Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2025**; 30(12): 45321
https://doi.org/10.31083/FBL45321

Review

Melatonin and Lipid Peroxidation: Antioxidant Shield and Therapeutic Potential

Octávio Antonio Jordan Volpe[1,†], Debora Aparecida Pires de Campos Zuccari[1,†], Luiz Gustavo de Almeida Chuffa[2], Russel J Reiter[3,*]

[1]Cancer Molecular Research Laboratory (CMRL), Faculdade de Medicina de São José do Rio Preto - FAMERP, 15090-000 São José do Rio Preto, SP, Brazil
[2]Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University (UNESP), 18618-689 Botucatu, SP, Brazil
[3]Department of Cell Systems and Anatomy, Joe R and Teresa Lozano Long School of Medicine, UT Health San Antonio, San Antonio, TX 78229, USA
*Correspondence: reiter@uthscsa.edu (Russel J Reiter)
[†]These authors contributed equally.

Academic Editors: Neven Zarkovic and Julia Kzhyshkowska
Submitted: 26 July 2025 Revised: 8 October 2025 Accepted: 22 October 2025 Published: 18 December 2025

Abstract

Melatonin, a highly conserved indoleamine produced by the pineal gland and also in the mitochondria of many, perhaps all, extrapineal tissues, has emerged as a powerful antioxidant molecule. This review explores its role in counteracting lipid peroxidation (LP), a process that damages cellular membranes through the oxidative degradation of lipids. LP is involved in numerous pathological conditions, including neurodegenerative diseases, cancer, cardiovascular disorders, and aging. The article discusses how melatonin prevents, mitigates, or even reverses LP-induced cellular damage by acting as both a direct free radical scavenger and as an indirect regulator of antioxidant enzymes. A key point is melatonin's amphiphilic nature, which enables it to access both lipid and aqueous cellular compartments, allowing for broad protection and supporting its diverse antioxidant, cytoprotective, and regulatory functions within the cell. Melatonin and its metabolites, such as N^1-acetyl-N^2-formyl-5-methoxykynuramine and N^1-acetyl-5-methoxykynuramine, interact with reactive oxygen and nitrogen species (ROS and RNS), effectively reducing the LP chain reaction. This series of protective actions is known as the melatonin antioxidant cascade. This highlights that melatonin not only inhibits the initiation and propagation phases of LP but may also contribute to the repair of oxidized membrane components. We further summarize the experimental and clinical evidence supporting melatonin's therapeutic potential in conditions in which LP plays a central role. Its ability to cross the blood–brain barrier and its synthesis in multiple tissues, combined with its low toxicity and minimal side effects, make it a promising therapeutic candidate. Additionally, melatonin modulates mitochondrial function and membrane fluidity, offering additional protection against oxidative stress. This positions melatonin not just as a passive antioxidant, but as an active therapeutic agent against oxidative damage. We advocate for deeper exploration of melatonin-based therapies in LP-driven diseases, proposing it as a multifunctional molecule with significant clinical value.

Keywords: melatonin/metabolism; melatonin/pharmacology; lipid peroxidation/physiology; antioxidants/pharmacology; reactive oxygen species/metabolism; oxidative stress

1. Introduction

Melatonin, a pleiotropic indoleamine, plays a crucial role in maintaining redox homeostasis by directly scavenging reactive oxygen species (ROS) and enhancing endogenous antioxidant defenses. Beyond its well-established circadian function, melatonin regulates oxidative stress, inflammation, apoptosis, and autophagy, positioning it as a promising therapeutic candidate in various pathophysiological contexts, including cancer, neurodegenerative disorders, cardiovascular disease, and aging [1,2].

One of the key processes influenced by melatonin is lipid peroxidation (LP), an oxidative chain reaction triggered by ROS that targets unsaturated fatty acids in cellular membranes, producing reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) [3,4]. While excessive LP compromises membrane integrity and mitochondrial function, contributing to genomic instability and inflammation, regulated LP can act as a signaling mechanism, modulating adaptive pathways such as apoptosis and ferroptosis [5,6].

Melatonin exerts protective effects by neutralizing ROS, reducing the formation of cytotoxic aldehydes, and preventing protein and DNA adducts that disrupt cellular homeostasis [7]. Importantly, it not only suppresses harmful LP but also fine-tunes redox-dependent signaling, preserving the physiological functions of lipid-derived mediators. This dual action distinguishes melatonin from traditional antioxidants, which may blunt essential oxidative signaling.

In cancer, where LP has a paradoxical role—promoting both tumor progression and ferroptotic cell death—melatonin emerges as a modulator capable of tip-

ping the balance toward protective outcomes. By restoring redox balance and regulating ferroptosis, melatonin offers a unique therapeutic avenue for oxidative stress–related diseases [8,9].

2. Melatonin as a Multifunctional Antioxidant and Modulator

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous indoleamine synthesized in a circadian manner in the pineal gland and in a non-circadian manner in many other cells [10]. In the pineal, its secretion is tightly regulated by the suprachiasmatic nucleus and modulated by the light/dark cycle. Peripheral tissues where melatonin synthesis has been documented include the gastrointestinal tract, adrenal cortex, retina, immune cells, and others. The presence of melatonin synthesis in multiple tissues underscores its broad involvement in autocrine and paracrine signaling pathways [11,12]. More recently, compelling evidence has demonstrated that mitochondria represent the primary intracellular source of melatonin in many tissues [13,14]. Unlike pineal synthesis, which depends on photoperiod regulation, mitochondrial melatonin production responds directly to metabolic demands, bioenergetic status, and the cellular redox environment, acting as a relevant local defense mechanism, complementary to other antioxidant systems [9,13,15].

The biosynthetic pathway of melatonin begins with the essential amino acid tryptophan, which is converted into 5-hydroxytryptophan by the enzyme tryptophan hydroxylase (TPH), followed by decarboxylation into serotonin by aromatic L-amino acid decarboxylase (AADC). The rate-limiting step occurs through the activity of serotonin N-acetyltransferase (SNAT), also known as arylalkylamine N-acetyltransferase (AANAT), which catalyzes the conversion of serotonin into N-acetylserotonin. Subsequently, the enzyme hydroxyindole-O-methyltransferase (HIOMT)—currently designated as acetylserotonin-O-methyltransferase (ASMT)—methylates N-acetylserotonin to produce melatonin [9,13,15]. This biosynthesis depends on the availability of serotonin and cofactors such as zinc, an essential element that also participates in antioxidant defense, underscoring that melatonin production is interconnected with broader metabolic and redox pathways.

Although widely recognized for its pivotal role in circadian rhythm regulation, melatonin exerts effects that extend beyond chronobiology. It is a multifunctional molecule that contributes to cellular homeostasis, protection against oxidative stress, and modulates physiological and pathological processes, acting in synergy with classical antioxidant mechanisms. From an antioxidant perspective, melatonin exerts a dual mechanism of action: it directly scavenges reactive oxygen species (ROS) and reactive nitrogen species (RNS), and it also induces the expression of endogenous antioxidant enzymes [16,17]. Melatonin also inhibits lipid peroxidation (LP), stimulates antioxidant enzymes, and reduces metal toxicity; it stabilizes mitochondrial activity and suppresses inflammatory signaling [18]. Moreover, melatonin and its metabolites act collectively to provide versatile antioxidant protection: their combined presence amplifies the neutralization of ROS, facilitates metal chelation, radical adduct formation, and repair of oxidatively damaged biomolecules, positioning the melatonin family as a natural defense system against oxidative stress [19].

Its lipophilic nature facilitates diffusion across membranes, allowing melatonin to neutralize free radicals in mitochondria and other cellular compartments, thereby reducing lipid peroxidation, preventing DNA damage, and preserving mitochondrial integrity [9]. In addition to this direct antioxidant action, melatonin modulates gene expression of crucial antioxidant enzymes such as cytosolic Cu/Zn-superoxide dismutase (SOD1), mitochondrial Mn-SOD (SOD2), glutathione peroxidase (GPx), and catalase (CAT). These enzymes act in concert to detoxify ROS: SODs catalyze the dismutation of superoxide anions into hydrogen peroxide, which is subsequently degraded into water and oxygen by GPx and CAT [16,20]. By influencing these pathways, melatonin complements and amplifies the activity of the endogenous antioxidant network, rather than acting as an isolated regulator.

At physiological concentrations, melatonin predominantly exerts its antioxidant activity via gene regulation, whereas pharmacological levels potentiate its direct radical-scavenging effects. Recent studies reinforce that, beyond its recognized antioxidant activity, melatonin helps to preserve the structural integrity of cellular components, particularly membranes, mitochondria, and DNA [17,20,21]. A key example of this property is illustrated by the "antioxidant cascade" of melatonin, in which the indoleamine not only scavenges hydroxyl and peroxyl radicals to prevent the initiation and propagation of LP, but also generates metabolites such as cyclic 3-hydroxymelatonin (C3OHM), N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), and N^1-acetyl-5-methoxykynuramine (AMK). These products retain significant radical-scavenging activity, thereby extending melatonin's protective role against oxidative damage through successive reactions [22]. As shown in Fig. 1 (Ref. [10]), this cascade interrupts LP chain reactions and neutralizes reactive intermediates, highlighting melatonin's unique advantage over classical antioxidants [10].

Its ability to contribute to the limitation of LP helps maintain membrane fluidity and selective permeability, which are essential for cell function and signaling. At the mitochondrial level, melatonin cooperates in preventing dysfunction by mitigating ROS accumulation, thereby preserving the integrity of the respiratory chain and ATP synthesis efficiency. Furthermore, it supports genomic stability by protecting both nuclear and mitochondrial DNA from oxidative damage—either by directly scavenging radicals or by enhancing DNA repair mechanisms [20]. These

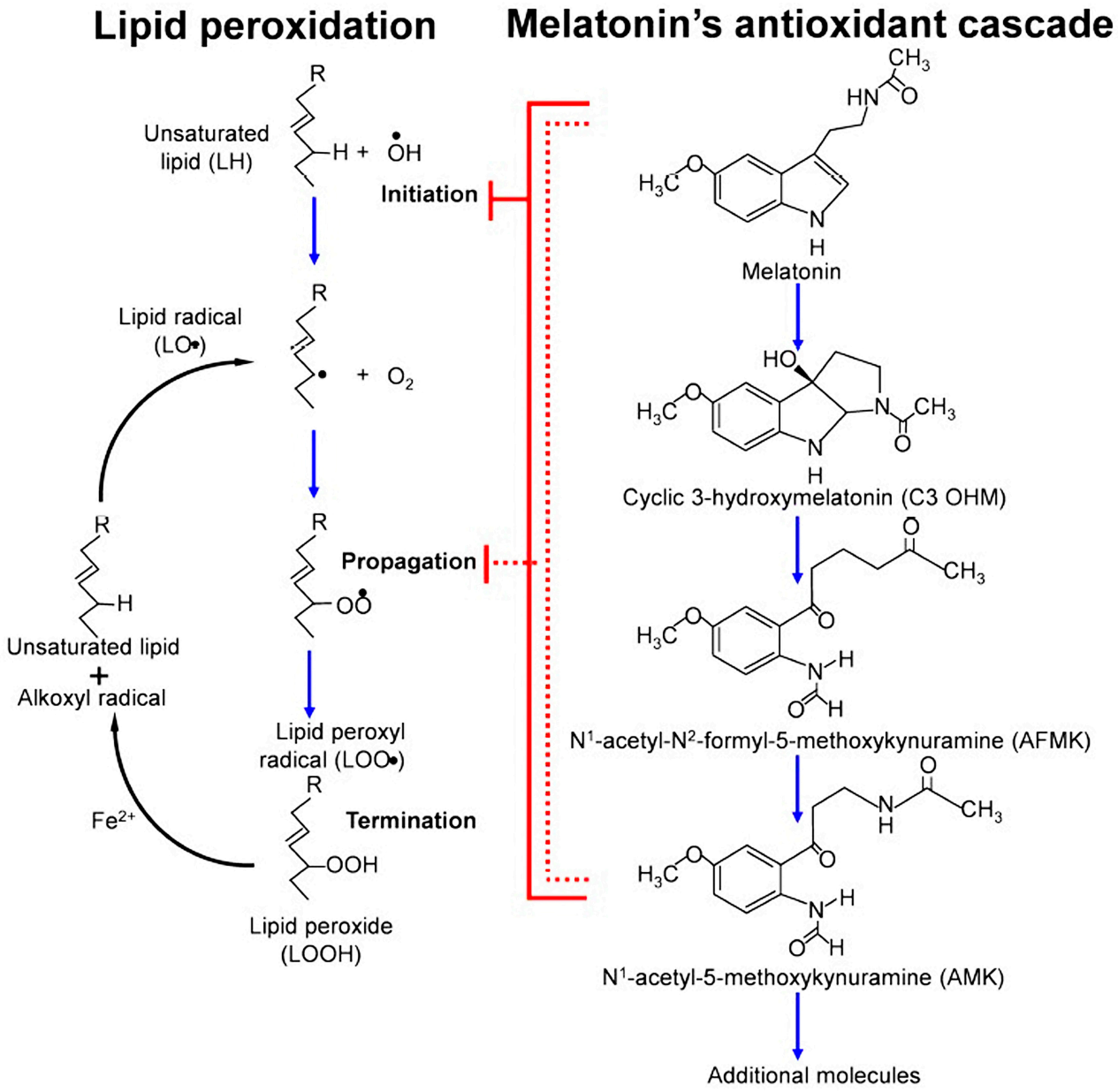

Fig. 1. Mechanism of lipid peroxidation (LP) and melatonin's antioxidant cascade. On the left, the initiation, propagation, and termination steps of LP are shown, leading to the generation of lipid radicals and lipid hydroperoxides (LOOH). On the right, melatonin's antioxidant cascade is represented, where melatonin not only directly neutralizes reactive oxygen and nitrogen species (ROS/RNS) but also generates bioactive metabolites such as cyclic-3-hydroxymelatonin (C3OHM), N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), and N^1-acetyl-5-methoxykynuramine (AMK). These metabolites further contribute to the suppression of oxidative stress, amplifying melatonin's protective role. Illustration adapted with permission from Reiter *et al.* [10], Dual sources of melatonin and evidence for different primary functions; published by Frontiers Media S.A., 2024.

protective mechanisms are fundamental for maintaining cellular homeostasis, particularly under conditions of oxidative stress, and have been associated with melatonin's protective effects on aging, neurodegeneration, and cancer [9,21].

Rather than functioning alone, melatonin interacts with multiple signaling and defense pathways, exerting modulatory actions across apoptosis, immunoregulation, and mitochondrial processes. It can exert anti-apoptotic effects in normal tissues, while promoting apoptosis in malignant cells, highlighting its context-dependent role [23]. In the hematopoietic system, for example, melatonin protects progenitor cells from chemotherapy-induced apoptosis by stimulating Th2 cells to release IL-4, which in turn activates stromal cells to produce GM-CSF, thereby promoting cellular regeneration. In tumor cells, such as MCF-7, melatonin

induces cell cycle arrest via the p53/p21^{WAF1} pathway, balancing mitosis and apoptosis—demonstrating its dual role as a cytoprotective agent in normal cells and an antiproliferative factor in cancer cells [23]. Additionally, Florido *et al.* (2022) [24] detailed the mechanisms through which melatonin induces reactive ROS production in cancer cells, including interactions with calmodulin to activate iPLA2, inhibition of the AKT pathway leading to NRF2 degradation, modulation of mitochondrial sirtuin 3 (SIRT3), and stimulation of the mitochondrial respiratory chain via reverse electron transport. By increasing ROS while reducing antioxidant defenses, melatonin selectively promotes apoptosis in tumor cells, highlighting its potential as a complementary anticancer therapy.

Beyond its antioxidative and apoptotic regulation, melatonin contributes to immunomodulation. It regulates the function of diverse immune cells, including T and B lymphocytes, macrophages, neutrophils, and dendritic cells [25]. This indoleamine finely adjusts the balance between pro-inflammatory and anti-inflammatory cytokines, being capable of either enhancing immune responses against pathogens or suppressing chronic inflammation [26]. Mechanistically, this occurs through modulation of key intracellular signaling pathways such as NRF2, NF-κB, and the NLRP3 inflammasome, leading to reduced expression of inflammatory mediators including TNF-α, IL-6, and IL-1β [27]. Such regulatory capacity is critically important in chronic inflammatory and degenerative diseases, where melatonin functions as part of a broader anti-inflammatory and immunoregulatory network.

A further aspect of melatonin's biological role is its ability to regulate apoptosis—a key process for maintenance of tissue integrity and the elimination of damaged or potentially malignant cells. Melatonin promotes apoptosis in tumor cells by modulating key regulatory proteins such as Bax, Bcl-2, caspases, and p53, while also affecting mitochondrial membrane permeability and triggering cytochrome c release [9,14]. Likewise, melatonin exerts antiapoptotic and cytoprotective effects in normal cells subjected to oxidative or toxic stress, preserving cell viability and preventing irreversible damage [28]. This duality emphasizes its complementary value within endogenous defense mechanisms, particularly in conditions of oxidative stress.

Adding to its multifaceted role, recent studies have highlighted melatonin's capacity to regulate mitochondrial biogenesis and function. By enhancing mitochondrial efficiency and reducing mitochondrial ROS production, melatonin further strengthens its antioxidant and cytoprotective effects [9,29]. Therefore, melatonin emerges as an important component of the antioxidant and regulatory network, acting alongside classical enzymatic defenses and trace elements such as zinc.

In summary, melatonin is a versatile molecule, endowed with a broad spectrum of bioactive properties that contribute to maintaining homeostasis and protecting against physiopathological insults, in concert with other endogenous mechanisms. Its therapeutic potential has been extensively explored in various medical fields, with significant relevance in the treatment of neurodegenerative diseases, cancer, cardiovascular disorders, sleep disturbances, and chronic inflammatory conditions. This underscores the importance of advancing research focused on elucidating its molecular mechanisms and expanding its clinical applications [20].

3. Melatonin as a Modulator of Cellular Redox Homeostasis and Apoptosis

Based on extensive experimental evidence, melatonin is considered a highly promising natural modulator of LP, a critical process mediated by ROS/RNS that compromises membrane integrity and generates toxic byproducts such as MDA and 4-HNE (Fig. 2). Melatonin exerts its antioxidant effect both directly, by neutralizing radicals such as •OH, ROO•, and $ONOO^-$, and indirectly, by enhancing the expression and activity of SOD, CAT, GPx, while inhibiting pro-oxidant enzymes such as NADPH oxidase (NOX) and iNOS. Its metabolites, including AFMK and AMK, retain significant antioxidant capacity which sometimes exceeds that of melatonin, providing a cascading protective effect against LP progression. Furthermore, melatonin attenuates ferroptosis by modulating ROS production and iron metabolism.

Upon entering the cell via PEPT ½ oligopeptide transporters, GLUT4 receptor and/or diffusion, melatonin scavenges ROS, enhances mitochondrial ATP production, maintains redox homeostasis, and promotes antioxidant defense via SOD1/2, GPx, CAT, and NRF2 signaling. GPX4 reduces lipid hydroperoxides, preventing accumulation of toxic LP products and ferroptotic cell death. Melatonin also regulates transferrin receptor expression and iron uptake (Fe^{3+}/Fe^{2+}), limiting the Fenton reaction and subsequent LP, thereby maintaining membrane integrity and protecting against iron-dependent oxidative damage.

LP can trigger regulated cell death pathways, particularly apoptosis and ferroptosis, depending on context, location, and intensity. During apoptosis, LP products like 4-HNE modify regulatory proteins such as caspases and members of the Bcl-2 family and facilitate cytochrome c release from mitochondria [30,31]. Cardiolipin, a highly unsaturated mitochondrial phospholipid, is especially sensitive to LP; its oxidation promotes Bax translocation and mitochondrial permeabilization, initiating intrinsic apoptosis [32]. LP can also act as a resolution signal, promoting non-inflammatory clearance of damaged cells, which is essential for tissue remodeling, embryogenesis, and immune responses [12]. When deregulated, LP leads to pathological cell death such as ferroptosis, characterized by iron-dependent accumulation of lipid peroxides and GPX4 failure [33]. Melatonin counteracts ferroptosis by reducing

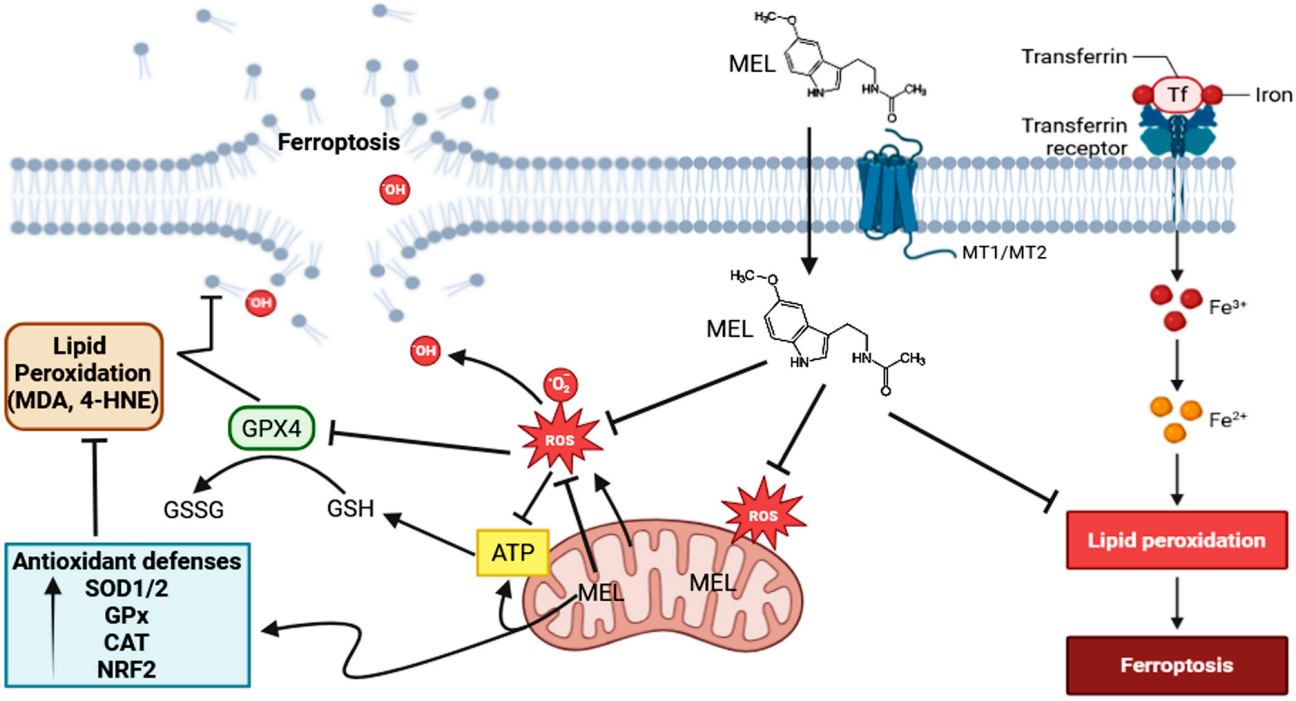

Fig. 2. Melatonin attenuates lipid peroxidation and ferroptosis through multiple antioxidant and regulatory mechanisms. Melatonin (MEL) exerts protective effects against ferroptosis by modulating reactive oxygen species (ROS) production and iron metabolism. Upon entering the cell via membrane receptors (MT1/MT2), melatonin directly scavenges ROS, including hydroxyl radicals (•OH) and superoxide anions (O_2•$^-$), reducing oxidative stress and mitochondrial dysfunction. MEL also enhances mitochondrial ATP production and maintains redox homeostasis. Through upregulation of antioxidant defense systems—including superoxide dismutase (SOD1/2), glutathione peroxidase (GPx), catalase (CAT), and the NRF2 signaling pathway—melatonin promotes the attenuation of lipid peroxidation. GPX4 reduces lipid hydroperoxides, thereby inhibiting the accumulation of toxic lipid peroxidation products (e.g., MDA, 4-HNE) and preventing ferroptotic cell death. Additionally, melatonin interferes with iron metabolism by modulating transferrin receptor expression and iron uptake (Fe^{3+}/Fe^{2+}), thus limiting the Fenton reaction and downstream lipid peroxidation. Collectively, these actions highlight melatonin's role in maintaining membrane integrity and preventing iron-dependent lipid damage and ferroptosis. GPX4, glutathione peroxidase 4; MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal; NRF2, nuclear factor erythroid 2–related factor 2; GSH, reduced glutathione; GSSG, oxidized glutathione; ATP, adenosine triphosphate. The Fig. 2 was created in BioRender.com. Chuffa, L. (2025) https://BioRender.com/fd436c4.

iron uptake, scavenging ROS, and reinforcing GPX4 activity, serving as a crucial regulator of ferroptotic sensitivity in cancer and degenerative diseases [34].

Mitochondria are both sources and targets of ROS, and their imbalance triggers oxidative stress, apoptosis, necrosis, and chronic inflammation [35,36]. Melatonin acts as a central protector against LP, both directly and indirectly. Its high mitochondrial concentration (~100× plasma) supports local organelle protection, and melatonin can be synthesized in mitochondria from tryptophan and metabolized to AFMK [14,35,37]. It preserves mitochondrial membrane potential, reduces ischemia-reperfusion-induced ROS, protects cardiolipin from oxidation, and regulates mitochondrial biogenesis and mitophagy [14,35,38]. In experimental models, melatonin decreased LP, preserved ATP, and enhanced antioxidant enzymes, while reducing protein carbonylation and maintaining membrane fluidity and function [1,2,39,40].

In HepG2 liver cancer cells, melatonin increases ROS and triggers mitochondrial dysfunction and cell death, while protecting neighboring healthy cells [41]. Similarly, Florido and co-workers [24] demonstrated that specific concentrations of melatonin can generate ROS in cancer cells, promoting programmed cell death and enhancing chemotherapy sensitivity, without affecting normal tissues. Chok *et al.* [42] further showed that melatonin induces ROS-mediated autophagy in colorectal cancer cells, causing endoplasmic reticulum stress and selective tumor cell death. These findings indicate that melatonin acts as a context-dependent redox modulator, preserving normal cell integrity while exploiting the oxidative vulnerability of cancer cells, supporting its potential as an adjuvant therapeutic agent in oncology.

4. Melatonin and LP at the Cellular Level

4.1 Metabolism

In addition to being synthesized locally in the mitochondria, melatonin is an amphiphilic molecule capable of freely crossing biological membranes, allowing its rapid and efficient distribution to different cellular compartments, including the nucleus, mitochondria, and plasma membranes [9]. Melatonin released into the blood is metabolized through different pathways. In the liver, it undergoes enzymatic metabolism predominantly via CYP1A2, producing 6-hydroxymelatonin, which is subsequently conjugated [9]. However, at the tissue level and especially in the mitochondrial microenvironment, melatonin is also metabolized through non-enzymatic pathways that occur in direct response to its interaction with ROS and RNS [13,15]. While melatonin possesses a notable ability to neutralize multiple reactive species, it functions alongside other endogenous and exogenous antioxidants, such as glutathione and vitamins C and E, contributing to a coordinated defense against oxidative stress [9,15]. A distinctive feature of melatonin, compared to some classical antioxidants, lies in its ability to perform sequential free radical scavenging. The metabolites generated during detoxification—such as AMK and cyclic 3-hydroxymelatonin—retain antioxidant activity, often exceeding that of native melatonin [13,15]. This cascade establishes melatonin as a central player in preserving cellular and mitochondrial integrity, particularly in high-ROS tissues, including tumor cells.

Beyond its direct radical scavenging activity, melatonin operates through a unique cascade defense system that plays a key role in preserving the integrity of cellular membranes, protecting nuclear and mitochondrial DNA, and maintaining mitochondrial homeostasis [14]. This property is especially relevant in tissues highly dependent on oxidative metabolism, such as the brain, heart, liver, and also in stressed cells, including tumor cells, where redox modulation plays a critical role in survival, proliferation, and progression [9,13].

Tanabe and colleagues (2015) [17] demonstrated that melatonin protects granulosa cells in ovarian follicles by crossing lipophilic membranes, reducing LP, preventing DNA damage, and maintaining mitochondrial integrity. Such protection includes the regulation of mitochondrial function, preservation of steroidogenesis, and inhibition of the activation of apoptotic enzymes caspases 3/7. In addition to its direct antioxidant functions, Mayo *et al*. (2002) [16] showed that, at physiological concentrations, melatonin acts indirectly by hormonal modulation of the expression of antioxidant enzymes, increasing mRNA levels of Cu-ZnSOD (cytoplasmic), MnSOD (mitochondrial), and GPx even in the absence of oxidative stress. This regulation involves receptor-dependent protein synthesis and modulation of mRNA stability, as well as interference with transcription factors such as AP-1, NF-κB, Sp1, and C/EBP. Melatonin's ability to act via multiple mechanisms—direct radical scavenging and genetic regulation at physiological doses—positions it as a critical endogenous modulator in the control of oxidative stress.

This dual antioxidant role has significant implications in aging, neurodegenerative diseases, and the maintenance of cellular function, especially in tissues exposed to high levels of ROS. In summary, melatonin stands out as a multifunctional molecule capable of preserving cellular, mitochondrial, and genomic integrity, modulating both the immediate response and adaptive antioxidant mechanisms, consolidating its role as a natural regulator of oxidative stress.

4.2 Molecular Aspects of Melatonin in Ferroptosis-Related Contexts

Dysregulated LP underlies ferroptosis, a regulated necrosis-like death characterized by the lethal accumulation of iron-dependent lipid peroxides and impaired glutathione peroxidase 4 (GPX4) function [33]. Ferroptosis integrates iron metabolism, PUFA-rich phospholipid oxidation, and antioxidant defenses, serving as a double-edged sword in cancer biology. Iron promotes ferroptosis via the Fenton reaction, generating ROS that oxidize polyunsaturated fatty acids (PUFAs) into cytotoxic aldehydes such as 4-HNE, MDA, and acrolein [43,44]. These adducts modify mitochondrial proteins, deplete reduced glutathione (GSH), inhibit SIRT3, and activate oncogenic pathways contributing to tumor growth and angiogenesis [44]. LP can also influence tumor immunity: it enhances $CD8^+$ T cell activity via IFN-γ release, while cholesterol-driven CD36 expression paradoxically promotes ferroptosis in T lymphocytes, facilitating immune evasion [45]. At the molecular level, ferroptosis is tightly regulated by the cystine/glutamate antiporter system Xc^-, GSH synthesis, and GPX4 activity [43]. Inhibition of Xc^- (e.g., by erastin) reduces cystine uptake, impairing GPX4 and facilitating ferroptosis, whereas GPX4 overexpression in ovarian, gastric, and colorectal cancers confers resistance to therapy [45]. Other regulators, including SLC7A11 modulated by YAP/TAZ or SOX2, further enable tumor cells to evade ferroptotic signals [45]. Importantly, ferroptosis induction sensitizes tumor cells to chemo- and radiotherapy, positioning it as a promising therapeutic target [43,46].

As illustrated in Fig. 2, melatonin counteracts ferroptosis by reducing iron uptake, scavenging ROS, and reinforcing GPX4 activity, thereby confirming it as a crucial regulator of ferroptotic sensitivity in cancer and degenerative diseases [34]. Ferroptosis arises when cellular homeostasis is disturbed, either by heightened susceptibility to ferroptotic signals or by impairment of the antioxidant defense machinery. As shown in Fig. 2, melatonin counteracts ferroptosis by reducing iron uptake, scavenging ROS, and reinforcing GPX4 activity, thereby suggesting that it is a crucial regulator of ferroptotic sensitivity in cancer and degenerative diseases [34]. Melatonin has emerged as a

candidate for mitigating iron overload and restoring iron homeostasis. Its protective role involves both direct iron chelation and regulation of iron metabolism, limiting Fenton reaction and attenuating oxidative stress linked to free iron ions. It upregulates hepcidin in hepatocytes via MT1 activation [47], decreases the expression of iron importers (TFR1, DMT1), and enhances iron export in the mouse hippocampus by upregulating ferroportin via MT2–NRF2 signaling, thereby counteracting memory loss induced by sleep deprivation [48]. It also promotes ferritin expression, particularly FTH, through MT2 activation in neuroinflammation and ferroptosis following brain injury [49], while inhibiting ferritinophagy by downregulating NCOA4 in age-related cataract [50]. Considering the involvement of LP and ferroptosis, evidence suggests that melatonin interferes with this process by modulating enzymes linked to PUFA-PL synthesis (e.g., ACSL4, LPCAT3) and by limiting both enzymatic and non-enzymatic LP; the latter was already demonstrated in rat kidney, brain, and liver. As an antioxidant, it preserves long-chain PUFAs such as DHA and AA, while also suppressing pro-ferroptotic enzymes like 12-LOX and 5-LOX [51–53]. Exogenous melatonin has consistently shown protective efficacy against ferroptosis in numerous cell and animal models, although clinical validation remains necessary. Importantly, melatonin exerts beneficial effects across a wide spectrum of noncancerous conditions, mitigating ferroptosis-related injury in the eyes, brain, cardiovascular system, lungs, liver, kidneys, and bones. The underlying mechanisms, effective dosages, experimental models, and disease-specific pathologies are comprehensively addressed in the study presented by Zhang *et al*. (2023) [52].

A recent review provided evidence that melatonin counteracts ferroptosis by modulating multiple protective signaling cascades, including SIRT6/p-NRF2, NRF2/ARE/HO-1/SLC7A11/GPX4/PTGS2, ERK/NRF2, ferroportin (FPN), Hippo/YAP, PI3K/AKT/mTOR, and SIRT6/NCOA4/FTH1 pathways, thereby preserving redox balance, iron homeostasis, and cell survival in diverse disease contexts [54]. While melatonin is widely recognized for its protective role against ferroptosis in noncancerous diseases affecting the eyes, brain, heart, lungs, liver, kidneys, and bones, high pharmacological doses can exert the opposite effect in cancer. Melatonin's ability to act as either an antioxidant or a pro-oxidant depends on the administered dose, the cellular context, and the length of exposure. By enhancing ROS generation, promoting LP, elevating intracellular iron through ferritin degradation and TFR1 upregulation, and mediating pathways such as AKT/GSK-3β–NRF2 signaling, melatonin triggers ferroptosis in tumor cells, including oral squamous cell carcinoma and lymphoma. This dual activity highlights melatonin's context-dependent role and underscores its potential as a complementary therapeutic approach in oncology.

4.3 Melatonin, Mitochondria, and LP: Redox Mechanisms Associated With Disorders

Melatonin preserves the fluidity and selectivity of cell membranes by reducing LP, preventing pore formation and functional alterations that would lead to cell dysfunction, inflammation, and cell death [17,22]. In the mitochondrial environment, melatonin preserves respiratory function and prevents the opening of the mitochondrial permeability transition pore (mPTP), which, when activated by LP or Ca^{2+} overload, promotes the activation of the intrinsic apoptosis pathway [55]. Mitochondrial melatonin production may be upregulated by oxidative stress, independently of the light-dark cycle. Mitochondrial melatonin acts in an autocrine manner, reinforcing *in situ* antioxidant defense and reducing vulnerability to LP. In situations of SIRT3 dysfunction—a mitochondrial deacetylase that activates SOD2—such as in aging, obesity, or high-fat diets, melatonin administration restores SIRT3 activity, increasing antioxidant capacity and reducing lipid peroxide formation [35,56].

As already pointed out, melatonin exhibits a dual and context-dependent redox behavior, acting as an antioxidant in normal cells while displaying pro-oxidant properties in certain malignant cells [24,57]. This dualism is closely linked to cellular metabolic state, mitochondrial function, and the regulation of intracellular metal ions, which together dictate its functional outcome. In normal cells, melatonin primarily scavenges reactive oxygen and nitrogen species, reduces LP, and maintains mitochondrial membrane potential ($\Delta\Psi$m) [58,59]. By preserving $\Delta\Psi$m, melatonin prevents mPTP opening, cytochrome c release, and the initiation of intrinsic apoptotic pathways. It also upregulates antioxidant enzymes such as SOD, CAT, and GPx, reinforcing cellular defenses against oxidative stress [13,20]. In contrast, in malignant cells, melatonin can act as a selective pro-oxidant, a behavior modulated by the cell's metabolic profile, mitochondrial membrane potential, and intracellular iron or copper levels [24]. Tumor cells often exhibit altered mitochondrial dynamics and higher basal ROS levels, making them more susceptible to LP. Under these conditions, melatonin can intensify LP, inhibit specific antioxidant enzymes, and promote apoptosis selectively in malignant cells. This effect is facilitated by increased mitochondrial uptake via PEPT ½ transporters, which enhances local melatonin concentration and oxidative pressure within tumor mitochondria [57]. Experimental evidence supports this dualism in multiple cancer models. For instance, melatonin combined with chemotherapeutics in leukemia and cervical cancer cells promoted enhanced mitochondrial LP, respiratory dysfunction, and increased cell death, indicating potential therapeutic synergism [55,60].

As shown in Fig. 3, melatonin protects cardiovascular tissues by reducing LP and preserving membrane integrity, thereby preventing ferroptosis-associated cardiomyocyte

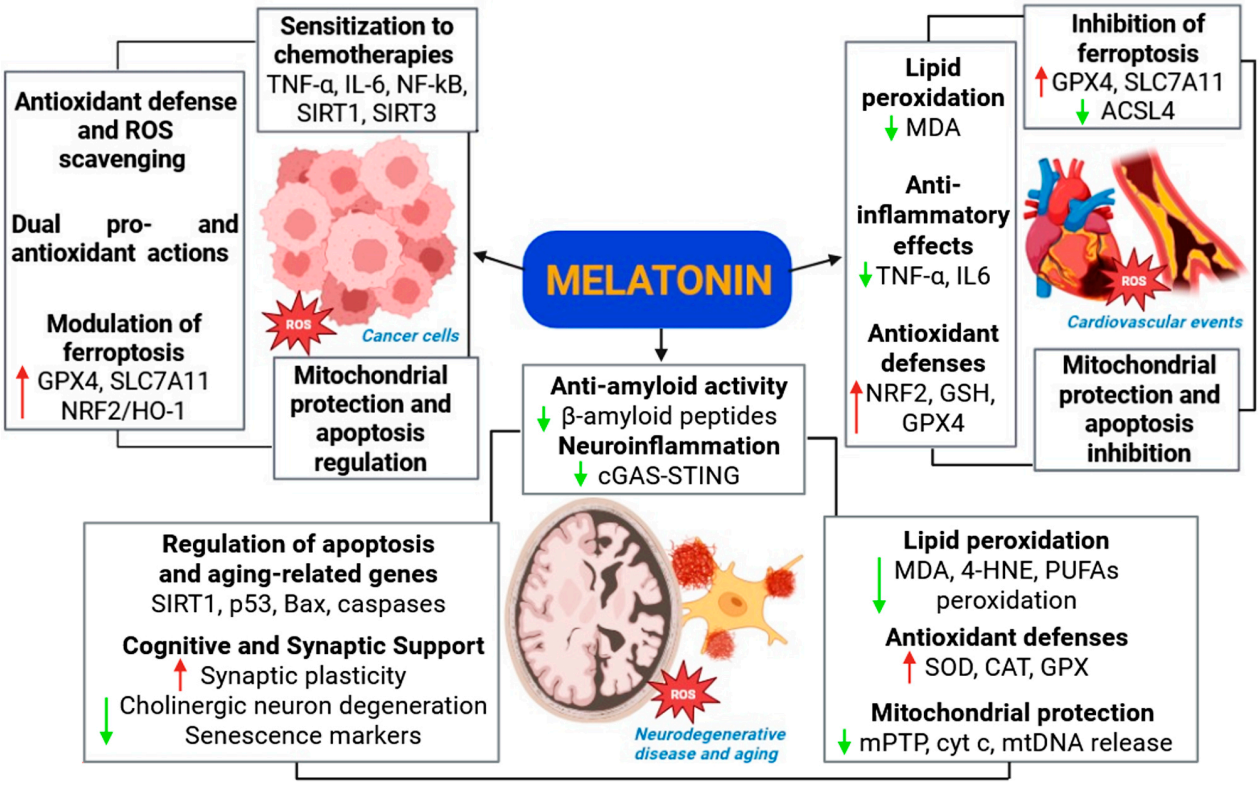

Fig. 3. Multifaceted roles of melatonin in modulating lipid peroxidation and oxidative stress across cancer, neurodegenerative diseases, and cardiovascular events. Melatonin exerts antioxidant, anti-inflammatory, and mitochondrial-protective effects in various pathological contexts. In cancer, melatonin regulates antioxidant defenses, modulates ferroptosis, and sensitizes cells to chemotherapy via regulation of pro-inflammatory and apoptotic pathways. In neurodegenerative diseases and aging, melatonin reduces β-amyloid peptides, suppresses neuroinflammation, and protects mitochondria by inhibiting oxidative damage, while supporting cognitive and synaptic function. In cardiovascular events, melatonin limits lipid peroxidation, inhibits ferroptosis, and reduces inflammatory cytokines, preserving mitochondrial integrity and preventing apoptosis. These effects are mediated through the enhancement of endogenous antioxidant systems and reduction of lipid peroxidation products, positioning melatonin as a potent cytoprotective agent across multiple systems. GPX4, glutathione peroxidase 4; SLC7A11, solute carrier family 7 member 11; NRF2, nuclear factor erythroid 2–related factor 2; HO-1, heme oxygenase-1; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; NF-κB, nuclear factor kappa B; SIRT1, sirtuin 1; SIRT3, sirtuin 3; cyclic cGAS–STING, GMP-AMP Synthase–Stimulator of Interferon Genes; mPTP, mitochondrial permeability transition pore; Cyt c, Cytochrome c; mtDNA, mitochondrial DNA; MDA, malondialdehyde; ACSL4, Acyl-CoA synthetase long chain family member 4; 4-HNE, 4-hydroxynonenal; GSH, reduced glutathione; CAT, catalase; SOD, superoxide dismutase; PUFAs, polyunsaturated fatty acids; p53, tumor protein p53; Bax, Bcl-2–Associated X Protein. Green arrows = down regulation/Red arrows = up regulation. The Fig. 3 was created in BioRender.com. Chuffa, L. (2025) https://BioRender.com/uxh4voi.

death. A comprehensive review by Maity *et al.* (2023) [61] highlighted melatonin's protection against obesity- and diabetes-induced cardiovascular injury through multiple mechanisms: reducing oxidative stress and inflammation, inhibiting LP and cholesterol deposition, promoting anti-inflammatory macrophage polarization, and preserving cardiac integrity. In animal models of high-fat diet–induced metabolic stress, melatonin mitigates mitochondrial and endoplasmic reticulum stress, helping maintain cardiac and adipose tissue homeostasis. However, these protective effects are markedly compromised when SIRT1 expression is reduced, underscoring the dependence of melatonin's redox-regulatory functions on intact SIRT1 signaling [62]. In models of drug-induced toxicity, such as doxorubicin-treated cardiomyocytes, melatonin reversed mitochondrial dysfunction, apoptosis, and ferroptosis marker alterations (ACSL4 and GPX4), emphasizing its role in suppressing oxidative stress-mediated cell death [63]. Mechanistically, melatonin also regulates the NRF2/SLC7A11/GPX4 axis, reducing LP and ferroptosis in macrophages exposed to oxidized LDL, and modulates inflammatory signaling and oxidative damage in acute injury models, such as heat stroke-induced myocardial injury [64]. Melatonin supplementation protected rat cardiac tissue from exercise-induced oxidative stress by reducing LP and protein oxidation, boosting the activity and expression of key antioxidant enzymes

(e.g., CAT, GPx, SOD), and restoring redox homeostasis, particularly in the recovery phase after exhaustive exercise [65].

In summary, melatonin exerts a dual functional mechanism: directly scavenging radicals and modulating gene expression and enzyme activity to maintain cellular integrity under oxidative stress. These mechanisms are critical in metabolically active tissues, including the brain, heart, and tumor cells, positioning melatonin as a versatile agent for mitigating LP-associated pathologies, including cancer, cardiovascular, and neurodegenerative diseases [20,29,56].

Aging is associated with the progressive production of ROS, predominantly generated in the mitochondria during aerobic respiration. This excess of free radicals is an important causal factor in functional cellular decline and the onset of neurodegenerative diseases [58,66]. Among the main targets of oxidative stress are membrane lipids, especially in PUFAs which are abundant in neuronal membranes and in myelin. These changes alter neuronal physiology and promote neurotoxicity [67]. With aging, there is a reduction in membrane fluidity, especially in the mitochondria, as a result of the accumulation of oxidized lipids, which compromises bioenergetic function and favors mitochondrial dysfunction [58,66]. This process affects the central nervous system particularly severely due to its high energy demand, elevated lipid content, and relatively limited antioxidant activity. The oxidation of neuronal lipids is now recognized as an early marker and potential promoter of neurodegenerative diseases such as Alzheimer's and Parkinson's disease [13,15].

Melatonin counteracts these processes by lowering iron overload, preventing excessive LP, and reinforcing neuronal antioxidant defenses (Fig. 3). Through these mechanisms, it helps preserve mitochondrial bioenergetics, maintains neuronal integrity, and protects cognitive function, highlighting its therapeutic potential in aging-related neurodegeneration. A recent review further documented that melatonin exerts strong antioxidant and anti-inflammatory effects by scavenging free radicals, limiting LP, and enhancing mitochondrial stability [18].

Melatonin, whose endogenous synthesis declines significantly with age [59] can prevent or attenuate LP of the few molecules capable of preventing and interrupting LP. Produced primarily in the mitochondrial matrix of neurons [68], melatonin acts both as a direct antioxidant, neutralizing reactive species [58], and as a regulator of the expression of antioxidant enzymes such as SOD, CAT, and GPx [13,20]. When administered peripherally, its high lipophilicity facilitates penetration into cell and mitochondrial membranes, supporting protection of lipid bilayers against the initiation and propagation of LP [12]. Melatonin also preserves mitochondrial integrity by preventing the opening of the mPTP and the release of cytochrome c and mitochondrial DNA, events that activate apoptosis and inflammatory responses via the cGAS-STING pathway [68]. These mechanisms contribute to limiting chronic neuroinflammation characteristic of neurodegenerative diseases (Fig. 3).

It is important to highlight that LP by-products such as 4-HNE may not only damage membranes but also form adducts with neuronal proteins, promoting the formation of pathological aggregates such as β-amyloids in Alzheimer's disease [13]. Melatonin demonstrates potential anti-amyloid activity by inhibiting the formation and aggregation of these neurotoxic peptides. In experimental models of neural damage, melatonin supplementation improved cognitive function, reduced cholinergic degeneration, preserved synaptic plasticity, and lowered levels of oxidized lipids and senescence markers in the hippocampus [13,58]. Its ability to efficiently cross the blood–brain barrier makes it a promising candidate for neural protection [23]. Beyond structural protection, melatonin acts in the regulation of circadian rhythms and the expression of apoptosis-related genes such as SIRT1 [69,70], p53, Bax, and caspases. In neurodegeneration models, it inhibited the activation of caspases-9 and -3, reinforcing its potential antiapoptotic effect in neurons [23,55].

In summary, melatonin protects the brain against deleterious effects associated with LP, promoting membrane stability, mitochondrial integrity, and the inhibition of neuroinflammation [58]. Its multifunctional antioxidant, anti-inflammatory, and anti-amyloid actions position it as a relevant molecule in the prevention and treatment of aging-associated neurodegenerative diseases [13,20,57].

5. Melatonin as Therapy

Melatonin therapy can be administered orally, by injection, topically, or in nanoencapsulated form. Various pharmaceutical formulations of melatonin have been explored with the aim of improving its bioavailability, stability, and therapeutic efficacy, especially in conditions related to oxidative stress. Oral administration is widely used, particularly in the treatment of sleep disorders and in antioxidant protocols, due to its practicality and safety. Clinical studies have shown that co-supplementation with melatonin, magnesium, and zinc improves sleep quality in elderly individuals, highlighting the role of micronutrients supporting melatonin synthesis and function [71]. However, this route presents limited bioavailability (9% to 33%) because of the first-pass hepatic metabolism [72,73].

In experimental models, injectable administration—mainly via intraperitoneal or intravenous routes—has been used to achieve faster and higher plasma levels, being particularly effective in acute scenarios, such as in models of cerebral or hepatic ischemia-reperfusion [74]. Topical melatonin formulations, in turn, have shown efficacy in skin diseases and wound healing processes, benefiting from melatonin's ability to cross the epidermal barrier and exert localized antioxidant and immunomodulatory effects

[73]. More recent advances include the use of nanoencapsulated melatonin formulations, which enable controlled drug release, greater chemical stability, and preferential targeting to intracellular compartments, especially mitochondria. Nanoencapsulated melatonin has proven more effective in neutralizing reactive oxygen and nitrogen species, in addition to showing greater antitumor and neuroprotective activity, as evidenced by studies in experimental models [9,73].

6. Preclinical Models Involving Melatonin and Oxidative Stress

Several preclinical experimental models have demonstrated the efficacy of melatonin as an antioxidant agent and cellular protector against various pathophysiological conditions characterized by oxidative stress and LP. These studies cover hepatic, neurodegenerative, oncological, and metabolic models, strengthening the understanding of its molecular mechanisms and potential clinical applications (Table 1, Ref [66,74–77]).

Aykutoglu *et al*. [75] (Table 1) demonstrated that endothelial cells (HUVECs) exposed to homocysteine showed increased cell viability, reduced lipid peroxidation (TBARS/MDA) levels, and lower caspase activation, associated with an upregulation of Bcl-2 expression. Moreover, treatment with melatonin (10 µM) was more effective than vitamin E (50 µM) in reducing LP and apoptosis, documenting the superior antioxidant and antiapoptotic potential of melatonin.

In the hepatic context, Aranda *et al*. (2010) [76] (Table 1) investigated the effect of melatonin in rats exposed to carbon tetrachloride (CCl_4), a potent hepatotoxic agent that induces LP. Treatment with melatonin (10 mg/kg, intraperitoneally) promoted a significant reduction in MDA and 4-HDA levels, classic biomarkers of lipid damage. Furthermore, melatonin preserved the fluidity of hepatic cell membranes and prevented morphological alterations such as necrosis and cellular ballooning. Even at pharmacological doses, no side effects were observed, confirming its high safety profile. These findings demonstrate melatonin's protective action on tissues highly susceptible to oxidative injury by xenobiotics.

In models of brain aging, García *et al*. (2011) [66] (Table 1) used mice from the SAMP8 (accelerated senescence) and SAMR1 (normal aging) strains to evaluate the chronic effects of melatonin (10 mg/kg/day). Treated animals showed significant improvement in mitochondrial membrane fluidity, reduction in LP levels, and lower expression of cathepsin D, a lysosomal protease associated with neuronal apoptosis. Restoration of the GSH/GSSG ratio was also observed, indicating recovery of redox homeostasis. These data suggest that melatonin exerts neuroprotective effects associated with preservation of mitochondrial function and attenuation of oxidative aging.

In metabolic disorders, especially in the context of experimental diabetes, Winiarska *et al*. (2006) [77] (Table 1) compared the efficacy of melatonin (1 mg/kg) to N-acetylcysteine (NAC, 10 mg/kg) in alloxan-induced diabetic rabbits. Melatonin proved more effective in reducing hydroxyl radicals, restoring the GSH/GSSG ratio, and activating antioxidant enzymes such as GPx, GR, and GST. On the other hand, NAC only promoted an increase in GSH levels without significantly impacting other oxidative stress markers. This highlights the broader and mitochondria-targeted action of melatonin, especially relevant for tissues with high redox disruption, such as those often affected in renal and hepatic contexts.

In the setting of acute neurological injury, Sabbaghziarani *et al*. (2024) [74] (Table 1) used a cerebral ischemia-reperfusion model in Wistar rats subjected to middle cerebral artery occlusion (MCAO). Administering melatonin (5 mg/kg), NAC (50 mg/kg), or a combination of the two revealed that melatonin alone induced the antioxidant NRF2 pathway, increased SOD, GPx, and catalase enzymes, and significantly reduced infarct volume. Although NAC showed antioxidant effects, its action was less robust and limited to increasing total glutathione. The combined treatment produced the best results, with marked reduction in lipid peroxidation (MDA), decreased brain damage, and improved functional recovery, suggesting therapeutic synergy.

These experimental models confirm that melatonin consistently acts as a modulator of LP, with direct and indirect effects on mitochondrial biogenesis, enzymatic antioxidant pathways, apoptosis, and cellular plasticity. Its ability to cross biological barriers, such as the blood–brain barrier, and its mitochondrial affinity confer significant advantages over classical antioxidants. These findings support the translational potential of melatonin as a therapeutic agent in clinical conditions marked by oxidative stress, such as hepatic diseases, neurodegenerative disorders, cancers, and metabolic disorders.

7. Critical Perspectives on Melatonin Use

Despite the widespread use of melatonin as a sleep aid and therapeutic supplement, recent evidence identifies several likely concerns regarding its safety, efficacy, and regulatory oversight. Studies in pediatric populations have reported non-serious adverse effects such as somnolence, headache, and dizziness, emphasizing the need for careful dose management and monitoring in vulnerable groups [78,79]. Analyses of commercially available supplements have revealed substantial discrepancies between labeled and actual melatonin content, with some products containing several times the declared dose, raising serious safety concerns [80]. Regulatory data from the CDC's Morbidity and Mortality Weekly Report reinforce these findings, warning that unregulated melatonin products, especially

Table 1. Effects of melatonin on lipid peroxidation in different experimental models.

Experimental model	Mechanism of action related to lipid peroxidation	Melatonin dose	Peroxidation markers	Reference
Rats with CCl_4-induced liver damage	Reduction in membrane fluidity, ↓ MDA/4-HDA (−93.4%), ↓ carbonylation and hepatic necrosis	10 mg/kg (i.p.)	MDA, 4-HDA	Aranda *et al*., J Pineal Res. 2010 [76]
SAMP8 and SAMR1 mice (aging model)	Preservation of mitochondrial fluidity, ↓ cathepsin D, restoration of GSH/GSSG	10 mg/kg/day	MDA, 4-HNE	García *et al*., Neurobiol Aging. 2011 [66]
Diabetic rabbits (alloxan-induced)	↓ free radicals, ↑ GSH/GSSG, ↑ GPx, GR and GST; ↓ hydroxyl free radicals (HFR)	1 mg/kg (i.p.)	HFR, GSH	Winiarska *et al*., J Pineal Res. 2006 [77]
Rats with cerebral ischemia-reperfusion	↑ SOD, GPx, catalase and NRF2; ↓ MDA, ↓ infarct volume	5 mg/kg (i.p.)	MDA	Sabbaghziarani *et al*., IBRO Neurosci Rep. 2024 [74]
HUVECs exposed to homocysteine	↑ cell viability, ↓ TBARS (MDA), ↓ caspases, ↑ Bcl-2	10 µM (*in vitro*)	TBARS (MDA)	Aykutoglu *et al*., Mol Biol Rep. 2020 [75]
Endothelial cells treated with vitamin E or melatonin	Melatonin more effective in ↓ LPO and apoptosis than vitamin E	10 µM melatonin vs. 50 µM vitamin E	TBARS, caspases, Bax/Bcl-2	Aykutoglu *et al*., Mol Biol Rep. 2020 [75]
Rats treated with NAC vs. melatonin (induced diabetes)	Melatonin superior: ↓ hydroxyl radicals, ↑ GSH/GSSG, ↑ antioxidant enzymes	1 mg/kg melatonin vs. 10 mg/kg NAC	HFR, GSH/GSSG, GPx, GR	Winiarska *et al*., J Pineal Res. 2006 [77]

MDA, malondialdehyde; 4-HDA, 4-hydroxy-2-decenal; 4-HNE, 4-hydroxynonenal; GSH, reduced glutathione; GSSG, oxidized glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferase; SOD, superoxide dismutase; NRF2, nuclear factor erythroid 2-related factor 2; TBARS, thiobarbituric acid reactive substances; LPO, lipid peroxidation; NAC, N-acetylcysteine. ↑ up regulated/↓ down regulated.

Table 2. Clinical studies on melatonin: dosing, biomarkers, patient stratification, and key outcomes.

Study/Reference	Population/Context	Intervention/Dose/Duration	Biomarker(s) or stratification criteria	Key findings/Clinical outcomes
Optimizing the Time and Dose of Melatonin as a Sleep-Promoting Drug: A Systematic Review (2024) [82]	Individuals with insomnia or healthy volunteers	Multiple clinical trials; 0.5–10 mg; varying administration timing	Insomnia status, timing vs. desired sleep, dose	4 mg/day optimal for reducing sleep latency; earlier administration (3 h before bedtime) enhances effect
Significant potential of melatonin therapy in Parkinson's disease – meta-analysis (2023) [83]	Patients with Parkinson's disease	$\geq$10 mg/day, immediate-release, $\geq$12 weeks	Formulation type, dose, treatment duration	Improvement in motor symptoms (UPDRS) and sleep quality with $\geq$10 mg/day for $\geq$3 months
Melatonin therapy to improve nocturnal sleep in critically ill patients (2008) [84]	Critically ill patients on mechanical ventilation	10 mg at 21:00 for 4 nights via enteral tube	Pharmacokinetic profile, blood levels, tolerability	Increased nighttime sleep duration and efficiency; lower doses (1–2 mg) may prevent next-day effects
Melatonin levels in the Alzheimer's disease continuum: a systematic review (2021) [85]	Patients across Alzheimer's spectrum (preclinical to dementia)	Observational; melatonin measured in blood, CSF, saliva	Alzheimer's stages (Braak), fluid type, correlation with neuropathology	Melatonin levels decrease with disease severity; inversely correlated with Braak stages; potential biomarker of progression
Rethinking Melatonin Dosing: Safety and Efficacy at Higher-than-Usual Levels in Aged Patients (2022/2023) [86]	Older adults with sleep disturbances and metabolic/cardiac comorbidities	High doses 40–200 mg/day long-term	Comorbidities, lab profiles, tolerability	Supraphysiological doses tolerated; improved metabolic and cardiovascular parameters; further safety studies needed
Clinical significance of serum melatonin in predicting the severity of oral squamous cell carcinoma (OSCC) (2020) [87]	Patients with OSCC vs. healthy controls	Observational; serum melatonin measured by ELISA	Tumor grade/size, invasion depth, nodal metastasis	Lower serum melatonin in cancer patients; effective for stratifying tumor stages

those marketed for children, may pose health risks due to extreme variability in dosage [81]. Collectively, these studies underscore the importance of cautious clinical use, stringent quality control, and further research to establish safe and effective therapeutic protocols for melatonin administration.

8. Clinical Perspectives and Future Directions

LP is a central process in the onset and progression of numerous chronic diseases, including cancer, neurodegenerative disorders, and cardiovascular conditions. Melatonin emerges as a highly promising molecule in this context, due to its potent antioxidant properties and its capacity to modulate inflammatory and apoptotic pathways. Its amphiphilic nature and ability to cross cellular membranes and target mitochondria—organelles particularly susceptible to oxidative stress—further enhance its therapeutic potential.

Melatonin functions as a multifaceted antioxidant, neutralizing ROS, modulating antioxidant enzymes, and influencing inflammatory signaling, apoptosis, and autophagy. Importantly, it can restore redox balance without disrupting physiological processes mediated by LP, thus maintaining essential cellular signaling and homeostasis. The protective actions of melatonin and its metabolites, such as AFMK and AMK, suggest an efficient antioxidant cascade potentially surpassing the limitations of conventional antioxidants. Its excellent safety profile, including the ability to cross the blood–brain barrier, positions melatonin as an attractive candidate for managing neuroinflammatory, neurodegenerative, and oxidative-stress-associated disorders.

Although preclinical evidence strongly supports melatonin's antioxidant and lipid-protective actions, clinical research remains limited and fragmented. To enhance its translational potential, future clinical studies should focus on large-scale, well-controlled randomized trials (RCTs) with standardized LP biomarkers (e.g., MDA, oxLDL (oxidized low-density lipoprotein), 4-HNE). Stratification by disease type (metabolic syndrome, oncology, neurodegeneration), route of administration (oral vs. targeted delivery), and dosing regimen will be critical. Biomarker-guided approaches could identify patient populations most likely to benefit, paving the way for personalized therapeutic strategies.

Table 2 (Ref. [82–87]) summarizes key clinical studies that have investigated melatonin's effects on sleep, neurodegeneration, cardiovascular risk, and oncology, highlighting dosing strategies, biomarkers, stratification criteria, and relevant clinical outcomes. This compilation illustrates the current state of clinical translation and supports the rationale for precision medicine approaches with melatonin.

9. Conclusion

Melatonin is clearly a multifunctional molecule with significant potential in the prevention and treatment of diseases associated with oxidative stress, chronic inflammation, and LP. Its antioxidant, anti-inflammatory, and anti-apoptotic properties, combined with its ability to target mitochondria and cross biological barriers, provide a compelling rationale for its therapeutic application in cancer, cardiovascular disorders, neurodegeneration, and metabolic diseases.

Preclinical evidence strongly supports melatonin's role in modulating LP, preserving mitochondrial integrity, and regulating apoptotic and ferroptotic pathways. Moreover, its metabolites contribute to an efficient antioxidant cascade that may overcome limitations of conventional antioxidants. Clinical studies revealed the diversity of dosing strategies, patient populations, biomarker applications, and observed outcomes, highlighting the translational potential of melatonin.

Importantly, melatonin has an excellent safety profile, especially in adults, even at supraphysiological doses, making it a viable candidate for both adjunctive and standalone therapies. Future research should prioritize personalized approaches, considering dose, timing, route of administration, and patient stratification based on biomarkers and disease type. Integration with advanced delivery systems—such as nanoparticles, extracellular vesicles, or targeted carriers—may further enhance efficacy and adherence.

In conclusion, melatonin represents a promising molecule capable of bridging basic mechanistic insights and clinical applications, positioning it as a potential cornerstone of precision medicine approaches for oxidative-stress-related diseases. Future studies should focus on large-scale, biomarker-guided trials to establish robust clinical protocols and optimize therapeutic outcomes.

Author Contributions

OAJV and DAPCZ were primarily responsible for the writing and critical revision of the manuscript, ensuring conceptual clarity and cohesion throughout the text. LGAC and RJR contributed substantially to the critical review and enhancement of visual elements, refining both the scientific content and its presentation. OAJV, DAPCZ, LGAC, and RJR contributed to the conceptualization. RJR served as the original mentor of the project, guiding its development from the initial stages with scientific insight and supervision. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The authors are grateful to the São Paulo Research Foundation (FAPESP) and the National Council for Scientific and Technological Development (CNPq).

Funding

The authors received financial support as follows: LGAC (CNPq Process number 306117/2023-1; FAPESP Process number 2021/12971-7).

Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT in order to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

[1] Esteban-Zubero E, López-Pingarrón L, Ramírez JM, Reyes-Gonzales MC, Azúa-Romeo FJ, Soria-Aznar M, *et al*. Melatonin Preserves Fluidity in Cell and Mitochondrial Membranes against Hepatic Ischemia-Reperfusion. Biomedicines. 2023; 11: 1940. https://doi.org/10.3390/biomedicines11071940.

[2] Bermudez-Gonzalez JL, Sanchez-Quintero D, Proaño-Bernal L, Santana-Apreza R, Jimenez-Chavarria MA, Luna-Alvarez-Amezquita JA, *et al*. Role of the Antioxidant Activity of Melatonin in Myocardial Ischemia-Reperfusion Injury. Antioxidants. 2022; 11: 627. https://doi.org/10.3390/antiox11040627.

[3] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity. 2014; 2014: 360438. https://doi.org/10.1155/2014/360438.

[4] Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochemical and Biophysical Research Communications. 2017; 482: 419–425. https://doi.org/10.1016/j.bbrc.2016.10.086.

[5] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biology & Medicine. 1991; 11: 81–128. https://doi.org/10.1016/0891-5849(91)90192-6.

[6] Butterfield DA, Halliwell B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nature Reviews. Neuroscience. 2019; 20: 148–160. https://doi.org/10.1038/s41583-019-0132-6.

[7] Kennedy L, Sandhu JK, Harper ME, Cuperlovic-Culf M. Role of Glutathione in Cancer: From Mechanisms to Therapies. Biomolecules. 2020; 10: 1429. https://doi.org/10.3390/biom10101429.

[8] Yang WS, Stockwell BR. Ferroptosis: Death by Lipid Peroxidation. Trends in Cell Biology. 2016; 26: 165–176. https://doi.org/10.1016/j.tcb.2015.10.014.

[9] Reiter RJ, Rosales-Corral S, Tan DX, Jou MJ, Galano A, Xu B. Melatonin as a mitochondria-targeted antioxidant: one of evolution's best ideas. Cellular and Molecular Life Sciences: CMLS. 2017; 74: 3863–3881. https://doi.org/10.1007/s00018-017-2609-7.

[10] Reiter RJ, Sharma R, Tan DX, Chuffa LGDA, da Silva DGH, Slominski AT, *et al*. Dual sources of melatonin and evidence for different primary functions. Frontiers in Endocrinology. 2024; 15: 1414463. https://doi.org/10.3389/fendo.2024.1414463.

[11] Reiter RJ, Tan DX, Galano A. Melatonin: exceeding expectations. Physiology. 2014; 29: 325–333. https://doi.org/10.1152/physiol.00011.2014.

[12] Hardeland R. Aging, Melatonin, and the Pro- and Anti-Inflammatory Networks. International Journal of Molecular Sciences. 2019; 20: 1223. https://doi.org/10.3390/ijms20051223.

[13] Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, *et al*. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Archives of Toxicology. 2023; 97: 2499–2574. https://doi.org/10.1007/s00204-023-03562-9.

[14] Tan DX, Manchester LC, Qin L, Reiter RJ. Melatonin: A Mitochondrial Targeting Molecule Involving Mitochondrial Protection and Dynamics. International Journal of Molecular Sciences. 2016; 17: 2124. https://doi.org/10.3390/ijms17122124.

[15] Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? Journal of Pineal Research. 2007; 42: 28–42. https://doi.org/10.1111/j.1600-079X.2006.00407.x.

[16] Mayo JC, Sainz RM, Antoli I, Herrera F, Martin V, Rodriguez C. Melatonin regulation of antioxidant enzyme gene expression. Cellular and Molecular Life Sciences: CMLS. 2002; 59: 1706–1713. https://doi.org/10.1007/PL00012498.

[17] Tanabe M, Tamura H, Taketani T, Okada M, Lee L, Tamura I, *et al*. Melatonin protects the integrity of granulosa cells by reducing oxidative stress in nuclei, mitochondria, and plasma membranes in mice. The Journal of Reproduction and Development. 2015; 61: 35–41. https://doi.org/10.1262/jrd.2015-052.

[18] Kołodziejska R, Woźniak A, Bilski R, Wesołowski R, Kupczyk D, Porzych M, *et al*. Melatonin-A Powerful Antioxidant in Neurodegenerative Diseases. Antioxidants. 2025; 14: 819. https://doi.org/10.3390/antiox14070819.

[19] Galano A, Reiter RJ. Melatonin and its metabolites vs oxidative stress: From individual actions to collective protection. Journal of Pineal Research. 2018; 65: e12514. https://doi.org/10.1111/jpi.12514.

[20] Galano A, Tan DX, Reiter RJ. Melatonin: A Versatile Protector against Oxidative DNA Damage. Molecules. 2018; 23: 530. https://doi.org/10.3390/molecules23030530.

[21] Acuña-Castroviejo D, Rahim I, Acuña-Fernández C, Fernández-Ortiz M, Solera-Marín J, Sayed RKA, *et al*. Melatonin, clock genes and mitochondria in sepsis. Cellular and Molecular Life Sciences: CMLS. 2017; 74: 3965–3987. https://doi.org/10.1007/s00018-017-2610-1.

[22] Hardeland R. Melatonin and the pathologies of weakened or dysregulated circadian oscillators. Journal of Pineal Research. 2017; 62: 10.1111/jpi.12377. https://doi.org/10.1111/jpi.12377.

[23] Ferreira CDS, Maganhin CC, Simões RDS, Girão MJBC, Baracat EC, Soares JM, Jr. Melatonin: cell death modulator. Revista Da Associacao Medica Brasileira (1992). 2010; 56: 715–718. https://doi.org/10.1590/s0104-42302010000600024.

[24] Florido J, Rodriguez-Santana C, Martinez-Ruiz L, López-Rodríguez A, Acuña-Castroviejo D, Rusanova I, *et al*. Understanding the Mechanism of Action of Melatonin, Which Induces ROS Production in Cancer Cells. Antioxidants. 2022; 11: 1621. https://doi.org/10.3390/antiox11081621.

[25] Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine. 2005; 27: 189–200. https://doi.org/10.1385/ENDO:27:2:189.

[26] Tan DX, Xu B, Zhou X, Reiter RJ. Pineal Calcification, Melatonin Production, Aging, Associated Health Consequences and

Rejuvenation of the Pineal Gland. Molecules. 2018; 23: 301. https://doi.org/10.3390/molecules23020301.

[27] Sayed RK, Fernández-Ortiz M, Fernández-Martínez J, Aranda Martínez P, Guerra-Librero A, Rodríguez-Santana C, *et al*. The Impact of Melatonin and NLRP3 Inflammasome on the Expression of microRNAs in Aged Muscle. Antioxidants. 2021; 10: 524. https://doi.org/10.3390/antiox10040524.

[28] García JJ, López-Pingarrón L, Almeida-Souza P, Tres A, Escudero P, García-Gil FA, *et al*. Protective effects of melatonin in reducing oxidative stress and in preserving the fluidity of biological membranes: a review. Journal of Pineal Research. 2014; 56: 225–237. https://doi.org/10.1111/jpi.12128.

[29] Acuña Castroviejo D, López LC, Escames G, López A, García JA, Reiter RJ. Melatonin-mitochondria interplay in health and disease. Current Topics in Medicinal Chemistry. 2011; 11: 221–240. https://doi.org/10.2174/156802611794863517.

[30] Zarkovic N. 4-hydroxynonenal as a bioactive marker of pathophysiological processes. Molecular Aspects of Medicine. 2003; 24: 281–291. https://doi.org/10.1016/s0098-2997(03)00023-2.

[31] Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Progress in Lipid Research. 2003; 42: 318–343. https://doi.org/10.1016/s0163-7827(03)00014-6.

[32] Kagan VE, Tyurin VA, Jiang J, Tyurina YY, Ritov VB, Amoscato AA, *et al*. Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. Nature Chemical Biology. 2005; 1: 223–232. https://doi.org/10.1038/nchembio727.

[33] Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, *et al*. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell. 2017; 171: 273–285. https://doi.org/10.1016/j.cell.2017.09.021.

[34] Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. Nature Reviews. Molecular Cell Biology. 2021; 22: 266–282. https://doi.org/10.1038/s41580-020-00324-8.

[35] Reiter RJ, Tan DX, Rosales-Corral S, Galano A, Zhou XJ, Xu B. Mitochondria: Central Organelles for Melatonin's Antioxidant and Anti-Aging Actions. Molecules. 2018; 23: 509. https://doi.org/10.3390/molecules23020509.

[36] Waseem M, Tabassum H, Parvez S. Melatonin modulates permeability transition pore and 5-hydroxydecanoate induced K_{ATP} channel inhibition in isolated brain mitochondria. Mitochondrion. 2016; 31: 1–8. https://doi.org/10.1016/j.mito.2016.08.005.

[37] Venegas C, García JA, Doerrier C, Volt H, Escames G, López LC, *et al*. Analysis of the daily changes of melatonin receptors in the rat liver. Journal of Pineal Research. 2013; 54: 313–321. https://doi.org/10.1111/jpi.12019.

[38] Paradies G, Petrosillo G, Paradies V, Reiter RJ, Ruggiero FM. Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. Journal of Pineal Research. 2010; 48: 297–310. https://doi.org/10.1111/j.1600-079X.2010.00759.x.

[39] Shi X, Zhang J, Gao J, Guo D, Zhang S, Chen X, *et al*. Melatonin attenuates liver ischemia-reperfusion injury via inhibiting the PGAM5-mPTP pathway. PLoS ONE. 2024; 19: e0312853. https://doi.org/10.1371/journal.pone.0312853.

[40] Ximenes VF, Silva SDO, Rodrigues MR, Catalani LH, Maghzal GJ, Kettle AJ, *et al*. Superoxide-dependent oxidation of melatonin by myeloperoxidase. The Journal of Biological Chemistry. 2005; 280: 38160–38169. https://doi.org/10.1074/jbc.M506384200.

[41] Wang J, Wang X, He Y, Jia L, Yang CS, Reiter RJ, *et al*. Antioxidant and Pro-Oxidant Activities of Melatonin in the Presence of Copper and Polyphenols In Vitro and In Vivo. Cells. 2019; 8: 903. https://doi.org/10.3390/cells8080903.

[42] Chok KC, Koh RY, Ng MG, Ng PY, Chye SM. Melatonin Induces Autophagy via Reactive Oxygen Species-Mediated Endoplasmic Reticulum Stress Pathway in Colorectal Cancer Cells. Molecules. 2021; 26: 5038. https://doi.org/10.3390/molecules26165038.

[43] Zuo YB, Zhang YF, Zhang R, Tian JW, Lv XB, Li R, *et al*. Ferroptosis in Cancer Progression: Role of Noncoding RNAs. International Journal of Biological Sciences. 2022; 18: 1829–1843. https://doi.org/10.7150/ijbs.66917.

[44] Barrera G, Gentile F, Pizzimenti S, Canuto RA, Daga M, Arcaro A, *et al*. Mitochondrial Dysfunction in Cancer and Neurodegenerative Diseases: Spotlight on Fatty Acid Oxidation and Lipoperoxidation Products. Antioxidants. 2016; 5: 7. https://doi.org/10.3390/antiox5010007.

[45] Gong D, Chen M, Wang Y, Shi J, Hou Y. Role of ferroptosis on tumor progression and immunotherapy. Cell Death Discovery. 2022; 8: 427. https://doi.org/10.1038/s41420-022-01218-8.

[46] Jelic MD, Mandic AD, Maricic SM, Srdjenovic BU. Oxidative stress and its role in cancer. Journal of Cancer Research and Therapeutics. 2021; 17: 22–28. https://doi.org/10.4103/jcrt.JCRT_862_16.

[47] Park WR, Choi B, Kim YJ, Kim YH, Park MJ, Kim DI, *et al*. Melatonin Regulates Iron Homeostasis by Inducing Hepcidin Expression in Hepatocytes. International Journal of Molecular Sciences. 2022; 23: 3593. https://doi.org/10.3390/ijms23073593.

[48] Wang X, Wang Z, Cao J, Dong Y, Chen Y. Melatonin Alleviates Acute Sleep Deprivation-Induced Memory Loss in Mice by Suppressing Hippocampal Ferroptosis. Frontiers in Pharmacology. 2021; 12: 708645. https://doi.org/10.3389/fphar.2021.708645.

[49] Gao Y, Wang T, Cheng Y, Wu Y, Zhu L, Gu Z, *et al*. Melatonin ameliorates neurological deficits through MT2/IL-33/ferritin H signaling-mediated inhibition of neuroinflammation and ferroptosis after traumatic brain injury. Free Radical Biology & Medicine. 2023; 199: 97–112. https://doi.org/10.1016/j.freeradbiomed.2023.02.014.

[50] Mi Y, Wei C, Sun L, Liu H, Zhang J, Luo J, *et al*. Melatonin inhibits ferroptosis and delays age-related cataract by regulating SIRT6/p-Nrf2/GPX4 and SIRT6/NCOA4/FTH1 pathways. Biomedicine & Pharmacotherapy. 2023; 157: 114048. https://doi.org/10.1016/j.biopha.2022.114048.

[51] Zhang F, Lin B, Huang S, Wu P, Zhou M, Zhao J, *et al*. Melatonin Alleviates Retinal Ischemia-Reperfusion Injury by Inhibiting p53-Mediated Ferroptosis. Antioxidants. 2023; 12: 1173. https://doi.org/10.3390/antiox12061173.

[52] Zhang D, Jia X, Lin D, Ma J. Melatonin and ferroptosis: Mechanisms and therapeutic implications. Biochemical Pharmacology. 2023; 218: 115909. https://doi.org/10.1016/j.bcp.2023.115909.

[53] Wu C, Du M, Yu R, Cheng Y, Wu B, Fu J, *et al*. A novel mechanism linking ferroptosis and endoplasmic reticulum stress via the circPtpn14/miR-351-5p/5-LOX signaling in melatonin-mediated treatment of traumatic brain injury. Free Radical Biology & Medicine. 2022; 178: 271–294. https://doi.org/10.1016/j.freeradbiomed.2021.12.007.

[54] Pourhanifeh MH, Hosseinzadeh A, Koosha F, Reiter RJ, Mehrzadi S. Therapeutic Effects of Melatonin in the Regulation of Ferroptosis: A Review of Current Evidence. Current Drug Targets. 2024; 25: 543–557. https://doi.org/10.2174/0113894501284110240426074746.

[55] Mafi A, Rismanchi H, Gholinezhad Y, Mohammadi MM, Mousavi V, Hosseini SA, *et al*. Melatonin as a regulator of apoptosis in leukaemia: molecular mechanism and therapeutic perspectives. Frontiers in Pharmacology. 2023; 14: 1224151. https://doi.org/10.3389/fphar.2023.1224151.

[56] Han L, Wang H, Li L, Li X, Ge J, Reiter RJ, *et al*. Melatonin protects against maternal obesity-associated oxidative stress and meiotic defects in oocytes via the SIRT3-SOD2-dependent path-

way. Journal of Pineal Research. 2017; 63: 10.1111/jpi.12431. https://doi.org/10.1111/jpi.12431.

[57] Mayo JC, Sainz RM, González-Menéndez P, Hevia D, Cernuda-Cernuda R. Melatonin transport into mitochondria. Cellular and Molecular Life Sciences: CMLS. 2017; 74: 3927–3940. https://doi.org/10.1007/s00018-017-2616-8.

[58] Unal O, Akgun-Unal N, Baltaci AK. Unveiling mysteries of aging: the potential of melatonin in preventing neurodegenerative diseases in older adults. Biogerontology. 2025; 26: 125. https://doi.org/10.1007/s10522-025-10254-7.

[59] Sack RL, Lewy AJ, Erb DL, Vollmer WM, Singer CM. Human melatonin production decreases with age. Journal of Pineal Research. 1986; 3: 379–388. https://doi.org/10.1111/j.1600-079x.1986.tb00760.x.

[60] Baburina Y, Lomovsky A, Krestinina O. Melatonin as a Potential Multitherapeutic Agent. Journal of Personalized Medicine. 2021; 11: 274. https://doi.org/10.3390/jpm11040274.

[61] Maity J, Dey T, Banerjee A, Chattopadhyay A, Das AR, Bandyopadhyay D. Melatonin ameliorates myocardial infarction in obese diabetic individuals: The possible involvement of macrophage apoptotic factors. Journal of Pineal Research. 2023; 74: e12847. https://doi.org/10.1111/jpi.12847.

[62] Favero G, Golic I, Arnaboldi F, Cappella A, Korac A, Monsalve M, *et al*. Cardiometabolic Changes in Sirtuin1-Heterozygous Mice on High-Fat Diet and Melatonin Supplementation. International Journal of Molecular Sciences. 2024; 25: 860. https://doi.org/10.3390/ijms25020860.

[63] Sun X, Sun P, Zhen D, Xu X, Yang L, Fu D, *et al*. Melatonin alleviates doxorubicin-induced mitochondrial oxidative damage and ferroptosis in cardiomyocytes by regulating YAP expression. Toxicology and Applied Pharmacology. 2022; 437: 115902. https://doi.org/10.1016/j.taap.2022.115902.

[64] Tao Y, Zhao Q, Lu C, Yong W, Xu M, Wang Z, *et al*. Melatonin suppresses atherosclerosis by ferroptosis inhibition via activating NRF2 pathway. FASEB Journal. 2024; 38: e23678. https://doi.org/10.1096/fj.202400427RR.

[65] Ishihara R, Barros MPD, Silva CMD, Borges LDS, Hatanaka E, Lambertucci RH. Melatonin improves the antioxidant capacity in cardiac tissue of Wistar rats after exhaustive exercise. Free Radical Research. 2021; 55: 776–791. https://doi.org/10.1080/10715762.2021.1939024.

[66] García JJ, Piñol-Ripoll G, Martínez-Ballarín E, Fuentes-Broto L, Miana-Mena FJ, Venegas C, *et al*. Melatonin reduces membrane rigidity and oxidative damage in the brain of SAMP8 mice. Neurobiology of Aging. 2011; 32: 2045–2054. https://doi.org/10.1016/j.neurobiolaging.2009.12.013.

[67] Acuña-Castroviejo D, Escames G, Venegas C, Díaz-Casado ME, Lima-Cabello E, López LC, *et al*. Extrapineal melatonin: sources, regulation, and potential functions. Cellular and Molecular Life Sciences: CMLS. 2014; 71: 2997–3025. https://doi.org/10.1007/s00018-014-1579-2.

[68] Suofu Y, Li W, Jean-Alphonse FG, Jia J, Khattar NK, Li J, *et al*. Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. Proceedings of the National Academy of Sciences of the United States of America. 2017; 114: E7997–E8006. https://doi.org/10.1073/pnas.1705768114.

[69] Atacak A, Baltaci SB, Akgun-Unal N, Mogulkoc R, Baltaci AK. Melatonin protects retinal tissue damage in streptozotocin-induced aged rats. Archives of Gerontology and Geriatrics. 2023; 112: 105035. https://doi.org/10.1016/j.archger.2023.105035.

[70] Akgun-Unal N, Ozyildirim S, Unal O, Gulbahce-Mutlu E, Mogulkoc R, Baltaci AK. The effects of resveratrol and melatonin on biochemical and molecular parameters in diabetic old female rat hearts. Experimental Gerontology. 2023; 172: 112043. https://doi.org/10.1016/j.exger.2022.112043.

[71] Rondanelli M, Opizzi A, Monteferrario F, Antoniello N, Manni R, Klersy C. The effect of melatonin, magnesium, and zinc on primary insomnia in long-term care facility residents in Italy: a double-blind, placebo-controlled clinical trial. Journal of the American Geriatrics Society. 2011; 59: 82–90. https://doi.org/10.1111/j.1532-5415.2010.03232.x.

[72] Reiter RJ, Tan DX, Korkmaz A, Ma S. Obesity and metabolic syndrome: association with chronodisruption, sleep deprivation, and melatonin suppression. Annals of Medicine. 2012; 44: 564–577. https://doi.org/10.3109/07853890.2011.586365.

[73] Tan DX, Manchester LC, Reiter RJ, Qi WB, Karbownik M, Calvo JR. Significance of melatonin in antioxidative defense system: reactions and products. Biological Signals and Receptors. 2000; 9: 137–159. https://doi.org/10.1159/000014635.

[74] Sabbaghziarani F, Soleimani P, Eynshikh FR, Zafari F, Aali E. Reduced ischemia-reperfusion oxidative stress injury by melatonin and N-acetylcysteine in the male rat brain. IBRO Neuroscience Reports. 2024; 17: 131–137. https://doi.org/10.1016/j.ibneur.2024.07.004.

[75] Aykutoglu G, Tartik M, Darendelioglu E, Ayna A, Baydas G. Melatonin and vitamin E alleviate homocysteine-induced oxidative injury and apoptosis in endothelial cells. Molecular Biology Reports. 2020; 47: 5285–5293. https://doi.org/10.1007/s11033-020-05607-z.

[76] Aranda M, Albendea CD, Lostalé F, López-Pingarrón L, Fuentes-Broto L, Martínez-Ballarín E, *et al*. In vivo hepatic oxidative stress because of carbon tetrachloride toxicity: protection by melatonin and pinoline. Journal of Pineal Research. 2010; 49: 78–85. https://doi.org/10.1111/j.1600-079X.2010.00769.x.

[77] Winiarska K, Fraczyk T, Malinska D, Drozak J, Bryla J. Melatonin attenuates diabetes-induced oxidative stress in rabbits. Journal of Pineal Research. 2006; 40: 168–176. https://doi.org/10.1111/j.1600-079X.2005.00295.x.

[78] Shenoy P, Etcheverry A, Ia J, Witmans M, Tablizo MA. Melatonin Use in Pediatrics: A Clinical Review on Indications, Multisystem Effects, and Toxicity. Children. 2024; 11: 323. https://doi.org/10.3390/children11030323.

[79] Händel MN, Andersen HK, Ussing A, Virring A, Jennum P, Debes NM, *et al*. The short-term and long-term adverse effects of melatonin treatment in children and adolescents: a systematic review and GRADE assessment. EClinicalMedicine. 2023; 61: 102083. https://doi.org/10.1016/j.eclinm.2023.102083.

[80] Panjwani AA, Cowan AE, Jun S, Bailey RL. Trends in Nutrient- and Non-Nutrient-Containing Dietary Supplement Use among US Children from 1999 to 2016. The Journal of Pediatrics. 2021; 231: 131–140.e2. https://doi.org/10.1016/j.jpeds.2020.12.021.

[81] Lelak K, Vohra V, Neuman MI, Toce MS, Sethuraman U. Pediatric Melatonin Ingestions - United States, 2012-2021. MMWR. Morbidity and Mortality Weekly Report. 2022; 71: 725–729. https://doi.org/10.15585/mmwr.mm7122a1.

[82] Cruz-Sanabria F, Bruno S, Crippa A, Frumento P, Scarselli M, Skene DJ, *et al*. Optimizing the Time and Dose of Melatonin as a Sleep-Promoting Drug: A Systematic Review of Randomized Controlled Trials and Dose-Response Meta-Analysis. Journal of Pineal Research. 2024; 76: e12985. https://doi.org/10.1111/jpi.12985.

[83] Iftikhar S, Sameer HM, Zainab. Significant potential of melatonin therapy in Parkinson's disease – a meta-analysis of randomized controlled trials. Frontiers in Neurology. 2023; 14: 1265789. https://doi.org/10.3389/fneur.2023.1265789.

[84] Bourne RS, Mills GH, Minelli C. Melatonin therapy to improve nocturnal sleep in critically ill patients: encouraging results from a small randomised controlled trial. Critical Care (London, England). 2008; 12: R52. https://doi.org/10.1186/cc6871.

[85] Nous A, Engelborghs S, Smolders I. Melatonin levels in

the Alzheimer's disease continuum: a systematic review. Alzheimer's Research & Therapy. 2021; 13: 52. https://doi.org/10.1186/s13195-021-00788-6.

[86] Valiensi SM, Vera VA, Folgueira AL, Caporale S, Ponce de León M, Pino Fernández I, *et al*. Rethinking Melatonin Dosing: Safety and Efficacy at Higher-than-Usual Levels in Aged Patients with Sleep Disturbances and Comorbidities. Brain Sciences. 2025; 15: 1040. https://doi.org/10.3390/brainsci15101040.

[87] Stanciu AE, Zamfir-Chiru-Anton A, Stanciu MM, Stoian AP, Jinga V, Nitipir C, *et al*. Clinical significance of serum melatonin in predicting the severity of oral squamous cell carcinoma. Oncology Letters. 2020; 19: 1537–1543. https://doi.org/10.3892/ol.2019.11215.