 and 9 production (56, 58), in cells triggered by bacteria or LPS. Blueberry extracts decreased TNF-$\alpha$ gene expression induced by LPS in THP-1 monocytes (59) and U937 macrophages (57), but on the contrary, increased its expression in THP-1 differentiated macrophages (60). The regulatory effect reported in most studies was associated with a decrease in NF-$\kappa$B translocation in THP-1 cells (59). In PBMCs and THP-1 cells alternatively induced with either IFN-$\gamma$ or TNF-$\alpha$, the effects of a blueberry treatment were less robust (Table 1). Cytokine secretion and adhesion molecule gene expression were inhibited by a blueberry extract in IFN-$\gamma$-induced PBMCs, but the same blueberry extract further increased the proinflammatory marker secretion when the cells were induced with TNF-$\alpha$ (61). These observations were tentatively explained by looking at the pathways activated by the different cytokines (IFN-$\gamma$ or TNF-$\alpha$): the signal transducers and activators of transcription (STAT) pathway activation was inhibited by coincubation of IFN-$\gamma$ with the berry treatment, but NF-$\kappa$B was enhanced by the addition of TNF-$\alpha$ combined with a blueberry extract (61). A follow-up study demonstrated that the IFN-$\gamma$ receptor 2, responsible for transducing the signal conveyed by the proinflammatory cytokines, was inhibited by the blueberry anthocyanins (62). These observations on cell models induced with non-LPS ligands suggest that the immunomodulatory effects of blueberry compounds are context and pathway specific.

In summary, blueberry phenolic and polyphenolic extracts have been shown to dampen inflammation in RAW 264.7, U-937, BMDMs, and PBMCs challenged with inflammation inducers, through the reduction of proinflammatory cytokine gene expression and secretion, and inhibition of NF-$\kappa$B translocation to the nucleus. No specific fraction emerges as being more potent, suggesting a general effect of multiple phytomolecules rather than a single compound. More studies are warranted to better define molecular targets of blueberry-derived molecules and to assess the involvement of TLR-dependent and -independent pathways.

### Oxidative stress models.

ROS and free radicals are natural by-products of enzymatic reactions produced during metabolism (63, 64). When controlled, ROS production is used for signaling in metabolic processes (65). Environmental factors, lifestyle, and pathologies contribute to an unbalanced state, where ROS production overwhelms the defense capacity of the cells and induces oxidative stress (66). This state leads to protein and nucleic oxidation, and lipid peroxidation, which can impair enzymatic processes, induce breakage of DNA strands, and may lead to cell death (67–69). Endogenous antioxidant defense mechanisms exist in the body to limit the production and deleterious effects of ROS. Superoxide dismutase (SOD), found in the membrane or cytosolic fractions of cells, converts superoxide radicals ($O_2^-$) to $H_2O_2$ and $O_2$ (70). Glutathione peroxidase (GSH-PX), via the oxidation of glutathione *S*-transferase, reduces lipid peroxide and converts $H_2O_2$ to $H_2O$ (71). Enzymes, including DNA glycosylases, repair damaged DNA (72). Oxidation and inflammation are intricately related, as cytokines and chemokines secreted by inflammatory cells can trigger ROS production. In turn, ROS activate proinflammatory pathways, including NF-$\kappa$B, and sustain the cycle of oxidative and inflammatory stress (10, 65). These conditions favor the development of chronic pathologies such as cancer (73), cardiovascular (74), inflammatory (75), and neurodegenerative diseases (76).

The effect of blueberry phytochemicals on oxidative stress has been evaluated using several cell models, including neurons (77), fibroblasts (78, 79), hepatocytes (80, 81), enterocytes (82–86), and epithelial cells (82, 87). Despite the diversity in models used, studies overlap in terms of the endpoints measured, which focus on evaluation of the modulation in ROS production and lipid peroxidation, increases in antioxidant enzyme activities, and protection of DNA against oxidative damage (**Table 2**).

The induction of oxidative stress in a variety of cells was attenuated by treatment with blueberry extracts, principally through the decreased formation of ROS (77, 78, 80, 84, 85), but also increased scavenging activity (79), and/or reduction of lipid peroxidation (77, 79). This effect, however, was only partly explained by the regulatory effect of the blueberry compounds on antioxidant enzymes, which were upregulated in neuronal cells treated with blueberry juice (77) and Caco-2 cells incubated with a polyphenol-rich blueberry extract (84). Glutathione concentrations, however, remained unchanged in Caco-2 cells treated with an anthocyanin fraction (85). In addition, a blueberry pomace extract tested on Chinese hamster ovary (CHO) epithelial cells and a human colon cancer cell line (HT-29) failed to stimulate the transcription of detoxification enzymes such as heme oxygenase (HO-1) and NADPH quinone oxidoreductase-1 (NQO-1) (88), both involved in the antioxidant/oxidant balance (89, 90). In contrast with those observations made on blueberry parent compounds, phloroglucinol aldehyde (an anthocyanin degradation colonic product) increased the

**TABLE 2**  Cell-culture-based studies on the effect of blueberry phytochemicals on oxidative stress models

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| **Whole and/or polyphenolic extracts:** | | | | | | | |
| SH-SY5Y | Lyophilized juice (25–100 μg/mL, 24 h) | $H_2O_2$ (100 μM, 30 min) | Vehicle, challenge | 3 | Viability (MTT), ROS (DCFDA), lipid peroxidation (TBARS), antioxidant enzymes activity (spectrophotometric assay) | ↓ intracellular ROS formation and lipid oxidation. ↑ catalase and SOD activity | (77) |
| L-929 | Lyophilized juice (0.05–12.5 mg/mL, 1–1.5 h[2]) | t-BuOOH (200 μM, 1–1.5 h) | Vehicle, challenge | 3 | ROS (DCFDA) | Dose-dependent ↓ ROS production (dose ≥6.25 mg/mL) | (78) |
| CHO and HT-29 | Pomace (50–200 μg/mL, 20 h) | n/a | Vehicle, positive (CDDO-imidazolide) | 4 | CHO: Nrf-2 luciferase expression (reporter gene assay); HT-29: phase II enzyme-transcription (qPCR) | No effect of extract on Nrf-2 luciferase activity nor relative transcription level of phase II enzymes | (82) |
| Caco-2 | Whole extract (1.8–7 mg/mL, 2–24 h) | t-BuOOH (100–150 μM, 0.5–1 h) | Vehicle challenge | 3 | Viability (trypan blue exclusion), DNA damage (Comet), free radical generation (electron spin resonance spectroscopy measurements), gene expression (qPCR) | Dose-dependent ↓ DNA damage. ↓ free radical formation at higher concentrations | (83) |
| **Phenolic and polyphenolic fractions:** | | | | | | | |
| V79-4 | Phenolic extracts, by enzymatic hydrolysates (25–100 μg/mL, 0.5–2 h) | $H_2O_2$ (1 mM, 5 min–24 h) | Vehicle challenge | 3 | Viability (MTT), ROS (DCFDA), lipid peroxidation (malondialdehyde measurement), DNA damage (Comet), nuclear morphology (Hoechst staining) | Dose-dependent ↑ ROS scavenging activity. ↓ lipid peroxidation, DNA damage ,and nuclear fragmentation | (79) |
| Caco-2 and HT-29 | Whole extract, anthocyanin, phenolcarbonic acid, polymeric fractions (5–500 μg/mL extract, or corresponding concentration of each fraction in the extract, 1 h) | t-BuOOH (250 μM, 40 min) or Menadione (Caco-2: 6 μM, HT29: 20 μM, 1 h) | Vehicle, challenge | 3–6 | Cytotoxicity (Alamar blue assay), ROS (DCFDA), tGSH concentrations (kinetic assay), oxidative DNA damage (Comet) | Caco-2: ↓ ROS production and DNA damage and ↑ tGSH after 24 h. Fractions: ↓ DNA damage by phenolcarbonic acid fraction only. No modulatory effects of the anthocyanin fraction on Caco-2 | (84) |

(Continued)

**TABLE 2** (Continued)

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| Anthocyanin fractions: Caco-2 and HT-29 | Anthocyanin (0.01–500 µg/mL, 1–24 h) | t-BuOOH (250 µM, 40 min), Menadione (Caco-2: 6 µM, HT29: 20 µM, 1 h) | Vehicle, challenge, positive (quercetin, 30 µM) | 3–6 | Cytotoxicity (Alamar blue assay), ROS (DCFDA), glutathione concentrations (kinetic assay), oxidative DNA damage (Comet) | HT-29: Slight ↓ ROS by berry extract after 24 h. No modulation of oxidative DNA damage or tGSH concentrations; Caco-2: ↓ ROS production at dose 50 µg/mL. Slight ↓ DNA damage, nonsignificant modulation of tGHS; HT-29: ↓ ROS production (dose 250 µg/mL) | (85) |
| IPEC-1 | Anthocyanin (1.6–200 mg/L, 3–24 h) | AAPH (1–15 mmol/L, 24 h) | Vehicle, challenge | 2 | Viability (MTT), cell cycle (flow cytometer), proliferation (BrdU), ROS (DCFDA) | Dose-dependent ↓ peroxyl radical formation. No protective effect on stress-induced cytotoxicity and cell cycle perturbation | (86) |
| HepG2 | Anthocyanin: water, ethanol, and methanol extracts (75 µg/mL, 12 h) | UV irradiation (4 mJ/cm²) | Vehicle, challenge | 3 | Viability (MTT), γH2AX (immunofluorescence staining), DNA damage (Comet), ROS (DCFDA), p53 and p21 gene expression (qPCR) and protein expression (WB) | Protective effect against DNA damage by methanol extract through ↓ γH2AX phosphorylation and ROS production. ↓ p53 and p21 gene and protein expression by all extracts | (80) |
| HepG2 | Anthocyanin (25–75 µg/mL, overnight) | UV irradiation (30 mJ/cm²) | Vehicle, challenge | 3 | Viability (MTT), morphology (SEM), nuclear morphology (Hoechst staining), cell cycle analysis (flow cytometry), mitochondrial potential (laser confocal scanning microscopy), DNA damage (Comet), Gadd45 and MDM2 gene expression (qPCR, Gadd45, MDM2, p21, and p53 proteins (WB) | ↓ UV damage on cell and nuclear morphology. Restoration of cell cycle and mitochondrial membrane potential. Dose-dependent protection against DNA damage. ↓ Gadd45, MDM2, p21, and p53 expression | (81) |

*(Continued)*

**TABLE 2**  (Continued)

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| HPAEpiC | Anthocyanin (75 µg/mL, 72 h) | Radiation (2 Gy/min, 7.5 min) | Vehicle challenge | — | Caspase-3, Bax, Bcl-2, PKR (qPCR and WB), viability (MTT) | Protective effect against cell death and modulation of Bcl-2, Bax, and caspase 3 gene expression in cells transfected with control small interfering RNA but not PKR-siRNA | (87) |

[1]N, number of experimental replicates, as stated by the authors. AAPH, 2,2-azobis(2-amidinopropane)dihydrochloride; Bax, B-cell lymphoma 2 (Bcl-2) associated X-protein; Bcl-2, B-cell lymphoma 2 (Bcl-2); BrdU, bromodeoxyuridine; CHO, Chinese hamster ovary; CDDO-imidazolide, 1[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole; DCFDA, 2′,7′-dichlorofluorescin diacetate; Gadd45, growth arrest and DNA damage-45; MDM2, mouse double minute 2; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide; Nrf-2, nuclear factor erythroid 2-related factor-2; PKR, protein kinase R; ROS, reactive oxygen species; SEM, scanning electron microscope; t-BuOOH, tert-butylhydroperoxide; tGSH, total glutathione; SOD, superoxide dismutase; WB, Western blot.
[2]Blueberry and challenge in coadministration. If no symbol is indicated, the cells were treated with the blueberry extracts first before challenge.

transcription activity of nuclear factor erythroid 2-related factor 2 (Nrf-2), which when induced by oxidative stress stimulates the transcription of HO-1 and NQO-1 (88). This suggests that blueberry metabolite derivatives may contribute to the antioxidant activity of the berries.

A consequence of oxidation is DNA damage (69) and potentially increases in cell death. Blueberry extracts demonstrated a protection against DNA damage induced by hydroxide peroxide (79) and tert-butylhydroperoxide (*t*-BuOOH) (83, 85), although in Caco-2 cells, only the blueberry phenolcarbonic acid fraction reduced DNA damage compared with whole blueberry extract, anthocyanin, and polymeric fractions (84). Protective effects in terms of DNA damage and cell death were attributed to blueberry anthocyanin fractions in liver cells (HepG2) and pulmonary epithelial cells (HPAEpiC) exposed to light or ionizing radiation, through modulation of apoptosis and cell cycle regulatory gene expressions (80, 81, 87). However, no improvement of cell cycle perturbation induced by 2,2-azobis(2-amidinopropane)dihydrochloride (AAPH) was reported in intestinal epithelial cells IPEC-1 treated with blueberry anthocyanins (86).

To summarize, blueberry extracts and phenolic fractions demonstrated protective effects against oxidative stress, which were mainly explained through reduction of ROS production and protection against DNA damage induced during oxidation. Additional studies comparing cell models and/or blueberry fractions using similar experimental parameters are necessary to demonstrate the effects of the treatment on antioxidant enzymes, and fully understand the antioxidant contribution of blueberry phytochemicals versus their metabolic by-products. There is also a need to evaluate the potential effects of blueberry volatiles in this regard.

### Blueberry phytochemicals, inflammation, and oxidative stress: perspectives on physiological functions
*Endothelial and vascular inflammation models.*
Atherosclerosis, a chronic inflammatory disease of the arterial wall (91), is characterized by the buildup of plaques in arteries and is the most frequent underlying condition for the development of cardiovascular diseases (92). Human umbilical vein endothelial cells (HUVECs) and human microvascular vein endothelial cells (HMVECs) provide a model to study normal as well as oxidation and inflammation-related dysfunctions. Studies on the effect of blueberry compounds in endothelial cell models are detailed in **Table 3**.

Risk factors including smoking, aging, hypercholesterolemia, and hyperglycemia (93) promote the retention of lipids, particularly LDL prone to oxidation in the vascular wall, causing the activation of inflammatory processes (94). The treatment of endothelial cells exposed to oxidative stress triggers with blueberry anthocyanins demonstrated protective effects toward ROS secretion and lipid peroxidation (95). A similar observation was reported with cells treated with blueberry exosome-like nanoparticles (ELN),

**TABLE 3** Cell culture-based studies on the effect of blueberry phytochemicals on endothelial and cardiovascular models

| Cell line (P[1]) | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[2] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| Whole and/or polyphenolic extracts: | | | | | | | |
| HUVEC | Whole extract (1000 $\mu$g/mL, 3–24 h) | P2X$_7$ agonist bzATP (300 $\mu$M) and antagonist AZ11645373 (100 nM, 24 h) | Vehicle, challenge | 3–4 | Viability and proliferation (Trypan blue), vesiculation (calcein staining), Akt, p38 (WB), P2X$_7$ (qPCR) | ↓ HUVEC vesiculation. ↓ Akt phosphorylation, but not p38. ↓ P2X$_7$ transcription. → or prevention of increase of genes involved in extracellular vesicle release | (104) |
| HUVEC (P#2) | Polyphenol extract (0.1–1 mg/mL, 24 h) | | Vehicle, negative (wortmannin, 30 nmol/L, 30 min) | 3 | Viability (Trypan blue), apoptosis (colorimetric assay), Akt (Alpha Screen SureFire Assay), migration (scratch assay), angiogenesis (microscopy) | Dose-dependent ↑ Akt activation. ↑ cell migration and capillary-like tube formation | (108) |
| HUVEC | Polyphenol extract (0.3–30 $\mu$g/mL, 4 h to 11 d[3] for tube formation assay) | VEGF-A (10 ng/mL) | Vehicle, challenge | 3–6 | Tube formation assay (coculture with fibroblast), proliferation (WST-8), migration (microscopy), ERK, PLC-$\gamma$, Akt (WB) | Dose-dependent ↓ tube formation. ↓ cell proliferation and cell migration. ↓ ERK and Akt phosphorylation but not PLC-$\gamma$ | (109) |
| HUVEC | Polyphenol extract (40 $\mu$g/mL, 3–24 h[3]) | LPS (100 ng/mL, 3–24 h) | Vehicle, challenge | 3 | Viability (MTT), PLC enzyme gene expression (qPCR), PLC enzyme distribution (immunofluorescence) | Time-dependent ↑ cell viability with blueberry treatment. No significant modulation of PLC expression | (110) |
| RAW 264.7 | Polyphenol extract (2.7–8.8 mg fresh blueberry equivalent/mL, 24 h) | LPS (100 ng/mL, 18 h) | Not described | 3 | miRNA expression (qPCR) | ↓ miRNA expression, generally upregulated during vascular inflammation | (42) |
| Polyphenolic fractions: | | | | | | | |
| HMVEC | Anthocyanin and hydroxycinnamic acids fractions (0.01–0.1 mg/mL, 30 min–2 h) | TNF-$\alpha$ (10–20 ng/mL, 6 h) or H$_2$O$_2$ (100 $\mu$M, 2 h) | Vehicle, challenge | — | ROS (DCFDA), lipid peroxidation (fluorescent membrane fatty acid probe), ICAM-1, MCP-1, IL-8 (ELISA), viability (MTT) | ↓ ROS production induced by H$_2$O$_2$. Time-dependent but not dose-dependent protection of membrane fatty acid. ↓ production of IL-8, MCP-1, ICAM-1 induced by TNF-$\alpha$ | (95) |
| HUVEC (+ THP-1) | Anthocyanin and phenolic acid fractions (0.01–10 $\mu$g/mL, 24 h) | TNF-$\alpha$ (100 ng/mL, 24 h) | Vehicle, challenge | 3 | Viability (MTT), THP-1 cell adhesion (fluorescence of labeled cells) | ↓ THP-1 adhesion by both fractions | (102) |

(Continued)

**TABLE 3** (Continued)

| Cell line (P#[1]) | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[2] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| Anthocyanin fractions: | | | | | | | |
| HUVEC (+ THP-1) | Anthocyanin (0.01–10 $\mu$g/mL, 24 h[4]) | TNF-$\alpha$ (100 ng/mL, 24 h) | Vehicle, challenge | 3 | THP-1 cell adhesion (fluorescence of labeled cells) | Dose-dependent $\downarrow$ of THP-1 adhesion | (103) |
| Others: | | | | | | | |
| EA.hy926 | Blueberry exosome-like nanoparticles (20–40 $\mu$g/mL, 3 h) | TNF-$\alpha$ (20 ng/mL, 2 h) | Vehicle, challenge, negative (NAC, 200 $\mu$M), positive (H$_2$O$_2$, 50 $\mu$M, 1 h) | 3 | Viability (SDB), ROS (fluorescent probe dihydroethidium), inflammatory genes (qPCR) | $\downarrow$ ROS production. $\downarrow$ endothelial activation and inflammation-related gene expression. $\uparrow$ antioxidant gene expression | (97) |

[1]P#, passage number used for the experiments (when specified); bzATP, 2′(3′)-O-(4-benzoylbenzoyl)adenosine-5′-triphosphate tri(triethylammonium); CAT, catalase; DCFDA, 2,7-dichlorofluorescin diacetate; ERK, extracellular-signal-regulated kinase; HMVEC, human microvascular vein endothelial cell; HUVEC, human umbilical vein endothelial cell; ICAM, intercellular adhesion molecule; MCP-1, monocyte chemoattractant protein-1; miRNA, microRNA; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide; NAC, N-acetyl-L-cysteine; PLC, phospholipase C; ROS, reactive oxygen species; SDB, Sulforhodamine B; THP-1, Tamm-Horsfall Protein 1; VEGF-A, vascular endothelial growth factor A; WB, Western blot; WST, water-soluble tetrazolium.

[2]N, number of experimental replicates, as stated by the authors.

[3]Blueberry and challenge in coadministration. If no symbol is indicated, the cells were treated with the blueberry extracts first before challenge.

[4]Blueberry treatment administered after challenge.

an extracellular messenger vesicle presents in plants that contains proteins, lipids, mRNA, and microRNA (miRNA) (96). In addition to reducing ROS production, blueberry ELN also regulated gene expression involved in endothelial activation and leukocyte recruitment [MAPK1 and intercellular adhesion molecule (ICAM)] and inflammation (IL-6, TLR8, and TNF) (97). The antioxidant effect of blueberry extract on endothelial cells is likely due to the activity of several phytochemicals, but the extent to which other compounds contribute to the effect remains unclear, particularly due to the low number of studies focusing on nonphenolic compounds.

The secretion of chemokines and adhesion molecules by the endothelium is primarily regulated by TNF-$\alpha$ and C-reactive protein (CRP) (98), and leads to monocyte recruitment (99). After infiltration, monocytes differentiate into macrophages and phagocytose LDL (100). Macrophages that accumulate lipids eventually turn into foam cells, becoming surrounded by smooth-muscle cells and a collagen matrix, ultimately resulting in plaque formation (101). Blueberry anthocyanins reduced the adhesion of THP-1 monocytes to HUVEC endothelial cells with a better efficacy than the phenolic acid fraction derived from the same extract (102). The action of blueberry anthocyanins was further investigated and the individual compounds malvidin and cyanidin 3-glucoside, protocatechuic, and gallic acid reduced THP-1 adhesion (103). Blueberry extracts also decreased platelet- and endothelial-derived microvesicles through the inhibition of P2X$_7$ transcription and Akt phosphorylation, both contributing to the release of extracellular vesicles (104) associated with monocyte interaction with endothelial cells (105). Su et al. (42) observed that blueberry extracts led to downregulation of noncoding miR-21, miR-146a, and miR125b, miRs typically increased in macrophages involved in plaque formation (Table 3).

In cardiovascular disease, endothelial cell migration and angiogenesis are reduced, leading to structural and functional alterations of the endothelium (106). Akt is a major signaling pathway in angiogenesis, regulating cell survival, cell cycle, and migration (107). Treatment with blueberry polyphenols increased angiogenesis in endothelial cells through the upregulation of the Akt pathway (108). However, abnormal angiogenesis promoted by vascular endothelial growth factor (VEGF) was counterbalanced by blueberry extract treatment through the inhibition of ERK and Akt phosphorylation (104, 109). In addition, blueberry polyphenol extract did not modulate polyphospholipase C (PLC) expression and phosphorylation, involved in angiogenesis, in HUVEC cells following induction with VEGF or LPS (109, 110). These results suggest that blueberry components may support angiogenesis under normal physiological conditions, but attenuate abnormal angiogenesis induced by overactive growth factors.

Blueberries exert protective effects on endothelial models by reducing oxidative stress and monocyte adhesion to the endothelium and modulating angiogenesis. The direct impact of blueberry compounds on endothelial dysfunction

in vivo remains to be elucidated, with few studies focusing on the molecular pathways involved in atherosclerosis.

### Brain and neuronal inflammation models.

Inflammation in the brain can be generated by the dysregulation of inflammatory pathways, molecular signals released by injured neurons, or the accumulation of protein aggregates (111). Brain inflammation has been associated with an impairment of neuron regeneration (112) and an increase in the incidence of neurodegenerative diseases such as multiple sclerosis (113), and Alzheimer's (114) and Parkinson's diseases (115). Mechanisms involved in neurodegeneration have been reviewed in detail by Jellinger (116).

The sensitivity of brain muscarinic receptors to oxidative stress increases with aging (117), and abnormal signaling is implicated in neurodegenerative diseases. Blueberry extracts alleviate oxidative stress in neuronal models through a variety of mechanisms (**Table 4**), including scavenging of ROS (118) and downregulation of iNOS gene expression (119–121). In COS-7 cells transfected with muscarinic receptors, treatment with a whole blueberry extract reduced activation of the cAMP response element-binding (CREB) pathway, which can be activated by protein aggregates and is involved in oxidative stress in neurons (122). Several blueberry fractions exerted antioxidant effects, although via different mechanisms: a blueberry extract polar fraction (containing high concentrations of polyphenols) and a nonpolar fraction (low in polyphenols) both reduced oxidative stress in TNF-$\alpha$- and PMA-induced human neuroblastoma (118). The polyphenolic-rich fraction exerted scavenging activity of ROS, whereas the nonpolar fraction disrupted NOX assembly, a mechanism that generates the production of ROS. Blueberry treatment also reduced proinflammatory cytokines in LPS-induced BV-2 cells, through modulation of COX-2 gene expression (119, 120) and the inhibition of NF-$\kappa$B translocation into the nucleus (123). The phenolic extracts increased concentrations of Arg-1 (124), a marker of the M2 phenotype of macrophages, which promotes a return to homeostasis (125) necessary to avoid chronic inflammation. In a model of microglial cells subjected to inflammation in different glycemic conditions, a blueberry extract was effective at inhibiting inflammatory markers, with a comparable effect to the insulin treatment used in the model (121).

Protein aggregations in the brain result from the abnormal deposition of misfolded proteins, leading to the accumulation of fibrils, including $\alpha$-synuclein ($\alpha$Syn) or amyloid-$\beta$ (A$\beta$) into plaques (126). Accumulation of A$\beta$ aggregates is associated with impairment of normal physiological activity of the neurons, increased production of ROS (127), and activation of microglia and astrocytes (128). Immune cells also generate A$\beta$ (129), therefore upregulating the inflammatory cascade. A blueberry extract and an anthocyanin fraction conferred protection against protein aggregate-induced cytotoxicity in mouse neuroblastoma and rat hippocampal neurons challenged with A$\beta$ species (130,

131), most likely through the inhibition of the CREB pathway but with no effect on ERK activation (131). $\alpha$Syn-challenged mouse microglia were also protected by polyphenol extracts, but not the cells challenged with glutamate, which is sometimes found in excess in pathological conditions (132).

Based on results in cell models, it is possible that the active compounds of blueberries could dampen the development of neurodegenerative diseases by reducing oxidative stress and inflammation, which limits the formation of protein aggregates and associated cell damage. It is important to remember that although cell culture studies of neurons and glial cells provide useful information to understand inflammation mechanisms in the brain, these models may be oversimplified due to the lack of a blood-brain barrier, which is critical in maintaining homeostasis (133).

### Dermal inflammation models.

The skin is subjected to constant challenge, including pollution, physical damage (wounds), and light irradiation, that play a role in the initiation of inflammation (134). Cell models include keratinocytes, found in the epidermis (135), and fibroblasts that synthesize the extracellular matrix, used alone or in coculture (136). Recently, 3D skin models and a human skin equivalent have been developed to provide more complex models for testing toxicity, absorption, and metabolism with the presence of several epidermal layers and stratum corneum (137, 138).

Blueberry extracts were used to treat human keratinocytes (HaCaT) and a human skin model ("EpiDerm") (**Table 5**) challenged with $O_3$ exposure, a toxic pollutant that can alter the redox status of the skin and induce inflammation (138). $O_3$ impaired wound healing of the cells, but function was recovered with a blueberry polyphenol treatment by activating cellular antioxidant defense systems, reducing $H_2O_2$ production (138). A blueberry polyphenol extract was compared with 2 anthocyanin and proanthocyanidin-rich extracts to investigate wound healing in human fibroblasts (HDFa), and anti-inflammatory and antioxidant effects on murine macrophages. The fractions were both effective at reducing ROS production and alleviated inflammation by reducing the expression of COX-2 and iNOS in the macrophages, but the protective effects of proanthocyanidins were more pronounced than for the other blueberry polyphenols (139). Blueberry treatments, obtained through several extraction methods, were also effective at reducing NO production of human foreskin fibroblast (HFF-1) induced by IL-2$\beta$ (140).

The major risk factor for developing skin cancer is sun exposure. DNA absorbs UV-B, which can induce the formation of dimeric pyrimidine bases (141). These mutations are usually repaired by nucleotide excision, although certain mutations, notably in the p53 gene, result in the loss of this repair function (142), possibly leading to the development of squamous or basal cell carcinoma (143). In cells exposed to UV-B, responsible for DNA damage and initiation of proinflammatory pathways, a blueberry extract protected

**TABLE 4** Cell-culture-based studies on the effect of blueberry phytochemicals on microglia and neuronal inflammation models

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| Whole and/or polyphenolic extracts: | | | | | | | |
| Microglia from C57BL/6 mice | Polyphenol extract (1 $\mu$L, equivalent to about 10 $\mu$g/mL, 24 h) | Glutamate (100 $\mu$M, 24 h) or $\alpha$-synuclein (100 ng/mL, 24 h) | Vehicle, challenge | 3 | Morphology (light microscopy), viability (DAPI staining) | Moderate effect on cell morphology (presence of both healthy and "fried egg" shaped cells). No neuroprotective effects by the treatment against glutamate-induced cell death, but protection against $\alpha$-synuclein damage | (132) |
| Hippocampal neurons from F344 rats | Whole extract (0.125 mg/mL, 10–30 min[2]) | A$\beta$ species (10 $\mu$M, 10–30 min) | Vehicle, challenge | 4 | Viability (staining exclusion), CREB, ERK, PKC (immunofluorescence), ROS (DCFDA), glutathione (immunofluorescence) | Protection against A$\beta$-induced cytotoxicity. No $\downarrow$ PKC$\alpha$ and PKC$\gamma$ in aging neurons. $\downarrow$ phosphorylation of CREB but no effect on ERK activation. $\downarrow$ ROS production and $\uparrow$ glutathione concentrations | (131) |
| COS-7 transfected with muscarinic receptors (M1 and M3) | Whole extract (2 mg/mL, 45 min + 4 h[2]) | Dopamine (1 mM, 4 h) | Vehicle, challenge, murine receptor cell control | — | PKC, CREB, MAPK (WB) | Further $\uparrow$ MAPK phosphorylation induced by dopamine in M1 cells but not M3. $\downarrow$ PKC$\gamma$ and CREB phosphorylation induced by dopamine in both cell types | (122) |
| BV-2 | Whole extract (50–500 $\mu$g/mL, 45 min + 4–16 h[2]) | LPS (100 ng/mL, 4–16 h) | Vehicle, challenge | 3 | NO (Griess), iNOS, and COX-2 gene expression (qPCR), iNOS and COX-2 proteins (WB), IL-1$\beta$ and TNF-$\alpha$ (ELISA), ROS (DCFDA) | $\downarrow$ NO production. Dose-dependent $\downarrow$ iNOS and COX-2 gene and protein expression. $\downarrow$ cytokines and ROS production for treatment dose 100 $\mu$g/mL and higher | (119) |
| BV-2 | Whole extract (0.25–2 mg/mL, 1 h) | LPS (100 ng/mL, overnight) | Vehicle, challenge | — | NO (Griess), iNOS, COX-2 protein expression (WB), TNF-$\alpha$ (ELISA) | $\downarrow$NO, TNF-$\alpha$ production and iNOS gene expression (doses $\geq$1 mg/mL). $\downarrow$COX-2 gene expression (dose 0.5 mg/mL) | (120) |
| BV-2 | Polyphenol extract (10 ng/mL, 1–6 h[2] for qPCR or 24–48 h[2] for other assays) | LPS (100 ng/mL, 1–6 h or 24–48 h) | Vehicle, challenge | 3 | Viability (MTT), morphology, iNOS and Arg-1 (immunofluorescence), migration (transwell chambers and scratch assay), Rho GTPases (WB), IL-1$\beta$, TNF-$\alpha$, IL-6 (qPCR) | $\downarrow$ migration and prevention of morphology changes. $\downarrow$ RAC-1-GTP. Modulation of M1 phenotype acquired after LPS challenge, towards M2 phenotype. $\downarrow$ cytokine production | (124) |

*(Continued)*

**TABLE 4** (*Continued*)

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| BV-2 | Polyphenolic extract (25–100 $\mu$g/mL, 45 min + 16 h[2]) | LPS (100 ng/mL, 16 h) | Vehicle, challenge | 3–4 | Viability (MTS), NO (Griess), iNOS, and COX-2 (WB, transient transfection/luciferase assay), NF-$\kappa$B (EMSA) | Dose-dependent ↓ NO production. ↓ iNOS and COX-2 protein and promoter activity. ↓ translocation of NF-$\kappa$B | (123) |
| HAPI | Whole extract (2 mg/mL, 24 h, alone or with 50 mM insulin) | LPS (100 mg/mL, overnight, in presence of 5, 25, or 50 mM glucose) | Vehicle, challenge | 3 | NO (Griess), TNF-$\alpha$ (ELISA), iNOS (WB), GLUT1, and NOX4 expression (DAPI staining) | Comparison with LPS control: ↓ all marker production or expression by blueberry treatment of all concentrations of glucose (higher inhibition in low glucose concentration). The inhibition effect was more pronounced when blueberry was used with insulin for NO and iNOS | (121) |
| Anthocyanin fractions: Neuro2a | Anthocyanin (0.5–8-fold molar ratio to A$\beta$, 15 min–3 h[2]) | A$\beta$ species (10 $\mu$M, 15 min–3 h) | Vehicle, challenge | 3 | Viability (MTS), amyloid formation (fluorescence assay), morphology A$\beta$ samples (microscopy), fibril formation (WB) | Dose-dependent ↓ amyloid formation and ↑ formation of low-molecular weight A$\beta$ species. ↓ cell cytotoxicity induced by A$\beta$ aggregates | (130) |
| Others: SH-SY5Y | Whole extract, polar (polyphenol-rich) and nonpolar (not polyphenol-rich) fractions (5 $\mu$g/mL, 1 h[2]) | TNF-$\alpha$ (200 ng/mL, 1 h) or PMA (400 ng/mL, 1 h) | Vehicle, challenge, negative: DPI (10 $\mu$M), NAC (1 mM), AEBSF (1 mM), GW4896 (13.8 $\mu$M), 1 h | 4 | ROS (DCFDA), scavenging capacity (choline oxidation assay), cytotoxicity (MTT), superoxide production (cytochrome C), P67$^{phox}$ and phosphor P40$^{phox}$ (ELISA), P67$^{phox}$ (WB), NOX assembly in lipid raft (confocal microscopy) | ↓ ROS formation by whole extract and nonpolar fraction. ↑ ROS scavenging activity of polar fraction only. ↓ superoxide production and plasma membrane-associated p67$^{phox}$. ↓ formation of large lipid raft by nonpolar fraction only | (118) |

[1]N, number of experimental replicates, as stated by the authors. AEBSF, 4-(2-aminoethyl) benzenesulfonyl fluoride; A$\beta$, amyloid $\beta$; COX-2, cyclooxygenase-2; CREB, cAMP-response element binding; DAPI, 4′,6-diamidino-2-phenylindole; DCFDA, 2′,7′-dichlorofluorescin diacetate; DPI, diphenyl iodonium; EMSA, electrophoretic-mobility shift assay; ERK, extracellular-signal-regulated kinase; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide; NAC, N-acetyl-L-cysteine; NOX, NADPH oxidase; PKC, protein kinase C; PMA, phorbol-12-myristate 13-acetate ester; ROS, reactive oxygen species; WB, Western blot.

[2]Blueberry and challenge in coadministration. If no symbol is indicated, the cells were treated with the blueberry extracts first before challenge.

**TABLE 5**  Cell-culture-based studies on the effect of blueberry phytochemicals on dermal inflammation models

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| **Whole and/or polyphenolic extracts:** | | | | | | | |
| HaCaT and 3D skin model "EpiDerm" | Polyphenol extract (HaCaT: 10 $\mu$g/mL, 24 h; 3D model: 100 $\mu$g/mL, 24 h) | $O_3$ (HaCat: 0.5 ppm, 1 h; 3D model: 0.5 ppm, 5 h) | Vehicle, challenge | 3 | Cytotoxicity (LDH), wound closure (scratch wound healing), migration (transwell), proliferation (BrdU), $H_2O_2$ (DCFDA), inflammasome activation (ASC oligomerization), ASC and NLRP1 (immunocytochemistry), caspase 1 (WB), ASC, caspase 1, and IL-18 gene expression (qPCR) | ↑ keratinocyte wound closure by increased migration and proliferation. ↓ inflammasome activation by limited induction of ASC, transcription of caspase 1 and IL-18, and ↓ oligomerization of ASC | (138) |
| HaCaT | Polyphenol extract (5–100 mg/L, 1 h + 4 h[2] or 4 h[2] only) | UV-A (10–40 J/cm²), $H_2O_2$ (0.3 mM, 4 h³) | Vehicle, challenge | 3–4 | Viability (LDH and neutral red retention), ROS (DCFDA), lipid peroxidation (TBARS), glutathione (DTNB) | Dose-dependent ↓ ROS production. ↓ membrane lipid peroxidation. Protection against glutathione depletion | (149) |
| HaCaT | Phenolic extract (5–50 mg/L, 1 h + 4–8 h² or 4/8 h² only) | UV-B (50–1000 mJ/cm²) | Vehicle, challenge | 3 | Cytotoxicity (LDH), proliferation (BrdU), DNA damage (gel electrophoresis), caspase-3 and -9 activity (fluorescent assay), caspase-3 activation (WB), RONS (dihydrorhodamine 123), IL-6 (ELISA) | ↓ LDH release and ↑ proliferation at higher treatment doses. ↓ DNA damage and apoptosis. ↓ RONS generation, better for cells treated after irradiation only. ↓ IL-6 production | (144) |
| HaCaT and HFF (alone and in coculture) | Phenolic extract (6–10 mg/mL, 2 h + 12 h) | UV-C (8 mJ, cm², 5 s, 270–290 nm) | Vehicle, challenge | 3 | Viability (methylene blue), DNA damage (DNA ladder assay), SOD and MDA content (kits), MMP-1 (ELISA), p38MAPK, c-fos, c-Jun, MMP-1, IL-1$\beta$, IL-8, TNF-a, and IL-6 (qPCR), 38MAPK, c-fos, c-Jun, MMP-1 (WB) | Protection against UV-C-induced cytotoxicity and ↓ DNA damage. ↓ MMP-1 only in coculture system. ↓ accumulation of MDA and ↑ activity of SOD. ↓ proinflammatory gene expression. ↓ MAPK pathway through ↓ MMP-1, c-fos, and c-Jun expressions in HFF and coculture but not HaCat | (150) |
| **Phenolic and polyphenolic fractions:** | | | | | | | |
| RAW 264.7 and HDFa | Polyphenol extract, anthocyanin and proanthocyanidin fractions (50 $\mu$g/mL, 24 h³) | RAW 264.7: LPS (10 $\mu$L, 24) | Vehicle, challenge | 3 | Cytotoxicity (MTT) RAW 264.7: ROS (DCFDA), NO (Griess), COX-2, iNOS (qPCR)<br><br>HDFa: cell migration (fluorescence) | RAW 264.7: No effect of polyphenol extract on ROS production. ↓ ROS by anthocyanin and proanthocyanidin fractions. Highest NO suppression with proanthocyanidin fraction. ↓ COX-2 and iNOS by all treatments HDFa: ↑ wound repair by proanthocyanidin B2 | (139) |
| **Anthocyanin fractions:** | | | | | | | |
| Human dermal fibroblast | Anthocyanin (1–10 mg/L, 48 h²) | UV-B (100 mJ/cm², 312 nm) | Vehicle, challenge | 3 | Viability (MTT), ROS (DCFDA), ASK-1, JNK, p38, c-Jun, p53, STAT-1, MMP-1, 8 and 13, I$\kappa$B, NF-$\kappa$B (WB), procollagen, collagen (immunocytochemistry), procollagen, MMP-1 (qPCR), TNF-$\alpha$, IL-8, IL-6, IL-1$\beta$ (ELISA) | Dose-dependent ↑ cell viability. Inhibition of reduction of procollagen and collagen induced by UV-B. ↓ MMP production and gene expression increased by the UV-B challenge. ↓ proinflammatory cytokine secretion, ↓ translocation of NF-$\kappa$B and ↓ activation of ASK-1-MAPK pathway | (145) |

*(Continued)*

**TABLE 5** (Continued)

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| Others: HFF1 | Blueberry extracts, comparison of extraction methods (12.5 µg/mL, 90 min) | IL-2β (10 µg/mL, 30 min) | Vehicle, challenge, positive (L-NIL, 1 µg/mL) | 4 | Viability (MTT), NO (Griess) | ↓NO production by all fruit extracts. Stronger inhibitory effect for extract obtained by decoction method | (140) |

[1]N, number of experimental replicates, as stated by the authors. ASC, apoptosis-associated speck-like; ASK, apoptosis signal-regulating kinase-1; BrdU, bromodeoxyuridine; COX-2, cyclo-oxygenase-2; DCFDA, 2,7-dichlorofluorescin diacetate; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); HFF, human foreskin fibroblast; iNOS, inducible nitric oxide synthase; IκB, NF κ B inhibitor; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; L-NIL, L-N⁶-(1-iminoethyl)lysine; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MMP-1, matrix metalloproteinase-1; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide; NLRP, nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing; RONS, reactive oxygen and nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; STAT-1, signal transducers and activators of transcription-1; WB, Western blot.

[2]Blueberry treatment administered after challenge.

[3]Blueberry and challenge in coadministration. If no symbol is indicated, the cells were treated with the blueberry extracts first before challenge.

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DNA against strand break formation (144). Blueberry extracts also demonstrated anti-inflammatory effects in UV-B-challenged cells and reduced cytokine gene expression through inhibition of NF-κB and MAPK activations (145). UV-A, in contrast, is less absorbed by DNA, but can interact with exogenous chromophores and induce lipid peroxidation, which generates ROS (146). These reactive species can lead to the oxidation of nucleic acid, DNA strand breaks (147), and increased MMP-1 activity (148), potentially increasing the risk of aggressive cancer. The increase of oxidative stress by UV-A on keratinocytes was partially reduced by a blueberry phenolic extract through decreased ROS production and lipid peroxidation (149). The third type of sunlight irradiation, UV-C, has the weakest penetration rate in the atmosphere, but has very high energy, possibly contributing to oxidative stress, inflammation, and DNA damage in the skin cells. A blueberry extract applied to HaCaT keratinocytes and HFF fibroblasts irradiated with UV-C showed protective effects against oxidative stress and DNA damage. The anti-inflammatory effects of the treatment were, however, only characterized in the keratinocyte and fibroblast coculture and the activation of MMP-1 expression by fibroblasts after irradiation was only observed in the presence of keratinocytes, suggesting interactions between the 2 types of cells (150). These observations support the need to use more complex models (coculture or 3D skin model) to investigate mechanisms mediated by the interaction between cell types in the skin. Limiting the studies to one type of cell may leave out crucial information regarding the physiological effects of the treatments on the skin. The results to date in cell models suggest that blueberry derivatives could have a protective effect on skin damage in response to sun and other stressors; however, the extent to which these molecules are stored and present in skin cells or associated tissue layers in vivo is not clear.

### Ocular inflammation models.

Artificial lights, environment, diet deficiencies, pathologies, and aging can impact oxidative stress and inflammation in the eyes, and may contribute to diabetic retinopathy (151) or age-related macular degeneration (152). The eye tissues and fluids contain little extracellular SOD, hence they have less protection against superoxide radical damage (153). Despite having higher SOD activity, the retina is at more risk of oxidative stress due to its high content of unsaturated fatty acids and high exposure to light (154) leading to lipid peroxidation, which can, in turn, react with DNA, proteins, and lipids, inducing cell damage (155).

Studies on blueberry compounds applied to cell-based models relevant to eye function have typically used light irradiation (UV, blue light, visible light) (156–159), a chemical challenge (e.g., $H_2O_2$, DHA) (160–162), or a combination of both (155, 163, 164) to induce inflammation (**Table 6**). The protective effect of blueberry compounds on oxidative stress induced by light in ocular models has been tentatively explained through a decrease in ROS production and lipid peroxidation. In murine photoreceptors (661 W line)

**TABLE 6** Cell-culture-based studies on the effect of blueberry phytochemicals on ocular inflammation and oxidative stress models

| Cell line (P#[1]) | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[2] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| Whole and/or polyphenolic extracts: | | | | | | | |
| 661 W | Polyphenol extract (1–30 $\mu$g/mL, 1 h + 20 min[3] + 24 h[4]) | UV-A light (4 J/cm$^2$, 20 min) | Vehicle, challenge, positive (NAC, 0.3–1 mM) | 6 | Cytotoxicity (fluorescent microscopy), ROS (DCFDA), MAPK, Akt, JNK (WB) | ↓ morphological defects induced by UV-A. Inhibition of reduction of metabolic activity. ↓ ROS production and ↓ phosphorylation of p38 MAPK and JNK. No downregulation of Akt | (156) |
| 661 W | Polyphenol extract (1–10 $\mu$g/mL, 1 h + 6 h[3]) | Blue light (460–470 nm, 2500 lx, 6 h) | Vehicle, challenge, extract, positive (NAC, 0.3–1 mM) | 6 | Viability (WST-8), cytotoxicity (fluorescent microscopy), ROS (DCFDA), MAPK, NF-κB, LC-3 (WB), caspase activity | Dose-dependent ↓ cell death and ROS production. ↓ p38 MAPK and NF-κB, and inhibition of upregulation of LC-3. ↓ the activation of caspase 3/7 | (157) |
| ARPE-19 | Polyphenol extract (0.01–100 $\mu$g/mL, 4 h) | H$_2$O$_2$ (500 $\mu$M, 2 h) | Vehicle, challenge, positive (BHT, 300–1000 $\mu$M) | 3 | Viability (MTT), ROS (DCFDA), HO-1, GST-pi (WB) | ↓ ROS production. ↑ HO-1 and GST-pi proteins and mRNA | (160) |
| Phenolic and polyphenolic fractions: | | | | | | | |
| ARPE-19 | Polyphenol extract, anthocyanin, phenolic acid, and flavonoid fractions (10–50 $\mu$g/mL, 24 h) | DHA (0.05 mmol/L, 24 h) + light irradiation (3500 lx, 12 h) | DHA + light challenge | 3 | Viability (MTT), ROS (DCFDA), lipid peroxidation (MDA) | No effect on ROS and lipid peroxidation for low doses of any fractions. 25 $\mu$g/mL: ↓ oxidative stress for polyphenol extract and flavonoid fraction. ↓ oxidative stress by all fractions (dose 50 $\mu$g/mL), better efficiency of polyphenol and flavonoid fractions | (155) |
| ARPE-19 | Polyphenol extract, anthocyanin, phenolic acid, and flavonoid fractions (5–50 $\mu$g/mL, 24 h) | DHA (0.05 mmol/L, 24 h) + light irradiation (3500 lx, 12 h) | DHA + light challenge | 3 | Viability (MTT), cytotoxicity (LDH), VEGF (ELISA), phagocytic index (fluorescent microspheres), senescence ($\beta$-galactosidase activity), lipid peroxidation (MDA), ROS (DCFDA) | ↑ cell viability and ↓ VEGF production. Dose-dependent amelioration of phagocytic function. Amelioration of senescence by all fractions, but better performance of polyphenol extract than fractions on viability and senescence. ↓ lipid peroxidation by all fractions, best effect for flavonoid fraction | (163) |

(Continued)

**TABLE 6**  (*Continued*)

| Cell line (P#[1]) | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[2] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| Anthocyanin fractions: | | | | | | | |
| ARPE-19 | Purified anthocyanin fractions (100 $\mu$M, 14–15 h[3]) | Bisretinoid fluorophore A2E (100 $\mu$M) + endoperoxide (A2E oxidant, 10 mM), 14–15 h or light (0.36 mW/mm$^2$, 10 min, 430 nm) | Vehicle, challenge | 3 | Viability (dye), A2E concentrations (HPLC), membrane permeability (DAPI) | ↓ photo-oxidation consumption and oxidation of A2E by anthocyanins. ↓ death of cells illuminated with blue light and pretreated with anthocyanins. ↓ number of permeabilized cells | (164) |
| ARPE-19 | Malvidin and delphinidin 3-glucoside extracted from blueberry (10–40 $\mu$g/mL[3]) | Visible light (2500 lx, 12 h, 420–800 nm) | Vehicle, challenge | 3 | Viability (MTT), ROS (DCFDA), VEGF (ELISA), $\beta$-galactosidase activity (staining) | Dose-dependent ↓ ROS production. ↓ VEGF by malvidin 3-glucoside but not delphinidin 3-glucoside. No effect on senescence | (158) |
| ARPE-19 | Anthocyanin (0.1–10 $\mu$g/mL, 24 h) | Light irradiation (2500 lx, 12 h) | Vehicle, challenge | 3 | Viability (MTT), cytotoxicity (LDH), senescence ($\beta$-galactosidase activity), VEGF (ELISA), ROS (DCFDA) | ↓ cell senescence after light exposure. ↓ ROS production. ↑ cell viability (doses 1 and 10 $\mu$g/mL) and ↓ VEGF expression for lower doses of blueberry extract | (159) |
| ARPE-19 | Anthocyanin (5 $\mu$g/mL, 6 h) | $H_2O_2$ (800 $\mu$M, 2 h) | Vehicle, challenge | 3–4 | Viability (MTT), apoptosis (annexin V detection), ROS (DCFDA), CAT, GSH-PX, MDA, SOD, VEGF (ELISA), pro-caspase, Bcl-2, Bax, Akt, ERK, p38, VEGF (WB) | ↓ apoptosis induced by challenge. ↓ caspase-3 and Bax, ↑ Bcl-2. ↓ ROS production. No effect on lipid peroxidation and antioxidant enzymes (CAT and SOD) production but ↑ GSH-PX. ↓ VEGF content but not protein expression. ↑Akt and ↓ ERK activations | (161) |
| HRCEC (P3-5) | Anthocyanin (10 $\mu$g/mL, 24 h) | Glucose (30 mmol/L, 24–48 h) | Vehicle, challenge | 3 | Viability (MTT), ROS (DCFDA), CAT, SOD, NO, eNOS, ACE, VEGF, ICAM-1, NF-$\kappa$B (ELISA), NOX-4, Akt, VEGF (WB) | ↓ ROS secretion. ↑ antioxidant enzyme activity. ↓ NOX-4 protein expression after 48 h. No significant reduction in NO production but ↓ eNOS activity at 24 h. ↓ VEGF production. No effect on ICAM-1 and NF-$\kappa$B | (162) |

[1] P#, passage number used for the experiments (when specified). ACE, angiotensin-converting enzyme; BHT, butylated hydroxytoluene; CAT, catalase; DAPI, 4′,6-diamidino-2-phenylindole; DCFDA, 2,7-dichlorofluorescin diacetate; eNOS, endothelial nitric oxide synthase; ERK, extracellular-signal-regulated kinase; GSH-PX, glutathione-peroxidase; GST-pi, glutathione S-transferase-pi; HO-1, heme-oxygenase; ICAM-1, intercellular adhesion molecule-1; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide; NAC, N-acetyl-L-cysteine; NO, nitric oxide; NOX, NADPH oxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; VEGF, vascular endothelial growth factor; WB, Western blot; WST, water-soluble tetrazolium.

[2] N: number of experimental replicates, as stated by the authors.

[3] Blueberry and challenge in coadministration. If no symbol is indicated, the cells were treated with the blueberry extracts first before challenge.

[4] Blueberry treatment administered after challenge.

induced with UV and blue light, blueberry extracts exerted protective effects through regulation of ROS production, limiting cell death induced by the light through inhibition of p38-MAPK, JNK, and NF-$\kappa$B pathways (156, 157).

The effects of blueberry extracts on alternatively challenged cells (H$_2$O$_2$ and glucose) were also reported (Table 6), but more research is needed to confirm the mechanisms behind their antioxidant and anti-inflammatory effects. Oxidative stress was alleviated by blueberry compounds in chemically challenged (H$_2$O$_2$) retinal pigment epithelial cells through an increased production of antioxidant enzymes (160). Blueberry anthocyanins modulated oxidative stress through a decrease in ROS production and increase in glutathione peroxidase activity in ARPE-19 cells induced with H$_2$O$_2$ (161). In 661 W cells challenged with high glucose, anthocyanins reduced ROS secretion and NOX protein expression, but limited anti-inflammatory effects were reported with no clear modulation of NF-$\kappa$B (162).

Several studies have reported the effects of an array of phenolic fractions (anthocyanins, flavonoids, and phenolic acid). Blueberry anthocyanins tested on light-induced ARPE-19 cells helped mitigate lipid peroxidation (159), whereas malvidin 3-glucoside, purified from a blueberry extract, decreased ROS and VEGF production in irradiated cells (158). However, a blueberry flavonoid fraction and whole polyphenol mixture were more effective than the anthocyanin and phenolic acid fractions at reducing lipid peroxidation (155, 163). These observations support the evidence for a general effect to reduce cell stress markers in ocular cell models when using multiple blueberry fractions and phytochemicals, with a potentially stronger activity of the total polyphenol extracts as opposed to individual compounds. Thus, testing the effects of dietary blueberries or their derivatives on eye function is an interesting avenue for future research.

*Intestinal inflammation models.*
Inflammatory bowel diseases (IBDs) encompass chronic inflammation-related disorders in the gastrointestinal tract. Chronic inflammation, associated with oxidative stress, increases the risk of developing colorectal cancers (165). Even though genetic predispositions have been documented (166), environmental factors such as the gut microbiota composition, influenced by lifestyle and diet, and its interaction with the intestinal mucosa, are key factors in increasing the prevalence of IBD (167, 168).

The effects of blueberry compounds on intestinal models are reported in **Table 7**, along with experimental conditions. IBDs are characterized by a loss of intestinal barrier integrity through the alteration of tight junction proteins (169). Although it is not clear if this loss of permeability is a cause or consequence of IBD, it promotes the chance of pathogens to enter the mucosa, triggering inflammation (170). Blueberry extracts were tested on Caco-2 cell permeability using a transepithelial electrical resistance (TEER) measurement. The anthocyanin and total polyphenolic fractions decreased permeability of the cell monolayer induced by *Escherichia coli*

challenge, but the proanthocyanidin fraction did not restore TEER values. The authors suggested an interaction between proanthocyanidins, cell surface proteins, and the ability of *E. coli* to adhere to the cells (171).

Regarding oxidative and inflammatory markers, a number of cytokine gene expressions have been found to increase in colonic mucosa of ulcerative colitis patients due to excessive immune response and immune cell reactivity (172, 173). In T84 cells, the increase in TNF-$\alpha$ and IP-10 gene expressions by a cytokine cocktail were counteracted by a blueberry extract and the production of IL-8 protein was slightly, but not significantly inhibited (174). ROS production in a similar cell model was also decreased by blueberry compounds (175). However, the effects of blueberry compounds on the NF-$\kappa$B pathway remain unclear, with contradictory effects reported in intestinal models of inflammation. The response induced by cytokines was dose-dependently alleviated by a blueberry anthocyanin fraction in Caco-2 cells through the inactivation of NF-$\kappa$B, but a water extract from the same fruits did not modulate the inflammatory response (176). An anthocyanin extract used on T84 human colon cells decreased the activation of the STAT pathway, upregulated during IBD, but also upregulated the NF-$\kappa$B pathway (62).

A strong advantage of using an intestinal model is the possibility to study both the effect of polyphenol compounds and their colonic metabolites in a more physiologically relevant situation than for other systems in the body, as both parent molecules and metabolites are present in the intestinal epithelial cell environment despite the low bioavailability of polyphenolic compounds. The limited number of studies on blueberry phytochemicals and cell culture models of intestinal inflammation, the diversity of cell lines used, and parameters measured speak to the need for more studies to determine how blueberries modulate gut function and health.

## Inflammation and oxidative stress: summary of the bioactivity of blueberry phytochemicals

Because both inflammation and oxidative stress are frequently associated with the development of chronic diseases (10), it is important to understand how dietary factors impact these outcomes. Here, we have reviewed studies reporting the effects of blueberry phytochemicals on cell culture models of inflammation and/or oxidative stress (**Figure 3**). Blueberry phenolic compounds and more broadly, phytochemicals, exert regulatory effects including a decrease in proinflammatory gene expression/production in part through the modulation of the NF-$\kappa$B pathway. A modulation of the MAPK pathway by blueberry phytochemicals is less evident with contradictory observations reported but may also play a role. Blueberry phytochemicals decreased DNA damage in cells in vitro, via the reduction of ROS production, lipid peroxidation, and an increase in antioxidant enzyme activities.

Despite many in vitro studies on blueberry extracts, no specific compounds have emerged as singly responsible

**TABLE 7** Cell-culture-based studies on the effect of blueberry phytochemicals on intestinal inflammation models

| Cell line | Treatments (dose, duration) | Challenge (dose, duration) | Controls | N[1] | Parameters measured (method) | Effects of treatment compared to challenge | Ref. |
|---|---|---|---|---|---|---|---|
| **Whole and/or polyphenolic extracts:** | | | | | | | |
| T84 | Whole extract (2.5–25 $\mu$g/mL, 1 h + 4 or 16 h[2]) | Cytokine mixture: TNF-$\alpha$ (10 ng/mL), IL-1$\beta$ (5 ng/mL), TNF-$\alpha$ and IFN-$\gamma$ (10 ng/mL, 4 or 16 h) | Vehicle, challenge | 3 | Viability (reazurin reduction assay), IP-10, IL-8, TNF-$\alpha$ (qPCR), cytokines (proteome profiler human cytokine antibody array) | Dose-dependent $\downarrow$ TNF-$\alpha$ and IP10 gene expression. Complete inhibition of synthesis of IP-10, I-TAC, and sICAM, and slight $\downarrow$ GRO-$\alpha$ and IL-8, all increased by challenge | (174) |
| NCM 356 and CCD 841 CoN | Whole extract (25 mg/mL, 48 h[3]) | Proinflammatory cocktail TNF-$\alpha$ (100 ng/mL), IL-1$\beta$ (100 ng, mL), LPS (100 ng, mL), IFN-$\gamma$ (10 $\mu$g/mL), (5 $\mu$g/mL), 24 h | Vehicle, challenge, negative (PBS, 1:20 dilution) | 5 | ROS (fluorescence microscopy and flow cytometry), cell death (flow cytometry) | $\downarrow$ ROS production in nuclear and cytoplasmic compartment of both cell lines. $\downarrow$ cell death | (175) |
| **Phenolic and polyphenolic fractions:** | | | | | | | |
| Caco-2 | Polyphenol extract, anthocyanin and proanthocyanidin fractions (10–100 $\mu$g/mL, 24–72 h[2]) | E. coli (5 $\times$ 10$^4$ CFU/mL, 24–72 h) | Vehicle, challenge | 3 | Membrane permeability–surrogate marker (TEER) | $\uparrow$ TEER value after 48 h treatment with polyphenol and anthocyanin fractions. Further $\downarrow$ TEER after 24 h by proanthocyanidin | (171) |
| Caco-2 (transfected with plasmid pNiFty2-Luc) | Anthocyanin, phenolic (5–100 $\mu$g/mL, 4 h[2]) and water-soluble extracts (25–100 $\mu$g/mL, 4 h[2]) | IL-1$\beta$ (2 ng/mL, 4 h) | Vehicle | 8 | NF-$\kappa$B (luciferase assay), viability (MTS) | No effect of phenolic and water-soluble fractions on NF-$\kappa$B induction, dose-dependent $\downarrow$ by anthocyanin fraction | (176) |
| **Anthocyanin fractions:** | | | | | | | |
| T84 | Anthocyanin (10 $\mu$g/mL, 20 min + 20 min[2] for WB or 24 h[2] for other assays) | TNF-$\alpha$ or IFN-$\gamma$, 100 ng/mL, 30 min[2] or 24 h[2] | Vehicle, challenge | 4 | STAT1, STAT3, ERK, JNK, p38, NF-$\kappa$B phosphorylation (WB) | $\downarrow$ phosphorylation of STAT1 and STAT3. No effect on MAPK pathway. $\uparrow$ NF-$\kappa$B phosphorylation | (61) |

[1]N, number of experimental replicates, as stated by the authors. ERK, extracellular-signal-regulated kinase; GRO-$\alpha$, growth-related oncogene $\alpha$; ICAM-1, intercellular adhesion molecule-1; IP-10, IFN-$\gamma$-inducible protein-10; I-TAC, interferon-inducible T-cell $\alpha$ chemoattractant; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; ROS, reactive oxygen species; sICAM, soluble intercellular adhesion molecule-1; STAT, signal transducers and activators of transcription; TEER, transepithelial/endothelial electrical resistance; WB, Western blot.
[2]Blueberry and challenge in coadministration. If no symbol is indicated, the cells were treated with the blueberry extracts first before challenge.
[3]Blueberry treatment administered after challenge.
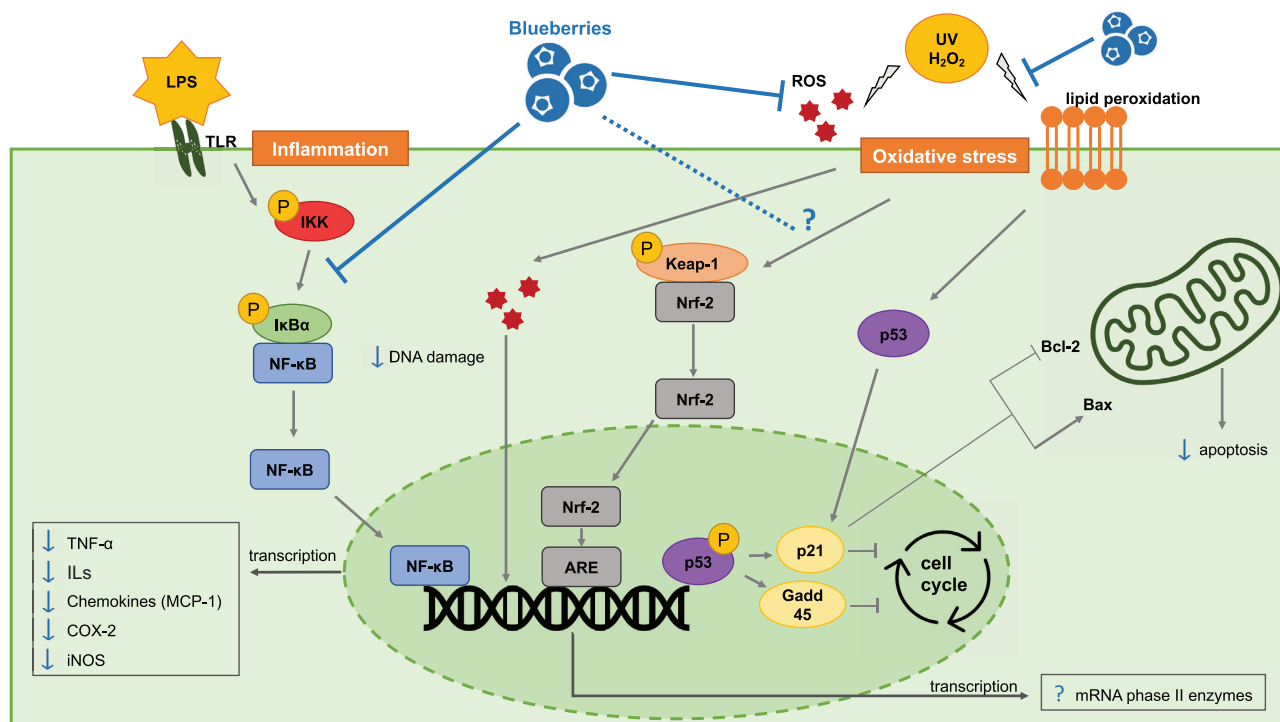
**FIGURE 3** Summary of proposed cellular regulatory mechanisms of blueberry extracts on inflammatory or oxidative stress pathways investigated through cell-based models. Blue lines and arrows show the modulatory effects of blueberry phytochemicals. ↓, downregulation or reduction; ⊢, inhibition; ?, contradictory results. ARE, antioxidant response element; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; COX-2, cyclooxygenase-2; IKK, inhibitory kappa B kinase; iNOS, inducible nitric oxide synthase; IκBα, inhibitor of nuclear factor kappa B-alpha; Keap-1, Kelch Like erythroid-derived CNC homology Associated Protein 1; Nrf-2, nuclear erythroid 2-related factor 2; P, phosphorylated; TLR, toll-like receptor.

for the regulatory effects on inflammation and oxidative stress. Virtually all studies have focused on blueberry (poly)phenolic extracts or fractions, with a large emphasis on anthocyanins. Health effects of dietary anthocyanins have been extensively reported and discussed (177–179), and berries provide an excellent vector for anthocyanin consumption. Blueberries have a complex anthocyanin profile and both major anthocyanidin derivatives, malvidin and delphinidin, have demonstrated a reduction of inflammatory markers in different in vitro models of intestinal inflammation (174) and endothelial dysfunction (103, 180). Although it is highly likely that anthocyanins largely contribute to the health benefits provided by blueberries, as supported by the number of studies focusing on those compounds, it is doubtful that they are entirely responsible for the bioactivities.

Several in vitro studies compared different fractions of blueberry phytochemicals, with reports of similar or better effects by other phenolic fractions and/or whole blueberry extract compared with anthocyanins (43, 84, 139, 158, 163). These different studies highlight that mechanisms of action of individual blueberry compounds and fractions are context and/or model specific. More studies comparing the effect on individual compounds and well-defined combinations

of molecules in different systems are needed to investigate the impact of a system's environment or system-specific regulation on the bioactivity of blueberries. Although the amplitude of the effect of individual compounds appears to be widely specific to the model studied, the use of whole fractions of the fruits seems to alleviate inflammation and/or oxidative stress more consistently across models, despite not always demonstrating the strongest effects compared with specific blueberry fractions.

As the health effects of polyphenols have been extensively described, more data on other phytochemicals should be gathered as they may also exert health benefits. Other notable phytochemicals in blueberries include ascorbic acid (181), polysaccharides (182), and volatile compounds (183) and could contribute to inflammatory or oxidative responses of cells to stimuli. A blueberry volatile extract, high in monoterpenes (linalool, linalool oxide, and $\alpha$-terpineol), modulated the inflammatory response in LPS-induced RAW 264.7 cells through inhibition of the NF-$\kappa$B pathway (38). Phenolic compounds, although carrying anti-inflammatory and antioxidant modulatory effects, may not be solely responsible for the health benefits of blueberries. Whether the phytochemicals act in synergy or target different molecular pathways remains to be elucidated.

Although the scope of this review is limited to blueberries, the anti-inflammatory and antioxidant effects and mechanisms are likely applicable to other commonly consumed berries. Berries are generally rich in polyphenols, particularly anthocyanins, flavonols, and proanthocyanidins, but the profile of each berry species, and even within varieties, harbors differences in terms of the individual compounds present and their respective concentration (184). Gasparrini et al. (15) reviewed in detail the anti-inflammatory effects of several berries in cellular models using LPS-induced inflammation, and consistently report alleviation of inflammation by berry phytochemicals through inhibition of NF-$\kappa$B and MAPK pathways. Other reviews also discuss and compare the anti-inflammatory properties of berries, in preclinical and human models (10, 16, 185). Moore et al. (186) and Gu et al. (38) have reported similar anti-inflammatory effects of berry volatiles compared with phenolic extracts for cranberries, blackberries, blueberries, red and black raspberries, and strawberries. Notably, the bioactivities of berry polyphenol extracts do not always explain the overall anti-inflammatory effects observed with whole berries (84, 187, 188), highlighting that potential health effects of berries as a group derived from highly diverse phytomolecules.

After consumption, blueberries and their phytochemicals undergo metabolism through phase II enzymatic reactions in the enterocytes and hepatocytes (189) or microbial metabolism in the gut (190). Metabolites are more likely to reach target sites inside the body and exert health benefits than their parent compounds (191). Evidence of the role of blueberry metabolites (e.g., protocatechuic acid and gallic acid, the catabolites for cyanidin and delphinidin 3-glucoside, respectively) in the modulation of inflammation and/or oxidative stress has also been established (103). Metabolites of elderberry were tested in RAW 264.7 and dendritic cells, and p-coumaric, homovanillic, 4-hydroxybenzoic, ferulic, protocatechuic, caffeic, and vanillic acids [also reported to be blueberry metabolites (25, 192)], exerted a dose-response inhibitory effect on NO (193). Studies regarding berry catabolites are less abundant than studies on berry parent phytochemicals but have gained interest in more recent literature. These studies of microbe- and host-modified phytochemicals are extremely important to fully understand the potential anti-inflammatory effects of blueberry consumption. Although most of the evidence focuses on the effect of individual compounds, it is essential to consider the potency of these metabolites in profiles similar to what occurs physiologically. To take the compound profile and physiologically available doses into account, Rutledge et al. (194) treated LPS-induced rat microglial cells with serum from subjects having regularly consumed blueberry, strawberry, or a placebo powder blends over 90 d. The blueberry consumption decreased NO production, TNF-$\alpha$ secretion, iNOS expression, and moderately modulated COX-2 protein expression in the cells (194). This type of design allows the integration of a more realistic profile of parent compounds and metabolites from blueberry consumption, at physiological doses, within a cell-culture-based model.

The current review summarizes the extensive amount of literature available on blueberry phytochemicals and inflammation using cell-based models. This choice comes with limitations, since it can be challenging to interpret results using specific concentrations of berry-derived molecules on cells when concentrations of these metabolites at the site of the target organs may not be established. There have been major differences in concentrations used to treat the cells, ranging anywhere from tens of $\mu$g/mL to mg/mL for total polyphenols and from tens of ng/mL to $\leq$1.2 mg/mL for anthocyanin fractions. Some of these concentrations are much higher than the blood concentrations that would be present in the body after consumption, as bioavailability of anthocyanins in the body is estimated to be lower than 2%, and peaking at 100 nmol/L after consumption of grape/blueberry juice (195). The relevance of the findings of cell-culture-based studies in complex human systems needs further investigation. These studies should comprise of well-controlled clinical trials, with the relevant choice of placebo controls and inclusion criteria depending on the specific blueberry phytochemical and physiological condition investigated. Future studies should also quantify the entire suite of berry-derived molecules and derivatives in key pools such as the blood, concurrently with physiologic indices of inflammation and oxidative stress.

## Conclusions

General anti-inflammatory and antioxidant outcomes are consistently reported for blueberry extracts or derivatives across many studies. However, results observed in diverse cell culture studies from different investigators are challenging to interpret due to the differences in protocol, treatment, cell line, and analyzed markers. More studies investigating the effects of blueberry extracts on different systems and using comparable conditions would be valuable. Cell-culture-based models are not suitable to draw definitive conclusions on the effects of blueberry compounds on complex physiological processes occurring in the human body. Limitations include the compartmentalization of the observations in space and time: the compounds are only available in the form they are distributed and to the type of cells tested, outside of any regulatory processes by surrounding local tissues or on the whole-body scale, and tested on a one-time, acute, and usually high-dose treatment. Thus, precautions should be taken when drawing conclusions from simplified models, especially when using pharmacological doses of compounds. Despite the limitations, cell-culture-based studies have yielded critical information regarding mechanisms of action of blueberry phytochemicals, and have provided consistent evidence that components of blueberries have anti-inflammation and antioxidant properties, which likely contribute to health and functional benefits attributed to blueberries.

## Acknowledgments

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