

REVIEW ARTICLE

Macrolide Antibiotics as Modulators of Cellular Oxidative Stress: Mechanisms, Pathways, and Therapeutic Implications

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ABSTRACT

Macrolide antibiotics, long recognized for antibacterial properties, have recently emerged as modulators of oxidative stress in eukaryotic cells, reflecting context-dependent effects and pharmacological activities distinct from their antimicrobial activities. The purpose of this review is to examine the capacity and outcomes of macrolides, using erythromycin as the most well-documented example, to modulate responses to stress from reactive oxygen species (ROS) and what this means for redox homeostasis. We compare evidence from studies of mammalian cells and the so-called PI3K/AKT/mTOR and PPAR γ /NF- κ B/Nrf2 signaling pathways to reveal erythromycin's suppressive effects towards oxidative stress-induced senescence, up-regulation of antioxidant enzymes (e.g., superoxide dismutase and catalase), and related effects that mitigate mitochondrial dysfunction. These redox outcomes of exposure to erythromycin are particularly relevant to therapeutic strategies targeting inflammatory and age-related diseases, including chronic obstructive pulmonary disease and neurodegeneration. Conversely, studies of aquatic species, including fish, algae, and invertebrates, suggest that erythromycin induces oxidative damage and genotoxicity. In this review, we discuss factors that are likely to shape this dualistic nature of macrolides in redox modulation such as levels and duration of antibiotic exposure, type of antioxidant system, tissue specificity, and organism complexity. Emerging research in oxidative modulatory properties of erythromycin in eukaryotic and prokaryotic microorganisms, and properties that are shared among macrolides are also discussed. While erythromycin's protective redox effects are therapeutically promising, concerns over chronic use, antioxidant suppression, and other context-dependent outcomes merit the need for systems-level multi-omics approaches to define therapeutic thresholds and ecological risks. This review provides a consolidative overview of our up-to-date understanding of macrolides as redox modulators, and advocates for future investigation into the broad physiological and environmental impacts.

Abbreviations: ARE, antioxidant response element; CAT, catalase; COPD, chronic obstructive pulmonary disease; CSE, cigarette smoke extract; GPx, glutathione peroxidase; H₂O₂, hydrogen peroxide; mTOR, mammalian target of rapamycin; NF- κ B, nuclear factor-kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; O₂⁻, superoxide; OH $\cdot$, hydroxyl radicals; PGC-1 α , PPAR γ coactivator 1-alpha; PI3K, phosphoinositide 3-kinase; PPAR γ , peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; SOD, superoxide dismutase.

1 | Introduction

Oxidative stress plays a critical role in the pathophysiology of numerous human diseases, including neurodegenerative disorders [1], cardiovascular conditions [2, 3], and chronic inflammatory diseases [2, 4, 5]. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the body's capacity to neutralize these species through both enzymatic and nonenzymatic antioxidant mechanisms [3, 6–8]. In living organisms, ROS are mainly produced as radical species like superoxide (O_2^-) and hydroxyl radicals ($OH^\bullet$) as well as non-radical species like hydrogen peroxide (H_2O_2). These derivatives of molecular oxygen can assume either destructive or regulatory roles depending on the cellular context [1, 9–15]. At controlled moderate to low concentrations, ROS play essential roles in cellular processes such as structural formation and signaling, apoptosis and immunity [14]. Free radicals are particularly important in host immunity, where phagocytes actively store and release ROS during infection to exert cytotoxic effects that inactivate enzymes and damage pathogenic DNA [9, 10, 14, 15]. In cell signaling, O_2^- acts as an intracellular messenger, while H_2O_2 facilitates both intra- and inter-cellular communication due to a greater capacity for diffusion within cells [12]. However, at high concentrations, ROS become harmful, compromising the structural integrity of vital cellular components, which, if unchecked, can contribute to disease progression. Prolonged oxidative stress has been linked to an array of dysfunctions such as cellular senescence [2], excessive apoptosis [14, 16], and tissue damage [14], ultimately worsening disease progression.

Macrolides are a class of antibiotics that primarily inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit and preventing the elongation of nascent peptide chains. Structurally, macrolides are characterized by a large macrocyclic lactone ring, typically containing 12–16 members [17]. Macrolides are widely used in treating respiratory, soft tissue, and sexually transmitted infections. Notable macrolides include erythromycin, clarithromycin, azithromycin, and roxithromycin, each varying in pharmacokinetics and spectrum of activity [18]. Erythromycin was first isolated in 1952 from the bacterium *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*). It has since been widely used as an alternative to penicillin for individuals with allergies and has applications in treating bacterial infections such as pneumonia, pertussis, and diphtheria [18]. Beyond the well-documented antibacterial mode of action, erythromycin has been shown to exert immunomodulatory and anti-inflammatory effects in mammalian cells [2, 5, 6, 13]. Recent studies suggest that erythromycin may influence oxidative stress pathways, either by upregulating antioxidant responses or mitigating ROS-induced cellular damage [2, 6, 13]. The ability of erythromycin to modulate cellular pathways as crucial as phosphoinositide 3-kinase (PI3K) and the mammalian target of rapamycin (mTOR), or PI3K/mTOR [2, 19] as well as nuclear factor-kappa B (NF- κ B) [13] further implicates it in oxidative stress modulation, presenting new therapeutic possibilities in addition to the typical antimicrobial role. The purpose of this review is to explore the ability of macrolides to modulate oxidative stress and associated regulatory mechanisms, with a particular focus on erythromycin due to well-documented interactions with key oxidative stress pathways. Although long associated with antimicrobial action,

the potential therapeutic implications of erythromycin's ability to influence oxidative balance in cellular environments are also explored. Additionally, this review highlights erythromycin's dichotomous effects towards antioxidant and pro-oxidant settings, depending on cellular context in eukaryotic and prokaryotic environments.

2 | Clinical Implications

The emergent association between some macrolides and modifications to oxidative stress conditions in mammalian cells has implications for several diseases where ROS and antibiotic therapy intersect. For example, a key part of the innate immune response to combat microbial pathogens during infection is the generation of ROS in neutrophils and other phagocytic cells to kill invading bacteria [9, 15, 20]; these ROS are likely to be present in tissues, cells and the extracellular milieu at times when antibiotic therapy has resulted in the distribution of the drug so as to be colocated [21]. In addition, ROS are central mediators of cellular homeostasis produced in epithelial cells and endothelial cells in various noninfectious diseases, such as chronic obstructive pulmonary disease (COPD) [2], cardiovascular [2] and neurodegenerative diseases [1].

2.1 | COPD and Inflammatory Lung Diseases

Erythromycin has been extensively studied for effects in COPD and other inflammatory lung diseases. COPD is characterized by progressive lung tissue damage and persistent inflammation, exacerbated by oxidative stress [3]. Studies have shown that erythromycin reduces oxidative stress-induced cellular senescence in human bronchial epithelial cells by inhibiting the PI3K-AKT-mTOR signaling pathway [2]. Additionally, erythromycin is shown to mitigate cigarette smoke extract (CSE)-induced inflammation in macrophages by modulating the PPAR γ /NF- κ B signaling pathway [13]. Overall, the findings of Xiaofei et al. [2] suggest that erythromycin may facilitate the reduction of oxidative stress-related lung tissue degeneration in COPD by modulating the PPAR γ /NF- κ B pathway, which in turn exerts anti-inflammatory effects. However, the viability of erythromycin as a therapeutic intervention for COPD remains uncertain due to the lack of a direct causal link between modulatory effects and tangible clinical benefits for COPD patients. Further research is needed to develop a more comprehensive understanding of the underlying mechanisms, but this study provides a conceptual basis for further investigation [2].

2.2 | Potential Neuroprotective, Cardiovascular, and Aging-Related Applications

Oxidative stress plays a central role in neurodegenerative diseases such as Alzheimer's and Parkinson's disease, where excessive ROS generation contributes to neuronal damage and cognitive decline [1]. Given erythromycin's ability to reduce inflammation and modulate oxidative pathways in mammalian cells, potential neuroprotective applications warrant further investigation. Similarly, in cardiovascular diseases, oxidative stress leads to endothelial dysfunction and atherosclerosis, conditions

where erythromycin's anti-inflammatory and antioxidant-modulating properties may have therapeutic relevance.

Biological aging is increasingly being framed as a disease [4, 22], a process characterized by cumulative oxidative damage and cellular senescence. As a result, the concept of targeting aging with pharmacological interventions has gained traction, and studies on erythromycin's effect on lifespan extension in yeast [23] indicate potential relevance to longevity research. Given the controversy surrounding the classification of aging as a disease, further research is required to establish whether erythromycin could be a viable candidate for age-related oxidative stress mitigation.

2.3 | Consequences of Long-Term Antioxidant Suppression

While erythromycin demonstrates promising antioxidant and anti-inflammatory properties, prolonged suppression of oxidative stress pathways may lead to unintended physiological consequences. Oxidative stress serves as a critical signaling mechanism in various cellular processes, including immune defense [9, 14, 24] and apoptosis [14, 16]. However, a study by He et al. [6] demonstrated that erythromycin suppresses the synthesis of glutathione, the body's primary antioxidant defense system that plays a crucial role in redox homeostasis, detoxification, and cellular protection [7, 8]. This raises concerns about whether long-term use could disrupt the oxidant-antioxidant balance [6]. Further research is needed to determine the clinical relevance of erythromycin exposure during various disease conditions.

3 | Mechanisms of Oxidative Stress Regulation by Erythromycin: Protective Antioxidant Effects in Mammals

Erythromycin exerts oxidative stress-modulating effects via multiple molecular pathways, mitigating oxidative stress, inflammation, cellular senescence, and mitochondrial dysfunction in eukaryotic cells exposed to exogenous stressors such as CSE. These mechanisms, detailed in Figure 1, involve the regulation of the PI3K-AKT-mTOR signaling pathway in relation to cellular aging, the axis of PPAR γ /NF- κ B/Nrf2 pro- and anti-inflammatory pathways, particularly important in generating and regulating antioxidant defense systems as well as mitochondrial dysfunction. This section integrates evidence from a range of in vitro and in vivo studies to investigate erythromycin's multifaceted role in oxidative stress regulation in the context of a chronic inflammatory disease such as COPD.

3.1 | Regulation of Enzymatic Antioxidants

Erythromycin enhances the enzymatic and nonenzymatic antioxidant defense systems to counteract ROS accumulation, a process critical for maintaining cellular redox balance [1, 6, 7]. Enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) detoxify ROS by converting cytotoxic free radicals into stable, nontoxic molecules, thereby neutralizing oxidative damage [25]. As a first line

of defense, SOD acts by dismutating superoxide radicals into H₂O₂, reducing ROS accumulation [15]. The mitochondrion is a primary site of excessive ROS generation in an instance of intracellular oxidative stress, where an overactivation of the electron transport chain (ETC) can lead to an overwhelming superoxide buildup caused by electron leakage. Detoxification of excess superoxides is managed by SOD2 within the mitochondria while the conversions are performed by SOD1 in the cytosol [26] (Figure 1). While the dismutation process does generate H₂O₂ that elevates cytosolic ROS levels, CAT and GPx further detoxify these molecules into water and oxygen, preventing damage to cellular components [25]. GPx plays a central role in this detoxification process by reducing lipid peroxides and free radicals by utilizing reduced glutathione (GSH), a major nonenzymatic antioxidant in the body [7]. In eukaryotic cells, erythromycin plays a key role as a regulator of these antioxidants which preserves cellular integrity by mitigating oxidative damage. Specifically, erythromycin has been shown to increase SOD and CAT expression, strengthening the cell's ability to neutralize ROS [2, 13]. This additionally prevents lipid peroxidation, a cascading process of free radicals attacking fatty acids in cell membranes, which leads to the formation of lipid peroxides that are capable of widespread damage to cellular structures. Erythromycin has also been shown to reduce peroxidation, as demonstrated by a decrease in cellular malondialdehyde (MDA) levels, a marker of oxidative damage to the cell membrane [2, 27]. The extent of these regulatory effects of enzymatic antioxidants will be explored in the context of discussing a variety of molecular mechanisms in in vitro and in vivo studies in the next few sections.

3.2 | PI3K-AKT-mTOR Inhibition: Suppressing Oxidative Stress-Induced Senescence

The intracellular signaling pathway PI3K-AKT-mTOR (PAM) serves as a central regulatory system in eukaryotic cells, controlling cellular growth, metabolism, and senescence (aging) [2, 28]. In response to an exogenous stressor such as CSE, enzyme PI3K phosphorylates membrane lipids to produce messenger molecules, initiating downstream signaling [29]. Protein Kinase B, now known as AKT is recruited to the membrane where it is activated, leading to the phosphorylation of a range of targets, one of which is mTOR Complex 1 (mTORC1), a central regulator of cell growth, metabolism, and autophagy [2, 28, 30]. However, when overactivated by CSE-derived ROS, the pathway promotes oxidative damage that leads to premature aging (Figure 1A). This mechanism is particularly relevant in chronic diseases such as COPD [2], where oxidative stress accelerates lung cell aging and inflammation [28]. Macrolides, including erythromycin and rapamycin are well-established in the treatment of inflammatory airway diseases. This was foundational to a study by Xiaofei et al. [2], which explored erythromycin's role in mitigating oxidative stress and cellular aging caused by CSE. Using a murine model of COPD and in vitro exposure of human lung epithelial cells to H₂O₂ (a known inducer of oxidative stress and aging) the study demonstrated that in both models, the PAM pathway was overactivated, causing cells to age faster. Notably, erythromycin's suppression of this activation paralleled the antiaging effects of rapamycin, a well-established cellular longevity-promoting drug. Cellular senescence occurs through replicative or stress-induced pathways, both controlled

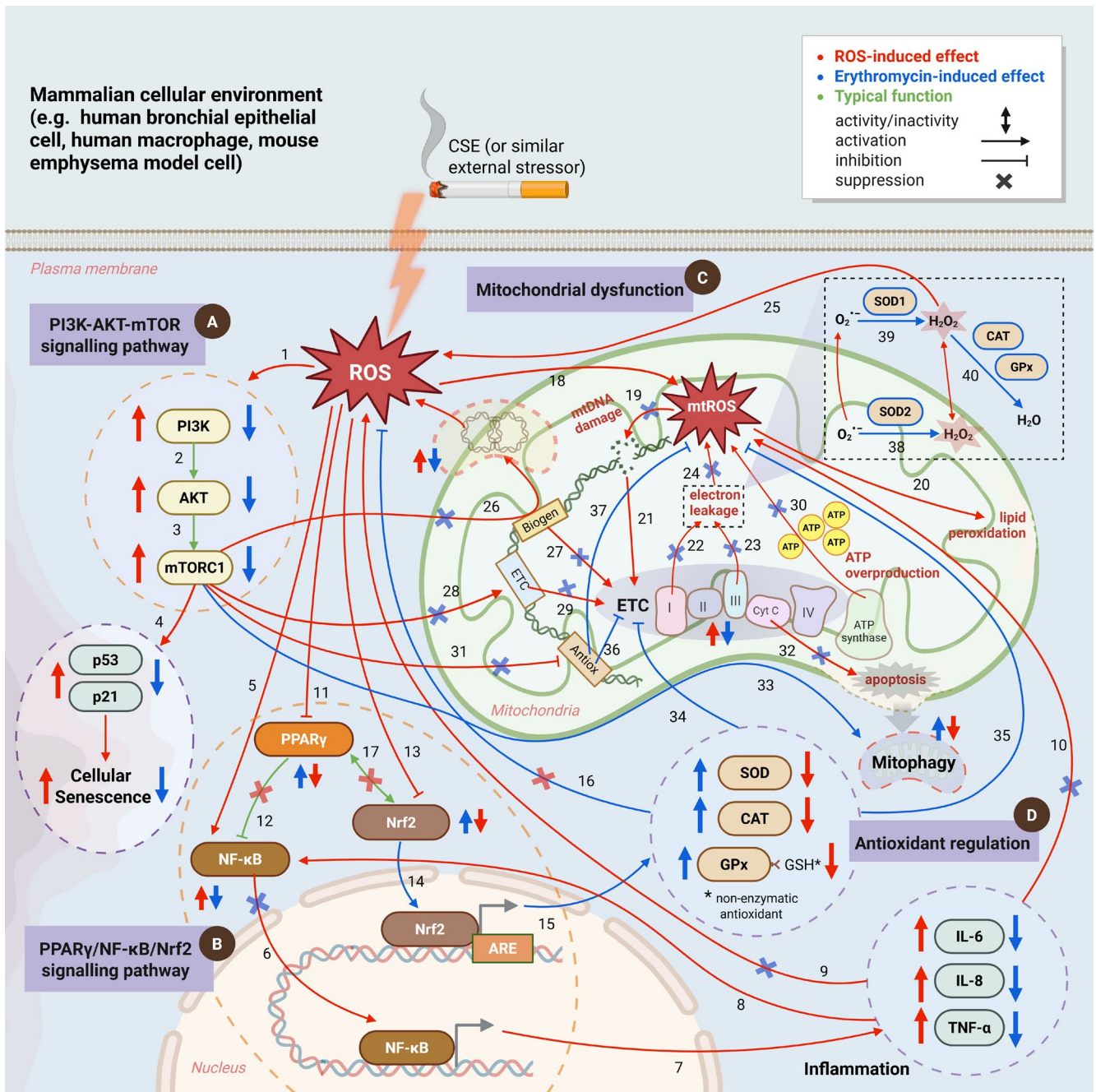


FIGURE 1 | Legend on next page.

by regulatory proteins p53 and p16INK4a, which activate p21, leading to cell cycle arrest. The antiaging effects of erythromycin were evidenced by a significant downregulation of p53 and p21, reducing oxidative-stress-induced premature aging [2].

The consequences of mTORC1's activity extend to mitochondrial dysfunction, where activation by ROS enhances mitochondrial biogenesis and ETC protein expression while suppressing mitochondrial antioxidant gene expression, leading to ATP overproduction and increased mitochondrial ROS (mtROS) [31, 32]. A critical downstream effector of mTORC1 in this context is the Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) coactivator 1-α (PGC-1α) axis, which regulates mitochondrial biogenesis and cellular energy metabolism [32].

When stimulated in response to an increase in CSE-derived ROS levels, mTORC1 activates PGC-1α through phosphorylation and transcriptional regulation, leading to upregulation of mitochondrial biogenesis genes (e.g., NRF1 and TFAM) [30, 31]. These genes (labeled as "Biogen" in Figure 1C), drive the production of new mitochondria to meet cellular energy demands. However, in the context of CSE-induced oxidative stress, this compensatory mechanism becomes maladaptive. Excessive ROS, amplified by mTORC1 activation, stimulates these biogenesis genes, leading to an overproduction of mitochondria; a process that is metabolically taxing especially during a state where energy homeostasis is compromised [31]. Moreover, the PAM pathway's activation by CSE-derived ROS impairs mitochondrial quality control by suppressing mitophagy, the selective

FIGURE 1 | Antioxidant modulatory effects of erythromycin against CSE-induced oxidative stress in mammalian cells. Molecular mechanisms by which erythromycin modulates oxidative stress, inflammation, and cellular senescence in mammalian cells following exposure to cigarette smoke extract (CSE) or a similar external stressor. (A) PI3K-AKT-mTOR signaling pathway illustrates CSE-derived ROS buildup¹ activating the PI3K-AKT-mTOR pathway through sequential phosphorylation of PI3K and AKT², leading to mTORC1 activation³. This cascade upregulates stress-responsive cell cycle regulators p53 and p21⁴, driving cellular senescence. Erythromycin inhibits this pathway by downregulating PI3K, AKT, and mTORC1 activity^{2,3}, reducing oxidative stress-induced senescence. (B) PPAR γ /NF- κ B/Nrf2 signaling pathway outlines the interplay between PPAR γ , NF- κ B, and Nrf2 in response to CSE-induced ROS. ROS activates NF- κ B⁵, which translocates to the nucleus⁶ and induces transcription of pro-inflammatory cytokines IL-6, IL-8, and TNF- α ⁷. These cytokines sustain NF- κ B activation via a positive feedback loop⁸ and amplify ROS generation in the cytosol⁹ and mitochondria¹⁰, promoting oxidative stress and mitochondrial dysfunction. Conversely, ROS suppresses PPAR γ ¹¹, which normally inhibits NF- κ B¹², lifting this anti-inflammatory checkpoint. Erythromycin counters this by upregulating PPAR γ ¹², restoring NF- κ B suppression, and activating Nrf2¹³, a key regulator of antioxidant defense. Nrf2 binds to the antioxidant response element (ARE)¹⁴, driving transcription of antioxidant enzymes superoxide dismutase (SOD1 and SOD2) catalase (CAT), and glutathione peroxidase (GPx)¹⁵. Under ROS overload, Nrf2 activity is suppressed¹³, but erythromycin overcomes this, enhancing antioxidant expression to restore redox balance by neutralizing excess ROS¹⁶. Nrf2 also promotes PPAR γ expression¹⁷, forming a positive regulatory loop. (C) Mitochondrial dysfunction is marked by mitochondrial ROS (mtROS) accumulation¹⁸, disrupting redox homeostasis. mtROS damages mitochondrial DNA¹⁹ and triggers lipid peroxidation²⁰, impairing membrane integrity. This destabilizes the electron transport chain (ETC)²¹, particularly at Complexes I (electron input from NADH)²² and II (electron transfer from quinol pool to cytochrome c)²³ causing electron leakage²⁴ and further superoxide production. This amplifies mtROS and contributes to cytosolic ROS²⁵, exacerbating oxidative stress. Concurrently, mTORC1, activated by cytosolic ROS³, worsens mitochondrial dysfunction by enhancing mitochondrial biogenesis through biogenesis-related gene expression^{26,27} (labeled “Biogen”) and upregulating ETC protein expression^{28,29} (encoding genes labeled “ETC”). This leads to excessive mitochondrial biogenesis²⁶, ETC overactivation²⁷, and ATP overproduction³⁰, further increasing mtROS. mTORC1 also suppresses mitochondrial antioxidant gene expression^{31,37,38} (labeled “Antiox”), reducing ROS detoxification capacity. This stress induces cytochrome c release³³, triggering apoptosis, and impairs mitophagy³⁴, allowing damaged mitochondria to accumulate and perpetuate ROS production. Erythromycin mitigates this cycle by suppressing mTORC1³, reducing mitochondrial biogenesis^{26,27} and ETC overactivity^{28,29}, while restoring mitophagy³³ to clear damaged mitochondria. Additionally, erythromycin-induced antioxidant upregulation¹⁵ stabilizes ETC function³⁴ and enhances mitochondrial antioxidant capacity³⁵, re-establishing redox balance. Both cytosolic antioxidant regulation (Section D) and mitochondria-specific Antiox^{31,36,37} reduce mtROS, though via distinct mechanisms. The former indirectly buffers mitochondrial ROS through global redox control, while the latter directly buffers ROS generation within mitochondria. (D) Antioxidant regulation centres on the enzymatic and nonenzymatic antioxidant defense system counteracting ROS accumulation. Antioxidants SOD, CAT, and GPx detoxify ROS intermediates in the cytosol¹⁶ and mitochondria, a primary ROS source^{34,35}. Within mitochondria, SOD2 converts superoxide radicals (O₂⁻), generated by ETC electron leakage, into hydrogen peroxide (H₂O₂)³⁸, while SOD1 performs this dismutation in the cytosol³⁹. This H₂O₂ can elevate cytosolic ROS levels²⁵, but CAT and GPx reduce it to water⁴⁰, mitigating oxidative stress. Legend keys of red arrows, crosses and text represent CSE-derived ROS-induced effects; blue arrows and crosses represent erythromycin-mediated protective effects, reflecting antioxidant and regulatory actions; and green arrows indicate typical cellular functions that may arise from physiologically elevated ROS levels, though not to the excessive extent induced by CSE. Terms *activity* and *inactivity* refer to up-regulation or downregulation of cellular functions, ranging from protein expression to broader physiological outcomes such as cellular senescence. *Activation* and *inhibition* denote interactions occurring at the level of gene-to-protein expression, where inhibition specifically refers to a direct blocking of molecular function or other biological activity. In contrast, *suppression* refers to a more indirect intervention that counters a pre-existing interaction—either due to excessive ROS or erythromycin's modulatory influence. This may even result from upstream effects triggered by other pathways. Orange dotted circles represent signaling pathways, while purple dotted circles highlight pathway outcomes or downstream consequences.

autophagic clearance of dysfunctional mitochondria. Mitophagy is regulated by the interplay between mTORC1 and autophagy-related pathways, where mTORC1 activation inhibits autophagy initiation by phosphorylating ULK1, a key autophagy regulator [33]. In CSE-exposed cells, mTORC1-driven suppression of mitophagy prevents the clearance of damaged mitochondria, which accumulate and continue to produce mtROS due to ETC dysfunction. This accumulation of dysfunctional mitochondria not only sustains oxidative stress but also triggers cytochrome c release, initiating apoptosis, further compromising cellular viability [34].

Erythromycin inhibits the PAM pathway by downregulating PI3K, AKT, and mTORC1 activity, reducing oxidative stress-induced senescence and mitochondrial dysfunction [35]. By suppressing mTORC1, erythromycin also reduces the maladaptive activation of the PGC-1 α axis, decreasing the expression of biogenesis genes and alleviating the metabolic burden of excessive mitochondrial production [35]. Furthermore, erythromycin's indirect influence could play a role in restoring mitophagy by

relieving mTORC1-mediated inhibition of autophagy, enabling clearance of dysfunctional, ROS-generating mitochondria [33]. These mechanistic observations are further supported by a recent proteogenomic study, which conducted a systems-level analysis to confirm erythromycin's downregulatory effects on the PI3K/AKT/mTOR axis. Notably, this study also highlighted erythromycin's ability to attenuate NETosis; a unique form of cell death involving the release of neutrophil extracellular traps (NET) and implicated in the progression of airway inflammation in COPD [35].

3.3 | Influence of PPAR γ /NF- κ B/Nrf2 Pathway on the Anti-Inflammatory State

Elevations of CSE-induced ROS within the intracellular environment have a significant impact on the PPAR γ /NF- κ B/Nrf2 signaling axis, which is the primary regulator of pro- and anti-inflammatory responses in this context (Figure 1B) [13]. The PPAR γ is a type of ligand-dependent transcriptional mediator

in the cell nucleus, involved in lipid metabolism, inflammation, and oxidative stress regulation [36]. This nuclear receptor plays an essential role in maintaining cellular homeostasis, particularly in lung and immune cells. Activation of PPAR γ promotes antioxidant responses, suppressing inflammatory pathways and reducing oxidative-stress-related cellular damage. As the primary driver of inflammation, NF- κ B is activated by ROS, leading to its translocation to the nucleus, inducing transcription of pro-inflammatory cytokines such as interleukin-6 (IL-6), IL-8, and tumor necrosis factor- α (TNF- α). These cytokines sustain NF- κ B activation through a positive feedback loop and amplify CSE-derived ROS generation in the cytosol and mitochondria, exacerbating oxidative tissue damage that contributes to chronic inflammatory diseases like COPD, as well as cardiovascular and neurodegenerative disorders [3]. Conversely, CSE-ROS suppresses PPAR γ function, which negates its normal role of inhibiting NF- κ B, worsening the inflammatory response. Erythromycin counters this imbalance by upregulating PPAR γ expression, which reinstates its suppressive control over NF- κ B, reducing pro-inflammatory cytokine production and preventing transcription of oxidative stress mediators [35]. Simultaneously, CSE-derived ROS suppresses the activation of nuclear factor erythroid 2-related factor 2 (Nrf2), a primary driver of antioxidant defense, which binds to the antioxidant response element (ARE) to initiate transcription of antioxidant enzymes such as SOD, CAT, and GPx [3, 24, 25, 37]. This suppression further weakens PPAR γ , as Nrf2 and PPAR γ typically engage in a positive feedback loop where Nrf2 enhances PPAR γ expression, and PPAR γ activation amplifies Nrf2 activity [34]. Erythromycin restores this feedback loop, enhancing Nrf2 activation and subsequently bolstering the expression of antioxidant enzymes [36]. This dual action on PPAR γ and Nrf2 highlights the significance of erythromycin's modulatory role in promoting anti-inflammatory and antioxidant effects, particularly as downstream consequences of this signaling axis include the detoxification of mitochondrial ROS, a key contributor to mitochondrial dysfunction [25, 32, 33, 36, 38].

4 | Mechanisms of Oxidative Stress Regulation by Erythromycin: Effects in Aquatic Species

4.1 | Redox Effects in Fish

While erythromycin generally exerts protective effects in mammalian systems in relation to ROS stress, studies in aquatic organisms highlight more complex and variable redox responses highlighting the ineffectiveness of generalizing such effects. Fish are a commonly studied organism for pharmaceutical pollution research, with various studies documenting oxidative stress effects in fish exposed to erythromycin. For instance, Rodrigues et al. [39] investigated the effects of environmentally relevant concentrations of erythromycin on Rainbow trout (*Oncorhynchus mykiss*), a freshwater species. The study found that erythromycin exposure induced mild but significant oxidative stress in gill tissue, as evidenced by altered antioxidant enzyme activities and increased lipid peroxidation. Specifically, acute exposure disrupted CAT and glutathione reductase (G_{Red}) activity in the gills, alongside elevated thiobarbituric acid reactive substances (TBARS) levels. TBARS is important as it measures lipid peroxidation by detecting the presence of MDA

in study samples. In contrast, chronic exposure led to a reduction in CAT activity and elevation in GPx activity and TBARS in gill tissue. Interestingly, the liver exhibited a distinct response, with TBARS levels decreasing following erythromycin exposure, suggesting tissue-specific differences in oxidative stress outcomes. Despite lower lipid peroxidation in the liver, erythromycin exposure resulted in significant DNA damage in blood cells, as indicated by genotoxicity assays. Taken together, these findings (summarized in Figure 2) suggest that erythromycin-induced oxidative stress is a likely contributor to the observed genotoxic effects in trout [39].

Similar pro-oxidative effects have been reported in Indian major carp (*Labeo rohita*), another freshwater species, following erythromycin exposure. In gill tissue, SOD activity initially declined during acute exposure but increased during prolonged exposure, whereas in the liver, SOD activity remained consistently elevated throughout the period of exposure. Catalase activity was significantly reduced in both gill and liver tissues, while GPx activity and lipid peroxidation levels increased, indicating a buildup of ROS-induced lipid damage [27].

In contrast, a multi-biomarker study by Rodrigues et al. [40] in 2019, examined the effects of erythromycin exposure on Gilthead seabream (*Sparus aurata*), a marine species. The study found only slight pro-oxidative effects following both acute and chronic erythromycin exposure compared to the more pronounced oxidative responses observed in freshwater fish [40]. This suggests that marine fish may exhibit greater resilience to erythromycin-induced oxidative stress, potentially due to species-specific physiological differences or environmental factors.

4.2 | Redox Effects in Aquatic Invertebrates

Aquatic invertebrates, such as crustaceans and mollusks (though studied less than fish and algae) also experience oxidative stress from erythromycin exposure to varying degrees. Bivalve mollusks, specifically the blue mussel (*Mytilus edulis*) exhibit an antioxidant defense response when challenged with high concentrations of erythromycin. This response is shown in the activities of GST and CAT in their tissues that increase significantly following exposure to high concentrations of erythromycin [41]. This is illustrated in Figure 2. Such upregulation of GST and CAT suggests that the mussel's cells recognize erythromycin as an oxidative stressor and activate enzyme systems to mitigate potential damage. At these elevated concentrations, mussels also show disturbances in normal metabolism, as evidenced by changes in metabolite profiles related to energy and osmotic regulation. Notably, levels of certain amino acids and osmolytes (alanine, glutamate, taurine, glycine, and betaine) rose in erythromycin-exposed mussels, indicating a shift in metabolic pathways to confront stress. Betaine, in particular, was identified as playing a protective role, likely stabilizing proteins and membranes under oxidative stress by adjusting cellular osmotic pressure. These biochemical adjustments support the interpretation that erythromycin does not inherently cause oxidative damage in mussels, but challenges cellular homeostasis and that these organisms can compensate to some extent by metabolic regulation and antioxidant deployment, at least

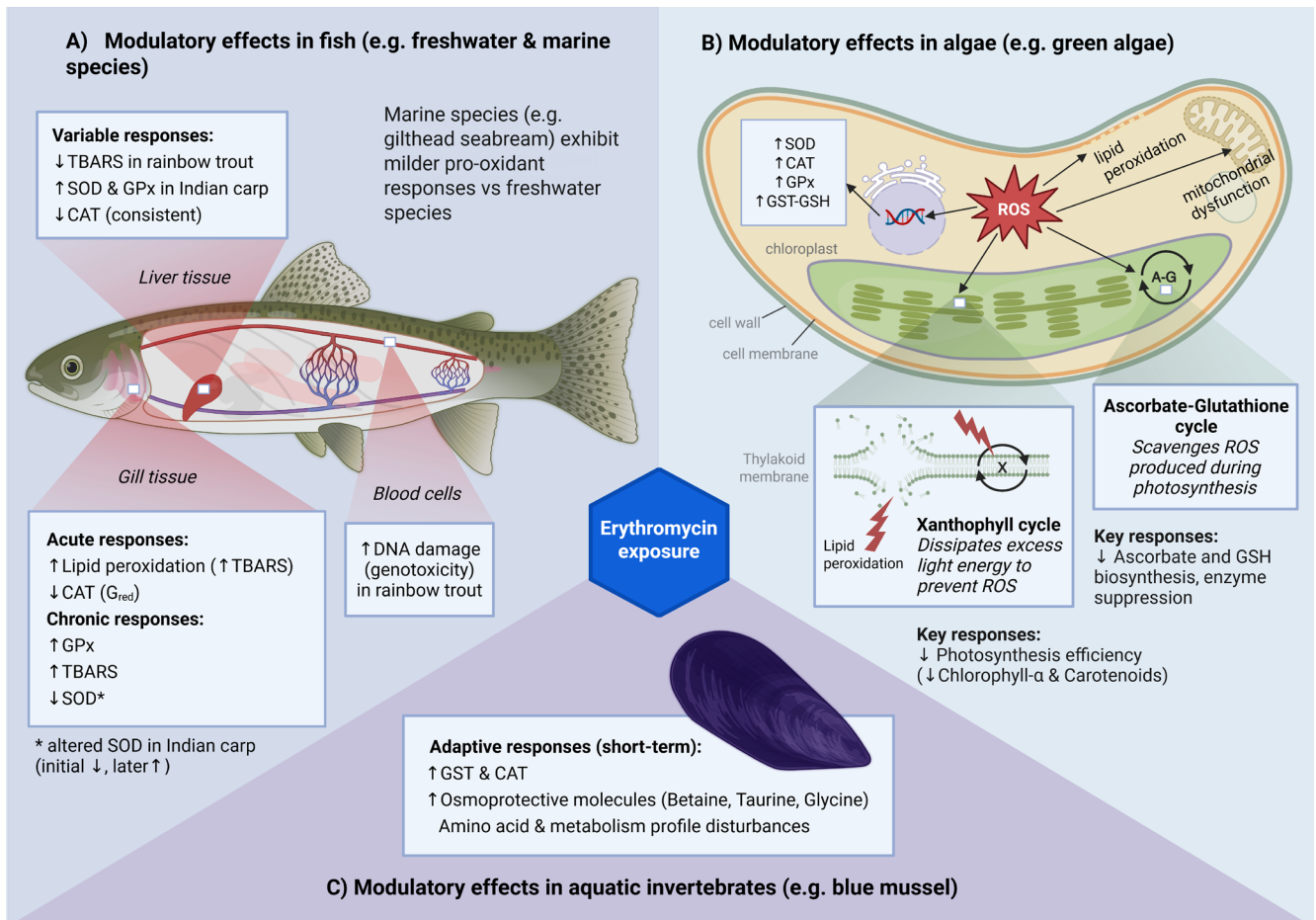


FIGURE 2 | Context-specific oxidative stress responses induced by erythromycin in aquatic eukaryotes. Erythromycin exposure elicits pro-oxidant effects across diverse aquatic taxa, mediated through tissue-specific and organism-dependent mechanisms. (A) In fish, erythromycin alters antioxidant enzyme activity and induces lipid peroxidation (↑ TBARS) and genotoxicity in blood cells. Responses vary between tissues (gill vs. liver) and between species, with freshwater species generally more sensitive than marine counterparts. (B) In *P. subcapitata* green algae, erythromycin disrupts photosynthetic function and redox balance by increasing intracellular ROS, indicated by upregulation of the cellular antioxidant system (signified by measured levels of SOD, CAT, GPx and GST-GSH), lipid peroxidation and mitochondrial dysfunction. Cytotoxic effects include impairment of the ascorbate–glutathione and xanthophyll cycles, and a decrease in photosynthetic efficiency marked by reduced production of chlorophyll-α and carotenoids. (C) Modulatory effects in aquatic invertebrates such as blue mussels indicate that erythromycin induces oxidative stress but also triggers short-term adaptive responses involving antioxidant enzyme upregulation and metabolic shifts to preserve homeostasis. The diagram highlights the context-dependent nature of erythromycin toxicity in environmentally significant eukaryotic organisms, emphasizing the role of ROS accumulation, enzyme modulation, and tissue- or organ-specific vulnerability across systems.

under short-term exposure [41]. Alternatively, these observations might reflect direct antioxidant effects of erythromycin in mussels that cause upregulation of GST and CAT activity akin to those reported in mammalian systems in which erythromycin induces antioxidant responses via the upregulation of SOD, CAT, and GPx (figure 1, part 15 [37]). Part of the challenge to these interpretations is the design of the studies of aquatic invertebrates, which exclude any direct ROS measures, instead relying on surrogate measures for oxidative stress, namely levels of antioxidant molecules. This means that it is difficult to confidently ascribe the effects of erythromycin in these systems as either driving stress from ROS or supporting antioxidant defense responses via the upregulation of detoxification systems. In other words, concluding whether erythromycin is the cause of the antioxidant response or the trigger of oxidative stress in the mussel, which leads to a normal antioxidant defense response, is a remaining challenge.

4.3 | Redox Effects in Algae and Bacteria

In pharmaceutical pollution research, primary producers such as algae and cyanobacteria are directly exposed to antibiotics in water and often exhibit sensitive oxidative stress responses [42–45]. This is well demonstrated in a study conducted by Wan et al. [46] where biphasic effects of erythromycin on the growth of the cyanobacterial algae *Microcystis flos-aquae* were investigated. Results show that at very low concentrations, erythromycin can stimulate algal growth and photosynthetic activity, showing no significant impact on ROS generation or antioxidant enzyme activity. However, as erythromycin concentrations increase to sub-milligram levels, this beneficial effect reverses. High erythromycin levels are shown to severely inhibit *Microcystis* growth and induce pronounced oxidative stress. Under high-dose exposure, the algae showed significant increases in SOD and CAT activities

along with elevated MDA content, indicating that oxidative damage to lipids was occurring. The simultaneous rise in intracellular ROS confirmed that the antioxidant defenses were unable to fully counteract the oxidative challenge at these concentrations [46]. The reporting of direct ROS measures in this study, unlike those for the aquatic invertebrates discussed above, lends strong support to the model that erythromycin induces ROS in these prokaryotes and that the antioxidant responses observed in these cells are a response to this stress. Chlorophylls play a key role in primary photosynthesis, which is one of the integral processes for metabolic activities in algae. In this study, erythromycin is shown to impair the photosynthetic apparatus of *M. flos-aquae*, as evidenced by declines in chlorophyll fluorescence parameters with increasing erythromycin dose and exposure time. These reductions are likely linked to oxidative damage of thylakoid membranes via lipid peroxidation, along with ROS-induced degradation or modification of chlorophyll and associated proteins [46], illustrated in Figure 2.

Pseudokirchneriella subcapitata (also known as *Raphidocelis subcapitata*), is a freshwater green alga that has also shown similar patterns of oxidative disruption, where a breakdown of the organism's antioxidant system is observed when exposed to erythromycin. A study by Zhang et al. [44] demonstrates that when exposed to erythromycin, this disruption occurs across multiple antioxidant pathways in *P. subcapitata*, more so than following exposure to some other common antibiotics like ciprofloxacin or sulfamethoxazole. The study highlights that erythromycin exposure led to reduced biosynthesis of ascorbate (Vitamin C) and GSH, two crucial nonenzymatic antioxidants in algal cells. Consequently, the normal function of the ascorbate-glutathione cycle and the xanthophyll pigment cycle that protect the photosynthetic machinery were disrupted. The major antioxidant enzymes of CAT, SOD, GPx, and glutathione s-transferase (GST) were also downregulated in *P. subcapitata* by erythromycin, preventing a sufficient antioxidant response. Instead, the compound overwhelms the algae's defenses, likely resulting in heightened oxidative damage to cellular components. Further experiments in the study confirm that *R. subcapitata* is quite sensitive to erythromycin, as high concentrations of it led to a decrease in chlorophyll- α and total carotenoid content, affecting the ability to photosynthesize and ROS scavenging capacity [44]. Overall, erythromycin is identified as a disrupter of redox homeostasis in photosynthetic organisms, impairing both growth and ecological function (primary production) by inducing oxidative stress conditions and impairing antioxidant defense mechanisms.

Interestingly, erythromycin's redox-modulating effects are not limited to eukaryotes, which have been a long-held assumption [2, 5, 6, 13, 17, 35] in this area of research. A recent study by Desai et al. [47] examined the protective effects of erythromycin in *Streptococcus agalactiae* when exposed to hydrogen peroxide-induced oxidative stress. These findings revealed that erythromycin alleviated H₂O₂-induced growth inhibition [47], raising questions about whether erythromycin alters streptococcal antioxidant defense mechanisms during oxidative stress.

5 | Mitochondrial Oxidative Stress Regulation by Erythromycin

Erythromycin appears to confer protective effects against mitochondrial dysfunction in mammalian systems exposed to oxidative stress, as outlined in Figure 1. However, contrasting evidence from studies on aquatic species and isolated mitochondrial systems suggests that erythromycin may impair mitochondrial function, highlighting a context-dependent duality in its modulatory role. Mitochondria are primarily characterized as major contributors to cellular metabolism due to substantial production of ATP through oxidative phosphorylation [38, 48, 49]. Additionally, mitochondria are also major producers of ROS, particularly at the electron transport chain (ETC) complexes I and III [50] that actively contribute to ROS signaling—a crucial function in oxidative stress regulation in cells [38, 49].

As outlined in previous sections, erythromycin's ability to suppress excess ROS-induced overactivation of intracellular signaling pathways carries both direct and indirect implications for mitochondrial health under oxidative stress (Figure 1C). In support of this, a study in *Saccharomyces cerevisiae* demonstrated that erythromycin enhanced mitochondrial efficiency by improving oxidative phosphorylation and reducing ROS leakage. More importantly, erythromycin was shown to extend the replicative lifespan of yeast by approximately 27%, an effect that was strictly dependent on functional mitochondrial respiration. However, such lifespan-extending effects were not observed in non-respiring yeast strains, which suggested that mitochondria are key mediators of erythromycin's longevity effects [23]. In this study conducted by Holbrook et al. [23], enhanced translational accuracy, reduced metabolic stress, and potentially lower ROS generation were theorized as factors that collectively contributed to a delay in cellular aging. Similar lifespan-extending benefits were also reported in *Caenorhabditis elegans*, where erythromycin analogues such as azithromycin improved mitochondrial function, reducing oxidative damage markers, and attenuating energy demands [51]. These observations may reflect a conserved mechanism among eukaryotic systems that enhances mitochondrial function in specific stress contexts.

Despite these protective effects, emerging evidence also suggests the contrary, where macrolides such as erythromycin are shown to disrupt mitochondrial activity. A study investigating erythromycin's impact on isolated rat heart mitochondria revealed that erythromycin exposure led to mitochondrial dysfunction, characterized by elevated ROS production and lipid peroxidation; an indication of oxidative stress-induced damage [52]. Similarly, in zebrafish cell lines, erythromycin reduced cellular viability and disturbed antioxidant balance, demonstrating pro-oxidant effects that are potentially linked to mitochondrial toxicity [53]. Additionally, a study by De Vries showed that erythromycin can inhibit protein synthesis in isolated mitochondria from renal-derived BHK-21 hamster cell lines. However, this inhibitory effect is not observed in intact mitochondria, suggesting that erythromycin's ability to penetrate the mitochondrial membrane is limited. Similar permeability limitations were observed with other macrolides used in this study, including tylosin tartrate, spiramycin, and

carbomycin. However, the extent of permeability varied, with the mitochondrial membrane acting as a barrier to erythromycin, while tylosin tartrate and spiramycin were limited by the cell membrane [54, 55].

6 | Context-Specificity of Erythromycin's Protective and Damaging Redox Effects

Taken together, erythromycin appears to induce context-dependent protective or damaging effects in response to oxidative stress across a range of biological systems in vitro and in vivo, from mammalian to nonmammalian aquatic species. This dichotomy can be interpreted as erythromycin functioning as a chemical stressor, challenging redox homeostasis by triggering adaptive activation of antioxidant defenses. The variability of redox effects seems to be dependent on a range of factors such as the intensity of oxidative stress on the system, the cellular or organismal capacity to neutralize ROS, and the efficiency of repair mechanisms. In various nonmammalian aquatic species such as fish, algae, and invertebrates, erythromycin exposure has been shown to upregulate antioxidant enzymes; indicating an attempt to counteract ROS accumulation. However, when erythromycin exposure is high, chronic, or affects sensitive tissues, antioxidant upregulation may fail to fully neutralize ROS or repair oxidative damage, leading to cytotoxic and genotoxic effects. In such cases, the increased expression of antioxidant defenses likely reflects cellular distress rather than successful adaptation, possibly due to limitations in enzymatic capacity, metabolic reserves, or redox buffering systems [56].

Understanding these factors that drive varying redox response will be crucial for assessing erythromycin's broader implications in both clinical and environmental contexts. Across all findings highlighted in this review, several factors appear to govern whether erythromycin's oxidative impact manifests as protective or damaging (Figure 2).

6.1 | Dosage and Exposure Duration

At low or therapeutic doses, erythromycin tends to promote protective effects by inhibiting inflammatory signaling (e.g., NF- κ B inhibition) and upregulating antioxidant enzymes [17]. However, high doses or chronic exposure have been linked to mitochondrial dysfunction, ROS accumulation, and lipid peroxidation in various models, including isolated rat heart mitochondria [52] and aquatic species [44], all displaying cytotoxicity. This dose-dependent modulatory effect is not unique to erythromycin as a study by Mao et al. [57] demonstrated that azithromycin (another macrolide) induces ROS accumulation and photosynthetic damage in microalgae, leading to impaired growth and energy metabolism.

Erythromycin's redox-modulating effects are dose-dependent across models discussed in this review. In human lung epithelial cells, pretreatment with 10 μ g/mL erythromycin (cell viability unaffected up to 100 μ g/mL) reduced H₂O₂-induced oxidative stress and senescence via PI3K-AKT-mTOR inhibition. In vivo, cigarette-smoke-exposed mice gavaged with

100 mg/kg/day erythromycin showed parallel anti-senescence and antioxidant effects [2]. The mouse dose corresponds to a human-equivalent dose (HED) of ~8 mg/kg (~560 mg for a 70 kg adult) using standard body-surface-area scaling [58]. In humans, standard oral doses of erythromycin (250–500 mg) typically produce blood concentrations in a low range (~1–2.5 mg/L) [59–61]. By comparison, the 10 μ g/mL concentration used in cell studies is higher than what is usually observed in patients [2]. This reflects a common practice where in vitro assays typically require drug levels multiple times higher than human peak plasma concentrations to induce measurable effects, due to the lack of pharmacokinetic processes such as distribution, metabolism, and clearance in these simplified systems [62]. Placing erythromycin's redox-modulatory effects in the context of pharmacologically relevant antimicrobial activity is important, as host-directed outcomes are most likely to be clinically meaningful when overlapped with bacteriostatic or therapeutic exposures. For example, erythromycin's bacteriostatic activity against susceptible Gram-positive pathogens occurs in the sub- to low- μ g/mL range, with MIC₅₀ and MIC₉₀ values for *S. pneumoniae* reported at 0.063 mg/L and 0.13 mg/L, respectively, while clinical breakpoints defined by EUCAST reported susceptibility at $\leq$ 0.25 mg/L and resistance at $>$ 0.5 mg/L [63, 64]. In this context, although the in vitro antioxidant effects were observed at concentrations above both bacteriostatic levels and typical plasma exposures, the in vivo murine dosages translated to human-equivalent exposures within the clinical range, highlighting the potential translational relevance.

6.2 | Cell or Tissue Type

Tissues associated with high metabolic activity (e.g., gills, photosynthetic cells) or those directly interacting with erythromycin-available environments tend to be more sensitive to oxidative stress [39, 46]. Even within an organism, different tissues (e.g., fish gills vs. liver) show variable antioxidant responses to erythromycin exposure (Figure 2A,B).

6.3 | Organismal Complexity

Systems as simple as yeast demonstrate lifespan extension under erythromycin exposure [23], which could be reflective of efficient systematic regulation of redox homeostasis. In contrast, complex multicellular organisms exhibit more organ- and tissue-specific variations, with some tissues being inherently more vulnerable than others (Figure 2). Apart from factors such as direct exposure to erythromycin-present environments, these variable effects could also be indicative of more intricate factors such as metabolic specialization of certain tissues. For example, photosynthetic cells in green algae are characterized by high oxygen flux and intense light-driven electron transport, both processes naturally generating significant amounts of ROS even under optimal conditions [65, 66]. Therefore, photosynthetic organisms rely on specialized antioxidant systems (e.g., the ascorbate-glutathione cycle and xanthophyll cycle) localized within chloroplasts to tightly regulate redox balance (Figure 2B). Due to this inherent predisposition to oxidative imbalance, exposure to erythromycin as

an environmental contaminant can easily result in a dramatic rise in intracellular ROS, overwhelming antioxidant defenses, leading to structural oxidative damage [44].

6.4 | Dependence on Antioxidant Systems

The type of antioxidant system that is dominant in an organism, whether enzymatic (SOD, CAT, GPx etc.) or glutathione-based, may influence the capacity to cope with erythromycin-induced stress. This is because systems heavily reliant on glutathione homeostasis may potentially be vulnerable to long-term erythromycin exposure that is linked to suppression of GSH synthesis [6], a process that might lead to further redox imbalance.

7 | Oxidative Stress Modulation by Other Macrolides and Broader Implications

While erythromycin has served as the central focus of this review, a variety of studies indicate that other macrolide antibiotics, most notably azithromycin, clarithromycin, and roxithromycin, also modulate oxidative stress in eukaryotic systems through shared mechanisms.

Azithromycin is one of the most extensively studied macrolides in the context of redox modulatory effects in eukaryotes. In nonmammalian organisms, such as green microalgae (*Chlorella pyrenoidosa*), azithromycin has been shown to induce biphasic oxidative responses [57]. At low concentrations, azithromycin enhanced photosynthetic pigment synthesis and upregulated expression of *psbA*, a gene encoding a photosystem II protein essential for maintaining efficient electron transport during photosynthesis, often used as a biomarker for photosynthetic health and promoting antioxidant capacity. However, at higher concentrations, it disrupted photosynthetic electron transport, increasing ROS accumulation and causing severe oxidative damage to thylakoid membranes, paralleling erythromycin's damaging effects on photosynthetic organisms. In mammalian models, azithromycin's oxidative modulation is similarly complex. Protective effects have been reported in studies involving COPD, CS exposure, and colitis, often attributed to Nrf2 activation and ROS suppression [67]. Conversely, in cancer models, azithromycin has been shown to induce mitochondrial dysfunction, accumulate ROS, leading to the activation of the integrated stress response (ISR) (a key intracellular signaling pathway that regulates protein synthesis and gene expression) enabling cells to adapt to a range of stresses including oxidative stress. Transcription factors TFEB and TFE3 are also activated downstream during ISR that regulate lysosomal biogenesis, autophagy, and metabolic adaptation in response to the redox imbalance [68]. These stress responses were confirmed to be ROS-dependent and were reversed by the ROS scavenger N-acetylcysteine (NAC), implicating azithromycin in pro-tumorigenic oxidative signaling under specific conditions [68].

Clarithromycin also demonstrates dose-dependent redox effects in both environmental and clinical models. For instance, clarithromycin induces oxidative stress and mitochondrial

impairment in liver cells [69], whereas in algae species such as *Chlorella vulgaris*, it triggers MDA accumulation and antioxidant enzyme modulation [70] similar to erythromycin and azithromycin. The context dependency of these dual effects by clarithromycin is further emphasized by a study that highlights how low-dose long-term clarithromycin exposure boosts cellular antioxidant defenses in bronchial epithelial cells [71]. Specifically, the cells were shown to increase Nrf2 expression and glutathione levels, while suppressing NF- κ B-driven IL-8 production in oxidant-challenged cells, paralleling erythromycin's mode of oxidative stress modulation. Furthermore, roxithromycin has garnered attention for anti-inflammatory and antioxidant properties. For instance, in rodent colitis models, roxithromycin treatment resulted in normalization of oxidative stress markers such as GSH and TBARS [72]. Furthermore, roxithromycin was shown to inhibit NF- κ B activity and attenuate redox-sensitive inflammation in a rodent hepatocarcinoma study [73].

As illustrated, macrolides generally exert context-dependent redox modulation through host-signaling pathways rather than direct free radical scavenging. Comparatively other antibiotic classes show varied and often pro-oxidant modulation profiles. For example, Kalghatgi et al. [74] demonstrated that bactericidal antibiotics such as fluoroquinolones, β -lactams, and aminoglycosides trigger mitochondrial dysfunction and ROS overproduction in mammalian cells and mice, leading to oxidative macromolecular damage, subsequently mitigated by N-acetyl-L-cysteine. Bacteriostatic tetracyclines, by contrast, did not elevate ROS under similar conditions [74]. Reinforcing these interclass variations, aminoglycosides are consistently characterized as pro-oxidant, particularly in the context of inducing ROS-dependent injury in hair cells and renal epithelia. Resulting ototoxic and nephrotoxic phenotypes have been shown to be attenuated by antioxidants, indicating contributions from iron-drug redox cycling and disruption of mitochondrial translation leading to oxidative stress [75, 76]. Fluoroquinolones likewise elevate ROS levels by disrupting electron transport, correlating with adverse outcomes such as tendinopathy [77, 78]. Conversely, β -lactams exhibit a relatively mixed modulation profile. In cell-based studies on β -lactams, increases in mtROS at therapeutic ranges are reported, while chemical assays indicate mild or balanced antioxidant potential, highlighting the intraclass variation [74, 79]. Tetracyclines comparatively occupy an intermediate position for exhibiting redox modulatory properties. Compounds such as minocycline and doxycycline display antioxidant and neuroprotective effects at low exposures yet can induce ROS through mitochondrial translation interference at higher or prolonged doses [80, 81]. Unlike cell-based work, a recent study by Maliar et al. [79] applied in vitro chemical assays that quantified intrinsic antioxidant or pro-oxidant capacity of drugs by monitoring radical-scavenging and reducing activity. Across the 18 clinically prescribed antibiotics tested, only six antibiotics that are tetracyclines, a rifamycin and β -lactams showed any measurable antioxidant capacity. Most other antibiotics, including a macrolide (azithromycin) did not show significant redox capacity. However, tetracyclines and rifampicin displayed dual antioxidant/pro-oxidant potential, while some β -lactams (e.g., tebipenem, selected cephalosporins) exhibited antioxidant-leaning effects [79]. These findings imply that direct chemical antioxidant activity is uncommon across antibiotics

and largely absent in macrolides, reinforcing the interpretation that redox-modulatory actions in mammalian systems derive from host-pathway regulation rather than radical scavenging.

8 | Future Directions and Concluding Remarks

Current and emerging evidence supports a context-dependent role for erythromycin and some other macrolides as modulators of oxidative stress conditions and responses in eukaryotic cells. Themes that are common threads among the mechanisms underlying these effects encompass the degree of activation of the Nrf2 pathway, NF- κ B suppression, and modulation of ROS levels. Studies alluding to distinct effects of azithromycin, clarithromycin, and roxithromycin compared to erythromycin suggest the possibility of class-level redox-modulating properties in macrolides. However, there remain challenges to interpreting studies so far reported and open questions necessitate work to better understand erythromycin's therapeutic potential and environmental impact in the context of ROS and oxidative stress defense.

From a therapeutic perspective, direct causal links between modulatory effects and clinical benefits remain uncertain. While protective outcomes are generally observed in mammalian systems at therapeutic levels, cytotoxicity is linked to chronic or high-dose exposure. This distinction remains ambiguous, as evidenced by variability in redox modulatory responses after exposure to different macrolides. A key question is what are the therapeutically relevant parameters for redox modulation by macrolides in terms of dose and duration thresholds to distinguish beneficial from harmful outcomes? These may differ for long-term usage in chronic diseases or for repurposing efforts in aging and inflammation-related disorders. The threshold at which antioxidant upregulation shifts from an indicator of resilience against oxidative stress to a marker of cell distress remains unclear. Evaluation of redox homeostasis across exposure gradients, biological scale (from cellular to organismal complexity), and trophic levels is thus warranted.

Erythromycin is the primary focus of this review, reflecting the available literature and research efforts to date; however, other macrolides exhibit some oxidative stress-modulatory effects at a class level. This warrants comparative work to gain insight into shared molecular responses to macrolides in distinct host systems, ISR involvement [68], pathway crosstalk, and perhaps mapping of a reactive species interactome [56]. The effectiveness of such approaches is apparent in a recent study by Ma et al. [35] that demonstrates how erythromycin attenuates NETosis by suppressing transcriptional and post-translational regulators within the PAM pathway. Extending such multi-omics approaches across different host cell types and physiological stressors may be the key to unraveling erythromycin's context-dependent effects as a modulator of ROS and oxidative stress conditions.

The recent report by Desai et al. [47] that erythromycin confers a protective effect against oxidative stress in *Streptococcus agalactiae* warrants an analysis of the effect of erythromycin on antioxidant responses in the bacteria and if these are up-regulated during oxidative stress [47]. Erythromycin activates

the antioxidant system and induces glutathione synthesis in *Chlorella vulgaris*, a eukaryotic microbe [82], and alters redox-regulating transcriptional activity in gram-positive enterococci bacteria [83]. Collectively, these observations lend merit to the notion that macrolide-mediated redox modulation is not a phenomenon exclusive to eukaryotic systems. With this view, other future avenues to explore include how macrolide-induced redox shifts might affect the survival of a diversity of bacterial pathogens and during host-pathogen interactions.

The effects of macrolides as modulators of oxidative stress in cellular systems extend beyond their canonical antimicrobial functions, highlighting context-dependent regulation of ROS and oxidative stress conditions that span protective, adaptive, and potentially detrimental effects in eukaryotic and prokaryotic systems. Moving into the future, addressing the complexities of the effects of macrolides will require integrated, multi-omic, and cross-disciplinary approaches targeted to evaluate relevant ROS and redox effects across biological and ecological contexts.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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