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Chapter 3

An Overview of Melatonin as an Antioxidant Molecule: A Biochemical Approach

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Abstract

Melatonin is an endogenous hormone derived from tryptophan that is mainly released from the pineal gland in the dark. Melatonin regulates many biological functions such as sleep, circadian rhythm, immunity, and reproduction. Melatonin has a free radical scavenger, anti-inflammatory, and antioxidant effects. It scavenges reactive oxygen and nitrogen species and increases antioxidant defenses, thus it prevents tissue damage and blocks transcriptional factors of pro-inflammatory cytokines. Due to its small size and amphiphilic nature, it increases the efficacy of mitochondrial electron transport chain and reduces electron leakage. Melatonin prevents degenerative changes in the central nervous system in models of Alzheimer's and Parkinson's disease and reduces free radical damage to DNA which may lead to cancer and many other situations. Consequently, melatonin has beneficial effects including stimulation of antioxidant enzymes, inhibition of lipid peroxidation, and so it contributes to protection from oxidative damages.

Keywords: melatonin, antioxidant, free radical, oxidative stress, anti-inflammatory, neurohormone, tryptophan, disease

1. Introduction

Melatonin, N-acetyl-5-methoxytryptamine, which was first isolated from bovine pineal glands [1], is an endogenous neurohormone derived from tryptophan [2]. Melatonin controls various physiologic processes, including circadian rhythms, mood regulation, anxiety, sleep, appetite, immune responses, and cardiac functions [3]. The sleep–wake cycle is the most overt circadian rhythm [4]. More or less sleep shows negative effects on biological and physiological processes including alterations in metabolic, endocrine, and immune pathways that lead to health problems

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involving obesity, diabetes, hypertension, and respiratory diseases [4–6]. Timing of melatonin secretion is closely associated with the timing of sleep propensity, and it also coincides with decreases in core body temperature, alertness, and performance [7]. Melatonin regulates memory formation by directly affecting hippocampal neurons. There are antinociceptive, antidepressant, anxiolytic, antineophobic, and locomotor activity regulating effects of melatonin [3, 8]. Melatonin plays important roles in neurogenesis, neuroprotection, maintenance of oxidant/antioxidant balance, modulation of cardiovascular and/or immune system, and diabetes control. It exerts a direct antioxidant effect on tissues/organs and antiapoptotic effects on cells [9]. Other actions of melatonin include inhibition of dopamine release in the hypothalamus and retina, involvement in the aging process and pubertal development, blood pressure control, and free radical scavenging [7]. Melatonin dysfunction may contribute to many divergent diseases, such as neurodegenerative diseases, circadian and mood disorders, insomnia, type 2 diabetes, and pain [3]. Low levels of melatonin have been shown in Parkinson's disease (PD), Alzheimer's disease (AD), insomnia, epilepsy, ischemic injury, and neuropsychiatric disorders; in addition, roles for melatonin in the development of cataracts, aging, and retinitis have also been reported [10]. Melatonin has been utilized in several countries for circadian rhythm disorders, sleep disturbances, jet lag, and sleep–wake cycle disturbances in blind people and shift workers [7, 11, 12].

Melatonin is secreted primarily by the pineal gland in response to darkness [2, 13, 14]. It was later found to be also present or synthesized in extrapineal sites such as retina, Harderian gland, lymphocytes, gastrointestinal tract, bone marrow cells, platelets and skin [13, 15–17]. The neurohormone melatonin is not stored in the pineal gland but rather is released into the bloodstream and can penetrate all body tissues [18]. The synthesis of melatonin shows a clear circadian rhythm with low levels during the daytime and its secretory peak at night [19, 20]. The nocturnal synthesis and release of melatonin by the pineal gland are strictly controlled by the suprachiasmatic nucleus (SCN) clock and inhibited by lighting conditions [19, 21]. In humans and other mammals, detection of light drives activity in retinal ganglion cells that project to the SCN in the hypothalamus, causing the release of inhibitory γ-amino butyric acid that suppresses the circuit controlling melatonin synthesis and release [22]. Serum melatonin reaches a peak value (80–150 pg/mL) between midnight and 3 a.m., while its concentration during the day is low (10–20 pg/mL) [23]. Both normal melatonin patterns and the influence of light can vary considerably between individuals, either in terms of personal characteristics or as a consequence of aging or a chronic disease [24]. Serum concentrations of melatonin vary considerably with age, and infants secrete very low levels of melatonin before 3 months of age. Amplitude of the nocturnal peak in melatonin secretion reaches the highest levels between the 4th and 7th year of age [15, 19]. Other factors that alter melatonin levels are nightwork, impaired light–dark cycles, and obesity. Additionally, some nutritional factors could change melatonin production [13].

Melatonin, hormone of darkness, is synthesized from tryptophan, which is an essential amino acid by the pineal gland. The synthesis of melatonin is a multistep process. Firstly, tryptophan is hydroxylated by tryptophan-5-hydroxylase (TPH) to form 5-hydroxytryptophan, which is subsequently decarboxylated to 5-hydroxytryptamine (serotonin) by L-aromatic amino acid decarboxylase (AADC). Serotonin is N-acetylated by arylalkylamine N-acetyltransferase (AA-NAT, also called "Timezyme," is the rate-limiting enzyme for melatonin synthesis), to form N-acetylserotonin, which is converted to N-acetyl-5-methoxytryptamine (melatonin) by N-acetylserotonin-O-methyltransferase (ASMT, also

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called hydroxyindole-O-methyltransferase or HIOMT). The last step is the rate-limiting step in the biosynthesis of melatonin (**Figure 1**) [18, 20, 25–28].

Melatonin synthesis depends on intact beta-adrenergic receptor function. Norepinephrine activates the N-acetyltransferase, and beta-receptor blockers depress melatonin secretion [29]. Both AA-NAT and ASMT activities are controlled by noradrenergic and neuropeptidergic projections to the pineal gland. The pineal gland receives input from postganglionic fibers, leading to the release of norepinephrine. Norepinephrine induces its α1/β-adrenoceptors that activate adenylate cyclase-cAMP system. Thus, intracellular levels of the second messengers include cAMP, Ca^{2+}, phosphatidylinositol, diacetylglycerol, and protein kinase C increase. These messengers induce the expression and activity of AA-NAT and HIOMT [7, 14, 15, 18, 30].

The pineal gland is located outside the blood brain barrier, and loses it connections with the central nervous system, having sympathetic innervation as its main source. This may explain for the pineal gland ability to have a large uptake of tryptophan leading to a high melatonin production and secretion in response to darkness [18]. Once synthesized, melatonin is quickly released into the systemic circulation to reach central and peripheral target tissues. The effects of melatonin depend on the localization and types of melatonin receptors [15]. Melatonin activates two high-affinity G-protein-coupled receptors, termed MT1 and MT2. The MT1 and MT2 lead to an inhibition of the adenylate cyclase in target cells and regulate a variety of cellular and physiological processes including neuronal firing, arterial vasoconstriction, cell proliferation, immune responses, and reproductive and metabolic functions [8, 16, 27, 31–33]. MT1 and MT2 receptors are 350 and 362 amino acids long, located on chromosome 4q35.1 and chromosome 11q21-q22, respectively. MT1 receptors are expressed in the brain, cardiovascular system, immune system, testes, ovary, skin, liver, kidney, adrenal cortex, placenta, breast, retina, pancreas, and spleen. MT2 has been found in the immune system, brain, retina, pituitary, blood vessels, testes, kidney, gastrointestinal tract, mammary glands, adipose tissue, and the skin [27, 31, 32]. The MT3 receptor has a low affinity, unlike MT1 and MT2; it is not coupled to G proteins; it has a nanomolar affinity for melatonin, and it is not sensitive

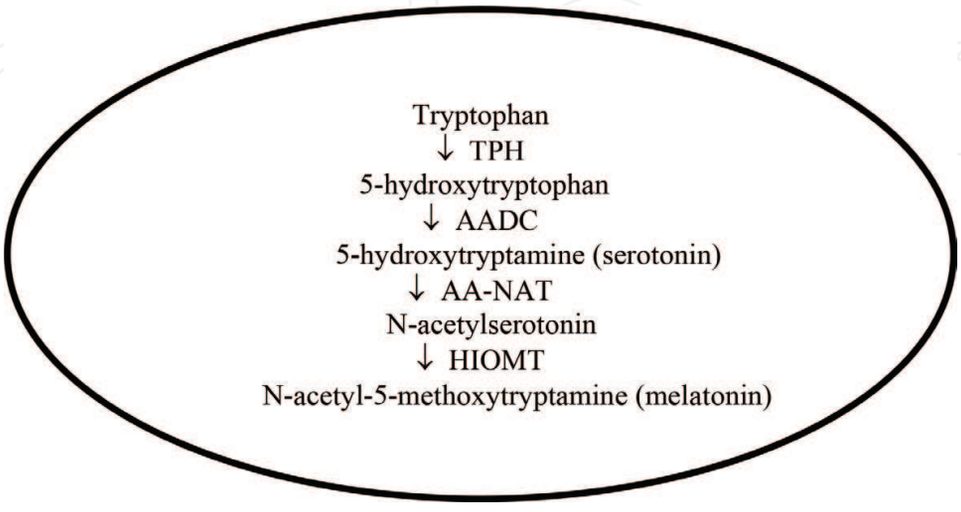

Figure 1. Biosynthesis of melatonin from tryptophan (TPH, tryptophan-5-hydroxylase; AADC, L-aromatic amino acid decarboxylase; AA-NAT, arylalkylamine N-acetyltransferase; HIOMT, hydroxyindole-O-methyltransferase).

to Na^{+2}, Mg^{+2}, and Ca^{+2}. The MT3 is equivalent to enzyme quinone reductase II [27]. The relationship between multiple physiological function of melatonin and this enzyme is possibly involved in the regulation of cellular redox status, although the exact role of this relationship remains unclear [16, 34]. Melatonin appears to be a natural ligand for the retinoid-related orphan nuclear hormone receptor family (RZR/ROR). RZR/RORα is expressed in a variety of organs, whereas RZRβ is specific for the brain and retina [35]. In addition, melatonin interacts with intracellular proteins such as calmodulin, calreticulin, or tubulin and antagonizes the binding of Ca^{2+} to calmodulin [7]. ROR/RZR has been proposed to work in coordination with the plasma membrane receptors MT1/MT2 to regulate gene expression. The low-affinity interaction between melatonin and calmodulin may be involved in its antioxidant action as well as other signaling processes [15, 16]. The membrane receptors have been defined in the central nervous system and in peripheral organs, such as liver, gastrointestinal tract, skin, kidney, heart, and adipose and lymphoid tissues in many mammalians [33]. Melatonin also acts through nonreceptor-mediated mechanisms, for example, serving as a scavenger for reactive oxygen species (ROS) and reactive nitrogen species (RNS) [27]. Melatonin and its metabolites have potent antioxidative and radioprotective properties [36]. Melatonin has been proven to be an efficient oxidant scavenger of a variety of radical and nonradical reactants [37].

In the circulation, melatonin is partially bound to albumin and can also bind to hemoglobin [38]. Melatonin metabolism is a rapid process, and its half-life in humans varies between 10 and 60 min following exogenous administration. It is deactivated mostly by the liver and excreted in the urine [13, 26]. There are three major pathways of melatonin degradation: (1) the classical hepatic degradation pathway that generates 6-hydroxymelatonin, (2) the alternative indolic pathway that produces 5-methoxyindole acetic acid (5-MIAA) or 5-methoxytryptophol (5-MTOL), and (3) the kynurenic pathway that produces the main brain metabolites of melatonin, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), and its deformylated product N1-acetyl-5-methoxykynuramine (AMK). These metabolites are highly remarkable and are generated enzymatically, pseudoenzymatically, by free radical, and via photochemical processes. Recently, it was reported that AFMK and AMK detoxify reactive species and preserve tissues from damage by reactive intermediates [39]. This chapter summarizes effects of melatonin and its metabolites as antioxidants and their clinical significance in several diseases.

2. Free radicals

Free radicals are atoms or molecules that containing one or more unpaired electrons in the external orbitals of the molecules, usually unstable and highly reactive. The free radical chemical reactivity is directly associated with the damage that they can inflict to biological molecules. In biology system, oxygen-derived radicals and nitrogen-derived radicals are two types of free radicals. Oxygen-derived radicals, such as superoxide ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), alkoxyl radicals ($RO^{\bullet}$), as well as nonradicals such as hydrogen peroxide (H_2O_2), ozone and hypochlorous acid, are defined as reactive oxygen species (ROS). ROS are produced during the oxygen metabolism. Nitrogen-derived radicals and nonradicals, such as nitrogen dioxide (NO_2), nitric oxide radicals ($NO^{\bullet}$), and peroxynitrite (ONOO), are known as reactive nitrogen species (RNS) which are derived from nitric oxide and superoxide by inducible nitric oxide synthase (iNOS) and NADPH oxidase, respectively [16, 40–44]. Oxidants are produced

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as a result of normal intracellular metabolism in mitochondria and peroxisomes, as well as from diverse cytosolic enzyme systems such as lipoxygenases, NADPH oxidase, and cytochrome P450. Furthermore, various external agents including ionizing radiation, ultraviolet light, environmental toxins, inflammatory, and cytokines can trigger ROS production [16, 44]. Mitochondria are the major source of ROS and RNS production [45]. Generation of $O_2^{\bullet-}$ during oxidative phosphorylation takes place mainly in the mitochondria. $O_2^{\bullet-}$ is quickly converted to H_2O_2 enzymatically by superoxide dismutases (SODs). After that, H_2O_2 is converted into water or highly toxic hydroxyl radical [16]. Although hydroxyl radical formation can occur in several ways, by far the most important mechanism in vivo is likely to be the transition metal-catalyzed decomposition of superoxide anion and hydrogen peroxide [46]. Hydroxyl radicals are generated from hydrogen peroxide during cellular oxygen metabolism via the Fenton and Haber-Weiss reactions (**Figure 2**) [47], in the presence of free iron or copper ions [48]. The $OH^{\bullet}$ is formed during the Fenton reaction when H_2O_2 interacts with transition metals (Fe^{2+}, Cu^{1+}, etc.) [16, 40, 41]. It can also be produced by ultraviolet and ionizing radiations [41].

Alkoxyl radicals that are formed from the reduction of peroxides, are less reactive than $OH^{\bullet}$ and significantly more reactive than ROO radicals, provided that R is the same in both species. Therefore, they are suggested to be ideal candidates to evaluate the efficiency of antioxidants and also the reactivity of any species reacting with ROS. As regards RNS, the chemical reactivity and direct toxicity of $NO^{\bullet}$ are quite low. However, it reacts with $O_2^{\bullet-}$ forming peroxynitrite, which is a powerful oxidant. NO_2 is a mild oxidant, and its reactivity is between those of $NO^{\bullet}$ and $ONOO^-$ [41, 42, 44].

In healthy organisms, there is a delicate balance between the production and the removal of free radicals, which guarantees that they remain in low/moderate concentrations. Under such conditions, free radicals have beneficial effects [41]. ROS and RNS play important roles in regulation of a wide variety of physiology functions like gene expression, cellular growth, differentiation, modulation of chemical reactions, and induction of transcription factors such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) and activation of signal transduction pathways. They also participate in blood pressure control, are mediators in the biosynthesis of prostaglandins, function in embryonic development, and act as signaling molecules within the individual cell and among cells during their life span [44–46]. The harmful and useful effects of ROS/RNS are associated with their concentrations, the cell type and the subcellular compartments that are produced, and their timing of production [16]. An imbalance between excessive ROS and RNS generation and rate of their elimination by the antioxidant capacity leads to oxidative stress [49, 50]. It has been shown that oxidative stress is involved in over 100 diseases, as their cause or consequence [51]. Oxidative stress results in macromolecular damage and is implicated in various disease states such as atherosclerosis, diabetes, cancer, neurodegeneration, and aging [52]. The cellular dysfunctions caused by excessive ROS and/or RNS might produce loss of energy metabolism, altered cell signaling

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet-} \text{ (Fenton reaction)}$$

$$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet-} \text{ (Haber-Weiss reaction)}$$

Figure 2. Fenton and Haber-Weiss reactions.

and cell cycle, gene mutations, and impaired cellular transport mechanisms. The oxidative stress promotes decreased biological activities, immune activation, and inflammation [50]. It seems that both high levels of ROS (oxidative stress) and excessively low levels of ROS (reductive stress) are deleterious and apparently play a causative role in the pathologies caused by malfunctioning processes related to the dramatic change of redox environment [53].

3. Antioxidants

Based on the oxidative stress related to free radical theory, the antioxidants are the first line of choice to take care of the stress [45]. Antioxidants act as free radical scavengers and can prevent oxidative reactions that lead to various diseases [54]. The antioxidant defense system includes endogenous (enzymatic and nonenzymatic) and exogenous (dietary) antioxidants that interact in establishing redox homeostasis in the body [49]. Endogenous antioxidants, which are products of the body's metabolism, may be enzymatic or nonenzymatic compounds localized generally in the cytoplasm and diverse cell organelles [45, 49]. In eukaryotics, various antioxidant enzymes, for instance, SOD, catalase (CAT), and some peroxidases, transform ROS into more stable molecules (e.g., water and O_2) via complex cascade of reactions [45]. One of the most effective intracellular enzymatic antioxidants is SOD. In humans, there are three forms of SOD: cytosolic CuZn-SOD, mitochondrial Mn-SOD, and extracellular SOD. SOD catalyzes the dismutation of $O_2^{\bullet-}$ to H_2O_2, decreasing the amount of $O_2^{\bullet-}$ and thereby lowering the formation of $ONOO^-$ [44, 50]. Other important enzymatic antioxidants include CAT, glutathione peroxidase (GPx), glutathione reductase (GR), and peroxiredoxins (Prxs). These enzymes neutralize hydrogen peroxide, yielding water (CAT, GPx) and oxygen molecule (CAT) [45, 49]. CAT which is found in the peroxisomes and cytoplasm [55] presents a molecule of ferric ion at its active site and converts two molecules of H_2O_2 into one molecule each of water and diatomic oxygen [37]. Glutathione peroxidase can be found in many subcellular compartments including the mitochondria and nucleus depending on the family member [55]. Selenium, as a selenocysteine, is a component of the active site of GPx [37, 55, 56]. GPx uses reduced glutathione (GSH) as a substrate to transfer electrons to H_2O_2 (and other peroxides), thereby converting it into two molecules of water [37]. When hydrogen peroxide is metabolized by glutathione peroxidase, reduced glutathione is oxidized to glutathione disulfide (GSSG) which is converted back to GSH by the enzyme GR [57–59].

Small molecular nonenzymic antioxidants (e.g., GSH, NADPH, thioredoxin, vitamin E (α-tocopherol), vitamin C (ascorbic acid), and trace metals, such as selenium) also function as direct scavengers of ROS [45]. In particular, glutathione plays a central role in defense against oxidative stress [54]. The antioxidant properties of GSH which is a tripeptide, γ-L-glutamyl-L-cysteinyl-glycine, depend on the presence of a peptide bond between the amino group of cysteine and the alpha-carboxyl group, which provide an excellent protection against aminopeptidases, and the expression of the thiol group which derive from the cysteine residue. Complexation of metal ions, participation in the oxidation reactions, and formation of thiol radicals and disulfides are the most important functions of thiol groups in the biological systems [49]. Maintaining or reestablishment of redox homeostasis are ensured by endogenous and exogenous antioxidants that act synergistically [49, 60], such as during the regeneration of vitamin E by GSH or vitamin C to prevent lipid peroxidation, which can affect membrane

fluidity and damage membrane proteins [60, 61]. Vitamin E and Vitamin C are the most frequently used antioxidant vitamins [62] that are thought to have a protective effect by either reducing or preventing oxidative damage [63]. Vitamin E belongs to the group of fat-soluble vitamins existing in eight different forms. The methylation pattern of the chroman ring determines the classification as α, β, γ, and δ tocopherols. These compounds have antioxidant properties. Vitamin E scavenges peroxyl radicals and hence acts to break the chain reaction of lipid peroxidation [64]. Besides its antioxidant role, vitamin E might also have a structural role in stabilizing membranes [46, 65, 66]. Vitamin C, which is readily water soluble, is an important antioxidant and thus works in aqueous environments of the body [46, 57, 67]. As an antioxidant, ascorbate is an efficient scavenger, or reducing antioxidant, capable of donating its electrons to ROS and eliminating them [44]. Loss of one electron generates the ascorbyl radical intermediate, and loss of two electrons generates dehydroascorbate (DHA, which can also be formed via dismutation of the ascorbyl radical) [61, 68]. It makes ascorbate a powerful important antioxidant [44]. Vitamin C serves as a co-antioxidant with vitamin E to regenerate α-tocopherol from α-tocopherol radicals in membranes and lipoproteins and protect protein thiol group against oxidation by increasing intracellular levels of GSH [46, 61, 69]. Vitamin C can also neutralize ROS (e.g., hydrogen peroxide) [46, 70]. Recently, toxicity of ascorbic acid has also been attributed to its autoxidation [45].

An efficient antioxidant should not only be ubiquitous but should also be present in adequate amounts in cells and easily reacts with a wide variety of free radicals which have short half-life due to high reactivity. A good antioxidant has the ability to cross physiologic barriers and to be quickly transported into the cells. Thus, it must be available to all cells. It is also important for an antioxidant to be available. Antioxidants should be available when needed. They should be easily acquired through the diet or produced in situ. Antioxidants should be suitable for regeneration. The reaction between an antioxidant and a free radical yields an oxidized form of the antioxidant which has less scavenging activity than the original compound. Therefore, many antioxidants have physiologically reducing mechanisms, or its oxidized forms can still efficiently react with new free radicals. An ideal antioxidant should be conserved by the kidneys. Otherwise, large urinary losses would occur and the half-life will be short. An important aspect to consider for evaluating the suitability of a compound as an antioxidant is its toxicity. It should be nontoxic prior to and after the free radical scavenging process takes place. In addition, it is also important to be aware of possible interactions with any drug that may be concurrently consumed [41, 71, 72].

Melatonin is a potent direct scavenger of free radicals. Unlike most of other radical scavengers, it is a multifunctional antioxidant. Melatonin can easily pass through cell membranes because of its high lipophilicity and hydrophilicity [73]. Melatonin is also widespread within cells. Its concentrations in human serum and cerebrospinal fluid vary widely. Melatonin is endogenously generated, and it is ingested in the food as it is widely available in fruits and vegetables. Hence, melatonin is produced internally and is also ingested in the diet. Only small amounts of melatonin are excreted into the urine in its unchanged form. It has minimal toxicity. Numerous in vivo studies on animals involving massive doses of melatonin have shown that acute and chronic toxicity of melatonin is extremely low [41, 74]. Unlike most small molecule biological antioxidants such as ascorbic acid, α-tocopherol, lipoic acid, etc., melatonin does not undergo redox cycling and, thus, does not promote oxidation. Melatonin

can be considered a suicidal or terminal antioxidant. It undergoes molecular rearrangement, effectively removing the free electron from the system. Each of these products of rearrangement is also a potent antioxidant in its own right. Furthermore, most of these processes involve more than one ROS per step, so that one melatonin molecule has the capacity to scavenge up to 10 ROS versus the classic antioxidants that scavenge one or less ROS [17, 20, 70, 74]. It has been found that melatonin promotes the repair of oxidized DNA. This is probably due to the melatonin's capability of transforming guanosine radical to guanosine by electron transfer [42]. It was shown that melatonin reduced the formation of 8-hydroxy-2′-deoxyguanosine (8-OH-dG), a damaged DNA product, 60–70 times more effective than some classic antioxidants (ascorbate and α-tocopherol) [75]. Additionally, the relative position of melatonin and its metabolites in the antioxidant "pecking order" (electrochemical potential) may contribute greatly to its utility in biological systems [76]. Melatonin protects lipids, proteins, and nuclear DNA from oxidative damage suggests that its intracellular distribution is wide [17]. Melatonin turned out to be considerably more efficient than the majority of its naturally occurring structural analogs, indicating that the substituents of the indole moiety strongly influenced reactivity and selectivity [77].

4. Melatonin and its metabolites as antioxidants

Melatonin is an indoleamine with two side chains, a 5-methoxy group and 3-amide group. Its molecular weight is 232.2 g/mol [42]. Melatonin has multifunctional activities in addition to its function as a synchronizer of the biological clock and seasonal reproduction [78, 79]. One such activity is its antioxidant capacity. Melatonin and its metabolites were found to have important antioxidant properties owing to their direct and indirect antioxidant actions. Melatonin can easily cross cell membranes [80] and the blood brain barrier [78] and protects various biomolecules against damage caused by free radicals by acting as a direct scavenger to detoxify reactive oxygen and nitrogen species. In addition, melatonin can indirectly reduce oxidative stress by increasing the activities of antioxidative defense systems; stimulating the expression and function of a number of antioxidant enzymes, as well as glutathione, another very important nonenzymatic, low molecular weight antioxidant; interacting synergistically with other antioxidants; and increasing the efficiency of the mitochondrial electron transport chain [40, 78–82]. Also, melatonin has a chelating property which may contribute in reducing metal-induced toxicity [83]. Melatonin was shown to be much more specific than its structural analogs in undergoing reactions, which lead to the termination of the radical reaction chain and in avoiding prooxidant, C- or O-centered intermediates [33, 38]. Moreover, it has been shown that it has an ability to scavenge free radicals, including hydroxyl radicals, hydrogen peroxide, peroxyl radicals, singlet oxygen, nitric oxide, and peroxynitrite. It was demonstrated that melatonin inhibits the activity of NO synthase, beside it's NO and peroxynitrite scavenging activity [84].

Melatonin, an endogenously produced indoleamine, is a highly effective antioxidant and free radical scavenger [82]. Melatonin has been reported to neutralize the most toxic oxidizing agents, hydroxyl radical and the peroxynitrite anion, generated within the cells. Moreover, melatonin reportedly scavenges singlet oxygen ($^1O_2$), superoxide anion radical, hydrogen peroxide, nitric oxide, and hypochlorous acid (HClO) [17]. Due to the electron-deficient nature of

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halide ions, haloperoxyl radicals are significantly more reactive than the alkylperoxyl radical; accordingly, the trichloromethylperoxyl radical ($CCl_3OO^{\bullet}$) was found to be potently trapped by melatonin [85]. Not only melatonin but also several of its metabolites that are formed when it functions as a direct free radical scavenger, i.e., cyclic 3-hydroxymelatonin (c3OHM), AFMK, AMK, etc., are also radical scavengers [57, 86]. Melatonin and its metabolites work in a "task-division" way, with some of them acting mainly as free radical scavengers, while others act as metal chelating agents and inhibitors of the hydroxyl radical ($OH^{\bullet}$) production [87]. The sequential scavenging of ROS by melatonin and its metabolites is known as melatonin's antioxidant cascade [16]. The efficiency of AMK for scavenging ROS and preventing protein oxidation has been reported to be higher than that of AFMK. Therefore, it seems that at least in general, their protective activities against oxidative stress follow the order AMK > melatonin > AFMK [88] (**Table 1**).

4.1. Effects of melatonin and its metabolites on reactive oxygen species

Electron donation is the principal mechanism by which melatonin detoxifies the free radicals [17]. While melatonin has the capability of donating one or more electrons to free radicals resulting in their detoxification, the metabolites that are formed during this process, i.e., c3OHM, AFMK, and AMK, also have similar capabilities [90]. After donating an electron to $OH^{\bullet}$, melatonin becomes a free radical itself, the indolyl radical cation. However, its reactivity is very low, and, therefore, it is not toxic to cells [41]. Oxidation of melatonin by hydroxyl radicals leads to several hydroxylated products which can be explained by interaction of melatonin with two hydroxyl radicals, one acting by hydrogen abstraction and the other by combining with the reaction partner especially, at the sides C2, C3, C6, and C7 [91].

6-Hydroxymelatonin (6OHM) is the major hepatic metabolite and photodegradation product of melatonin. It is an efficient metabolite for protecting against oxidative damage induced by UV irradiation. Due to its capability of scavenging $^1O_2$ and $O_2^{\bullet -}$, 6OHM can reduce neurotoxicity induced by quinolinic acid. It also lowers Fe(II)-induced neurotoxicity and iron-induced lipid peroxidation. It also inhibits the oxidative damage induced by this metal, UV radiation, thiobarbituric acid, and cyanide. It may be more efficient than melatonin in this capacity. Moreover, it inhibits oxidative stress induced by Cu^{+2}-ascorbate mixtures and $OH^{\bullet}$ production by sequestering Cu^{+2} ions. 6OHM also protects DNA damage induced by Fenton reagents and UV radiation [84, 92].

It was showed that the main hydroxylated metabolite of melatonin interaction with hypochlorous acid is 2-hydroxymelatonin (2OHM). Subsequently, 2OHM and its keto tautomer, melatonin 2-indolinone, were the oxidative products of melatonin's interaction with oxoferryl hemoglobin or $OH^{\bullet}$ [93]. 4-Hydroxymelatonin (4OHM) is an excellent peroxyl radical scavenger and also a preventing antioxidant by inhibiting Cu(II). This effect would reduce the Cu(I) availability, which is the redox state required for the $OH^{\bullet}$ to be formed, via Fenton-like reactions. 4OHM terminates the oxidant effects of copper-ascorbate mixtures. The key structural feature in the antioxidant activity of 4OHM is the presence of phenolic group, unlike 2OHM which has a relative low antioxidant protection [94]. 4OHM and 2OHM are generated during the UV-induced metabolism of melatonin. Further investigation needs to understand the antioxidant activity of these two compounds, as well as their potential role in protecting biomolecules against oxidative damage [87].

ROS/RNS neutralized by melatonin and its metabolites	Antioxidative enzymes that are stimulated by melatonin
Hydroxyl radical	Superoxide dismutase
Hydrogen peroxide	Glutathione peroxidase
Superoxide anion radical	Catalase
Nitric oxide	Glutathione reductase
Alkoxyl radical	Glutamyl-cysteine ligase
Peroxynitrite	Cyclooxygenase
Singlet oxygen	Heme oxygenase
Hydrogen peroxide	Nitric oxide synthase
Hypochlorous acid	Paraoxonase
Others	Myeloperoxidase
	Lipoxygenase

Table 1. Antioxidant effects of melatonin and its metabolites [89].

7-Hydroxymelatonin has been rarely considered, although the calculated activation energy for the respective reaction is as low as that for 6-hydroxylation. 3-Hydroxylation leads to an unusual compound cyclic 3-hydroxymelatonin (c3-OHM) [91]. c3-OHM is an intermediate metabolite of melatonin [16]. c3-OHM effectively scavenges $OH^{\bullet}$, $ABTS^{\bullet+}$ (2,20-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) [95], and peroxyl radicals [96] and can also chelate Cu(II), preventing its reduction and the consequent $OH^{\bullet}$ production via Fenton-like reactions [93, 97]. It is demonstrated that c3-OHM inhibits oxidative DNA damage and 8-OHdG lesions, induced by Fenton reagents, under in vitro conditions [98]. Indeed, c3-OHM is considered a footprint molecule, excreted in small amounts in the urine, and evidence of the in vivo scavenging activity of melatonin [41]. c3-OHM also undergoes oxidation resulting in the formation of AFMK [16, 77, 99].

AFMK is one of the metabolites of melatonin and can be formed by both enzymatic or pseudoenzymatic and nonenzymatic metabolic pathways [10, 88]. Pyrole ring cleavage of melatonin by varied enzymes including indoleamine 2,3-dioxygenase (IDO), myeloperoxidase (MPO), and hemoperoxidases, varied pseudoenzymatic catalysts such as oxoferryl hemoglobin and in varied reactions with ROS involving free radicals and singlet oxygen, generates AFMK [39, 88, 100]. Melatonin oxidation by MPO and IDO generally requires $O_2^{\bullet-}$ that produced in large amounts in inflammatory circumstances [100]. Besides, there are also multiple hydroxylations, which are formed in the peroxidase and peroxidase-like reactions and in the conversion of c3-OHM to AFMK [39]. Nonenzymatically, direct reaction of melatonin with highly reactive oxygen species (e.g., hydroxyl radical and singlet oxygen) formed AFMK [100]. The formation of AFMK by singlet oxygen deserves attention, as this reactive oxygen species is formed under the influence of UV light [101]. In light of these findings, it appears that AFMK is a product common to several interactions of melatonin with oxygen-based reactants [85]. The generation of AMK occurs via deformylation of AFMK [10, 16, 77]. These compounds are also major melatonin metabolites in detoxifying ROS and reducing oxidative stress

[10, 16]. AFMK is obviously more stable than many other oxidative metabolites or its secondary product, AMK [39]. AFMK reduces lipid peroxidation and oxidative DNA damage induced by a variety of oxidative stressors under various conditions [16]. It protects neuronal cell from injuries caused by hydrogen peroxide and amyloid-β (Aβ) peptide [85, 88, 93]. It has been suggested that neuroprotection of AFMK against radiation-induced oxidative damage to the brain is due to its free radical scavenging function [88].

Lipid peroxidation is a natural metabolic process under normal aerobic conditions, and it is one of the most investigated consequences of ROS action on membrane structure and function [44]. Alterations in the fluidity of membranes result in negative effects on their functions such as signal transduction processes and implicate in aging as well as in diseases [102]. Melatonin is known to be a stabilizer or protector of cell and organelle membranes because of its inhibitory effects on lipid peroxidation. Melatonin and its metabolites scavenge free radicals and thus terminate the initiation and propagation of lipid peroxidation [103]. Although melatonin and its metabolites, AFMK and AMK, are peroxyl radical scavengers, it is indicated that melatonin's ability to resist lipid peroxidation may also involve its metabolite, c3-OHM [104]. For the reaction with the peroxyl radical, c3-OHM was several orders of magnitude faster than melatonin, AFMK and AMK, and it was roughly 100-fold faster than water soluble vitamin E (Trolox) [96, 105]. Melatonin also directly scavenges the alkoxyl radical, a product resulting from the transition metal-catalyzed degradation of lipid peroxides. This is important for the control of lipid peroxidation since the alkoxyl radical can abstract a hydrogen atom from a polyunsaturated fatty acids; the resulting peroxyl radical can obviously continue the propagation of lipid degradation [104, 106].

4.2. Effects of melatonin and its metabolites on reactive nitrogen species

Reactive nitrogen species represent another category of potentially destructive substances, which react with melatonin [77]. $ONOO^{\bullet}$ itself is a very damaging species able to react with proteins, lipids, and DNA. Therefore, the reaction between two rather innocuous free radicals produces a much more reactive one [41]. Melatonin readily combines with a superoxide releasing NO, thus preventing the formation of peroxynitrite, a free radical even more harmful than NO. It has been described as a direct peroxynitrite scavenger [40].

Scavenging of nitric oxide by melatonin in a nitrosation reaction is well documented. Whether this can be regarded as a detoxification reaction keeping NO from forming, the more dangerous peroxynitrite is uncertain because nitrosomelatonin easily decomposes, thereby releasing NO. Melatonin also scavenges peroxynitrite, but it is difficult to discriminate direct reactions with peroxynitrite and with hydroxyl radicals generated by decomposition of peroxynitrous acid. The interaction with products from the peroxynitrite-CO_2 adduct ($ONOOCO_2^-$) which carbonate radicals ($CO_3^{\bullet -}$) and $NO_2^{\bullet}$ seems to be more important than direct scavenging of peroxynitrite [33, 77]. There is evidence for the formation of cyclic 2-hydroxymelatonin, cyclic 3-hydroxymelatonin, and 6-hydroxymelatonin about the reaction of melatonin with $ONOO^-$. It was suggested that one electron is transferred from melatonin to $ONOO^-$ in the melatonin $+ONOO^-$ reaction and/or nitrated intermediates occur in the oxidation. In addition, the 6-hydroxymelatonin is not generated in the presence of CO_2. Therefore, it was suggested that formation of 6-hydroxymelatonin required an activated peroxynitrite that can only exist in the absence of bicarbonate [41, 107, 108]. AFMK has the ability to interact with the ABTS

cation radical as well as with ROS/RNS to form AMK. When AMK interacts with the ABTS cation radical or with $ONOO^-$, it forms products that may also be ROS and RNS scavengers [59]. AMK was described as better a NO scavenger than melatonin or AFMK [88]. AMK effectively inhibits neuronal nitric oxide synthase activity and reduces intracellular NO levels [93].

4.3. Effects of melatonin and its metabolites on antioxidant enzymes

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes [70]. Antioxidative enzymes provide a major defense mechanism against free radical damage either by metabolizing them to less reactive species or to nontoxic by-products [85]. The activities of antioxidative enzymes depend on the duration and severity of oxidative stress. Under prolonged oxidative stress conditions, free radicals directly damage the antioxidant enzymes or reduce enzyme activities [90, 109]. Besides its ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, melatonin stimulates several antioxidative enzymes which increase its efficiency as an antioxidant [58]. The major antioxidative enzymes such as intracellular superoxide dismutases (CuZn-SOD and Mn-SOD), the selenium-containing glutathione peroxidases and catalase, are stimulated by melatonin under basal conditions [43, 75, 110]. Melatonin plays a significant role in maintaining indirect protection versus free radical injury by stimulating gene expression of antioxidant enzymes including those for SOD and GSH-Px [43, 58, 62, 111]. Melatonin affects both antioxidant enzyme activity and cellular mRNA levels for these enzymes under physiological circumstances and during increased oxidative stress, presumably through epigenetic mechanisms. These properties in a single molecule are unique for an antioxidant, and both actions protect against pathologically generated free radicals [43, 62].

The concentration of the intracellular antioxidant, glutathione, is very high in many cells. During high oxidative stress conditions total glutathione levels can be reduced [90]. Melatonin maintains the activities of enzymes that enhance intracellular levels of reduced GSH. The recycling of GSH may well be a major effect of melatonin in reducing oxidative stress. GSH is oxidized to its disulfide, GSSG, which is then quickly reduced back to GSH by GR, an enzyme which has been demonstrated to be stimulated by melatonin. The ability of melatonin to regulate the GSH/GSSG balance by modulating enzyme activities seems to involve an action of melatonin at a nuclear binding site [85, 112]. The other GSH-metabolizing enzyme, i.e., CAT, also increases its activity in response to melatonin [85]. Furthermore, one of the melatonin actions is stimulation of gamma-glutamylcysteine synthetase that is the rate-limiting enzyme in glutathione production, thus glutathione levels do not drop significantly [36, 43, 58, 75, 77, 85, 86, 90, 110, 112, 113].

There are a number of prooxidative enzymes in multicellular organisms which generate free radicals [90]. Melatonin not only upregulates the expression of genes involved in detoxifying free radicals, but it also suppresses the activity or expression of genes involved in the generation of free radicals [16, 113]. Melatonin inhibits the prooxidative enzyme nitric oxide synthase which generates $NO^{\bullet}$ and lipoxygenase which result in the formation of the superoxide anion [90, 113, 114]. Although $NO^{\bullet}$ is not a strong free radical, when it couples with $O_2^{\bullet -}$, it forms the peroxynitrite anion which is potently reactive and damaging [90]. Lipoxygenase reaction is another possible source of ROS and other radicals. It catalyzes the hydroperoxidation of polyunsaturated fatty acids [115]. The prooxidative enzymes inhibited by melatonin also include myeloperoxidase and eosinophil peroxidase [110]. As a result,

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free radical and/or toxic reactant generation is alleviated [90, 114]. In addition, AFMK and AMK also have the ability to downregulate prooxidative and pro-inflammatory enzymes including iNOS [102] and cyclooxygenase-2 (COX-2) and to carry out free radical avoidance functions [93].

4.4. Effects of melatonin and its metabolites on the mitochondria

Mitochondria are critical in the control of metabolism and responsible for orchestrating cellular energy production. Therefore, they are central to the maintenance of life and the gatekeepers of cell death [116]. The production of energy in the form of ATP is crucial to optimal cell function, including aiding in repairing any cellular damage that has occurred and in improving survivability of the cell, of the tissue, and of the organism [90]. Up to 95% of the ATP produced in aerobic cells is a result of mitochondrial oxidative phosphorylation [59]. The ETC which is coupled to oxidative phosphorylation [59] is a system of oxidoreductase protein complexes (complexes I, II, III, and IV) [85]. Deficiencies in the ETC can result in the leakage of electrons which thereafter generate free radicals and other toxic reactants which leads to molecular damage in mitochondria; this damage culminates in and promotes what are referred to as mitochondria-related diseases [85]. Mitochondria are the primary source of free radicals [44, 45]. Increased free radical generation, enhanced mitochondrial iNOS activity, enhanced NO production, decreased respiratory complex activity, impaired electron transport system, and opening of mitochondrial permeability transition pores have all been suggested as factors responsible for impaired mitochondrial function [117].

Melatonin has important actions at the level of mitochondria [85]. Melatonin exhibits remarkable functional versatility to protect the morphological and functional aspects of the cell membrane scavenging free radicals, enhancing the activity of the antioxidant enzymes, and optimizing the transfer of electrons through the ETC in the inner mitochondrial membrane [118]. Melatonin increases the efficiency of the ETC and thus reduces electron leakage and free radical generation [38, 75, 105] that is a consequence of the respiratory process by stimulating complex I and complex IV of the mitochondrial respiratory chain that are involved in oxidative phosphorylation [38, 58, 59, 118]. By directly detoxifying ROS/RNS, melatonin enhances ATP production via maintaining high levels of mitochondrial GSH, protects mitochondrial proteins and DNA from oxidative damage, and improves ETC activity [16, 90, 118]. Moreover, AMK, like its precursor melatonin, promotes mitochondrial complex I activity to elevate ATP production by lowering electron leakage and inhibiting the opening of the mitochondrial permeability transition pore [93].

4.5. Effects of melatonin and its metabolites on transition metals

Heavy metals are known to cause oxidative deterioration of biomolecules by initiating free radical-mediated chain reaction resulting in lipid peroxidation, protein oxidation, and oxidation of nucleic acid like DNA and RNA [119]. The ability of antioxidants to chelate and deactivate transition metals prevents such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal-catalyzed reaction [120]. Chemical mean of inhibiting metal-induced oxidation is chelation. This particular process is directly involved in the $OH^{\bullet}$-inactivating ligand (OIL) behavior of antioxidants. There are two different ways of action in the protection exerted by OIL species against $OH^{\bullet}$-induced oxidative damage: (i) inhibiting the reduction of metal ions; thus, their reduced forms are not available for Fenton-like reactions or (ii) deactivating $OH^{\bullet}$ after being produced by Fenton-like reactions [87].

Melatonin is able to prevent the oxidative actions of metals by neutralizing the produced ROS and capturing such metals to form chelates [83]. It was demonstrated that the interplay of melatonin with metals such as aluminum, cadmium, copper, iron, lead, and zinc depended on concentration. Melatonin chelates both iron(III) and iron(II), which is the form that attends the Fenton reaction. If iron is bound to a protein (e.g., hemoglobin), melatonin restores the highly covalent iron such as oxyferryl (Fe^{IV}-O) hemoglobin back to iron(III), thereby reestablishing the biological activity of the protein [89]. It is suggested that, under physiological circumstances, direct chelation mechanism would be the major chelation route for Cu(II). It was demonstrated that melatonin and its metabolites, 3OHM, AFMK, and AMK, fully inhibited the oxidative stress induced by Cu(II)-ascorbate mixtures, via Cu(II) chelation [97]. Melatonin decreases the Cu(II)/H_2O_2-induced damage to proteins and protects against copper-mediated lipid peroxidation, which led to the suggestion that the antioxidant and neuroprotective effects of melatonin may involve removing toxic metals from the central nervous system [42].

5. Melatonin and its metabolites as anti-inflammatory agents

Inflammation is an essential response to tissue injuries induced by physical, chemical, or biological insults [17]. The production of inflammatory cytokines including TNF-α (tumor necrosis factor-α), IL-1β (interleukin-1β), or IL-6 attenuates by melatonin in numerous experimental models of inflammation [2]. Melatonin has several additional anti-inflammatory effects, which are probably related to a direct interaction with specific binding sites located in lymphocytes and macrophages [103]. Anti-inflammatory activity of melatonin includes inhibition of the activation of COX-2 and iNOS, as well as blocking of the transcriptional factors that triggers proinflammatory cytokine production. These include not only NF-κB but also HIF, Nrf2, cAMP, CREB, STAT, PPARs, and AP-1 [2, 43, 121]. Melatonin may be useful for the treatment of inflammatory disease, as it reduces inflammatory injury by blocking transcription factors and NF-κB, thereby decreasing further ROS formation within cells [43]. In peripheral monocytes, melatonin and, even more, AFMK suppressed TNF-α and IL-8 production and, in macrophages, COX-2 and iNOS expression. Moreover, melatonin was found to be efficiently oxidized to AFMK by macrophages [91]. AMK was reported to downregulate COX-2—but not COX-1—expression in macrophages, an effect shared by its precursors AFMK and melatonin [122].

6. The clinical significance of melatonin

Melatonin plays important roles in neurogenesis, neuroprotection, maintenance of oxidant/antioxidant balance, and modulation of cardiovascular and/or immune system. It also exerts a direct antioxidant effect on tissues/organs and antiapoptotic effects on cells [9]. Melatonin has been investigated in a wide range of diseases, such as neurodegenerative, cardiovascular, liver, and kidney diseases, cancer, and diabetes [43].

Melatonin is a ubiquitously acting direct free radical scavenger and also an indirect antioxidant. Melatonin and its metabolites are efficient in scavenging ROS and RNS. It plays an effective role

in regulating mitochondrial homeostasis [33, 38]. Mitochondrial dysfunction, i.e., cell energy impairment, apoptosis, and overproduction of ROS, is a final common pathogenic mechanism in aging and in neurodegenerative disease [43, 123]. Melatonin may be possible to treat neurodegenerative disorders by inhibiting mitochondrial cell death pathways. It may easily protect brain mitochondrial membranes from free radical attack, stabilizing them. The ability of melatonin to prevent GSH loss probably reflects its effect on the activities of the GSH redox cycle enzymes [33, 38, 83, 103]. Moreover, several neurological diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, and Wilson's disease (hepatolenticular degeneration) are characterized by an overload of copper and/or other metals. Melatonin and its metabolites, c3OHM, AFMK, and AMK, have the copper sequestering ability [89].

Excessive and/or sustained increase in ROS generation plays a pivotal role in the initiation, progression, and clinical consequences of cardiovascular diseases (CVDs) [64, 124]. Clinically, melatonin is being increasingly recognized in the pathophysiology of CVD. Low levels of serum melatonin as well as its urinary metabolite, 6-sulphatoxymelatonin, have been reported in various CVDs including coronary heart disease, angina, congestive heart failure, and myocardial infarcts [125]. Melatonin plays an important role in the regulation of several parameters of the cardiovascular system, including blood pressure, and is considered to be a putative antihypertensive agent [126]. It may have cardio-protective properties via its direct free radical scavenger activity and its indirect antioxidant activity together with its significant anti-inflammatory properties [127, 128]. Mitochondrial respiration, mainly at the level of complex I and complex III, is an important source of ROS generation and hence a potential contributor of cardiac reperfusion injury [129]. Most of the beneficial actions of melatonin at the heart level may depend on its effect on mitochondrial bioenergetics mediated through various mechanisms including general antioxidant actions at the level of ETC dysfunction, electron leakage, and mitochondrial oxidative damage and also through a direct action of melatonin on mitochondrial permeability transition pore opening [127]. It was reported that melatonin protects against mitochondrial dysfunction associated with cardiac ischemia reperfusion, by preventing alterations to several parameters involved in mitochondrial bioenergetics [17].

Melatonin may also exhibit anticancer and protective oncostatic activity through several mechanisms, including inhibition of cancer cell proliferation, decrease in oxidative stress, and increase in immune system activity [130, 131]. Oxidative stress has complex and different effects on each type of cancer development [132]. Oxidation of cellular lipids and proteins can adversely affect several steps of the carcinogenic process through changes in a variety of cell regulatory functions, including signal transduction and gene expression. ROS are postulated to be involved in carcinogenesis process, especially in the stages of initiation and promotion [133]. It appears that the DNA damage is predominantly linked with the initiation process [132]. Free radicals and ROS generated by environmental carcinogens, or by metabolic alterations, cause DNA damage and genetic instability [134]. Furthermore, DNA damage, apoptosis resistance, enhanced proliferation, mutation, COX-2 upregulation, oxidative stress, tumor vascularity, and metastatic potential may be caused by nitric oxide synthase overexpression and increased nitric oxide and other RNS productions [132]. A growing body of evidence implicates melatonin's antioxidant/free radical scavenging actions in the inhibition of cancer development and growth [75]. Melatonin is a powerful scavenger of ROS, such as

hydroxyl radical, peroxyl radical, singlet oxygen, and nitric oxide, as well as a stimulator of the antioxidant enzymes, SOD, GPx, and CAT, all leading to a decrease in DNA damage [135]. Additionally, this indole stimulates antioxidant enzymes that remove ROS before they can inflict damage and aids in the repair of damaged DNA [136]. Melatonin could be an excellent candidate for the prevention and treatment of several cancers, such as breast cancer, prostate cancer, gastric cancer, and colorectal cancer [137].

A variety of antioxidants protect the liver from free radical-mediated damage, one of the best of which is melatonin. Clinical studies have confirmed that melatonin protects the liver from nonalcoholic liver disease and also during the surgical procedure of partial liver resection [138]. Melatonin is a well-known natural antioxidant and has many bioactivities. Melatonin exerts antioxidant effects in hepatocytes and epithelium of the liver by reducing lipid peroxidation and increasing the level of reduced liver glutathione. Melatonin is a highly valuable OH and H_2O_2 scavenger, during its metabolism to AFMK. It also induces several antioxidative enzymes such as glutathione peroxidase, glutathione reductase, and SOD and increases the synthesis of GSH [2, 88]. Melatonin exhibits potent anti-inflammatory, antioxidant, and fibrosuppressive activities against thioacetamide-induced hepatic fibrogenesis via the suppression of oxidative stress, DNA damage, pro-inflammatory cytokines, and fibrogenic gene transcripts [139]. Melatonin protects against lipid-induced mitochondrial dysfunction in hepatocytes and inhibits stellate cell activation during hepatic fibrosis in mice [140].

Inflammation and increased oxidative stress are also common features in chronic kidney disease patients [130, 141]. Oxidative stress and inflammation promote renal injury via damage to molecular components of the kidney by different mechanisms of action. ROS lead to the loss of significant functional properties, lipid peroxidation of cell membrane, decrease membrane viability, and cleavage, and cross-linking of renal DNA occurs leading to harmful mutations by oxidizing amino acids in the nephron. Furthermore, other ROS interactions in the nephron increase secondary radical production [130, 142]. Diabetes-associated hyperglycemia leads to mitochondrial ETC dysfunction culminating in a rise in ROS production [143]. Experimental evidence suggests that the indoleamine hormone melatonin is capable of influencing in development of diabetic complications by neutralizing the unnecessary ROS generation and protection of beta cells, as they possess low antioxidant potential and normalize redox state in the cell [144]. Melatonin acts as a cell survival agent by modulating autophagy in various cell types and under different conditions through amelioration of oxidative stress, ER stress, and inflammation [143].

7. Conclusion

Melatonin is a circulating neurohormone secreted predominantly at night, thereby called as hormone of darkness. It can cross all physiological barriers to exert widespread regulatory effects on body tissues. Melatonin is a universal antioxidant with multifunctional activities such as anti-inflammatory, antiapoptotic, and antioxidant effects in addition to its function as a synchronizer of the biological clock and seasonal reproduction. Melatonin and its derivatives have been shown to be powerful direct free radical scavengers. Besides direct scavenging of ROS/RNS, melatonin also stimulates antioxidant enzymes; suppresses prooxidant

enzymes; improves mitochondrial function, hence reducing radical formation; and reduces metal-induced toxicity. Results from previous studies support these effects on several diseases including cancer, diabetes, neurodegenerative, cardiovascular, liver; and kidney diseases.

Conflict of interest

The authors do not have any conflict of interest to declare.

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