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Review Article

An insight into the scientific background and future perspectives for the potential uses of melatonin

Md Jamir Anwar[*], *Bala Yauri Muhammad*, *Ahmed Abdulsabour Bader*, *Mahfoudh Abdulghani*, *Danish Mahmood*, *Mohammed Haider*

Department of Pharmacology and Toxicology, Unaizah College of Pharmacy, Qassim University, Al-Qassim, Kingdom of Saudi Arabia

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ABSTRACT

Melatonin is one of the most versatile and ubiquitous molecule widely distributed in nature has been reported to play a role in a wide variety of physiological responses including reproduction, circadian homeostasis, sleep, retinal neuromodulation, and vasomotor responses. In most vertebrates, including humans, melatonin is synthesized primarily in the pineal gland and is regulated by the environmental light/dark cycle via the suprachiasmatic nucleus. Melatonin is synthesized in all areas of the body such as gastrointestinal tract, skin, bone marrow, retina and in lymphocytes, from which it may influence other physiological functions through paracrine signalling. In addition to regulation of circadian rhythm of melatonin a variety of other physiological effects such as hypnotic, antidepressant, antiepileptic, oncostatic, immunomodulatory, antiosteoporotic, in cardiovascular disease, neuromodulatory and cerebral ischaemic condition have been reported. Moreover there is scarcity of literature that reviewed the scientific evidence for its use in these conditions. Therefore in this article we review recent advances in this research field, which is preceded by a concise account of general information about melatonin, melatonin receptors and intracellular signalling pathways for melatonin actions.

1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine) was isolated from the bovine pineal gland 55 years ago [1,2]. The rhythmic production of melatonin by the pineal gland was initially linked to the regulation of seasonal reproduction in photoperiodic species [3,4]. Subsequent studies have shown that melatonin's functions greatly exceed that of regulation of the waxing and waning of seasonal reproductive competence [5,6].

Seasonal reproduction is an adaptive physiological process utilized by animals that live under natural environmental conditions to anticipate annual changes in day length, temperature and food availability [7]. This allows them to make the necessary physiological adjustments in advance of the actual sexually quiescent interval or breeding period [8]. In

* *Corresponding author*. Tel.: +966 501071895.
E-mail addresses: m.anwar@qu.edu.sa, zameeranwar@gmail.com (M.J. Anwar).
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mammals, photoperiodic information is received at the level of the ganglion cells of the retina and is transmitted via a multi synaptic neural pathway to the pineal gland where the message modulates the rhythm of melatonin secretion [9]. A major function of the melatonin rhythm is to transmit information about length of the daily dark period to the circadian and circannual systems; thus it provides time-of-day and time-of-year information, respectively, to the organism [10]. This information is essential for sleep, temperature regulation, as well as for seasonal reproductive alterations [11–13].

Melatonin also is a powerful antioxidant and anti-apoptotic agent, which due to its direct scavenging of toxic oxygen derivatives and its ability to reduce the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) prevents oxidative and nitrosative damage to all macromolecules in all compartments of the cell [14–16]. Mammalian gametes and embryos are particularly vulnerable to oxidative stress [17–19] due to plasma membrane composition, the presence of higher levels of lipids and exposure to dramatic changes in the microenvironment, especially when used in artificial breeding techniques [20,21]. Melatonin has the ability to neutralize damaging ROS and RNS species in these cells, reduce lipid peroxide concentrations and DNA damage, and thereby improve the viability of germ and embryonic cells [22–24]. The role of melatonin in the production and preservation of mammalian gametes and embryos is summarized in this brief review.

2. Melatonin as an antioxidant

Despite of well known hormonal [25], sleep-inducing [26], and chronobiological [27] effects of melatonin, antioxidant properties have also been widely reported. Ianas and colleagues (1991) initially reported and claimed the antioxidant and prooxidant actions of melatonin [28]. Therein they generated free radicals using a combination of luminol and H_2O_2 and used chemiluminescence as an index of free radical production.

The free radical scavenging activity of melatonin was studied in a battery of *in vitro* tests. Wherein they reported that melatonin is devoid of prooxidant actions, at least in the series of tests they performed [29]. Their findings are in line with the previous observations, where they reported that melatonin is an excellent scavenger of trichloromethylperoxyl ($CCl_3O_2^{\bullet}$) free radical [30,31]. Furthermore, they also found that although melatonin reacts with hypochlorous acid (HOCI), though the reaction is slow but it does not directly scavenge the $O_2^{\bullet -}$ free radicals.

Recently several publications have reported the evidence for cardioprotective effects of melatonin via its direct free radical scavenger and its indirect antioxidant activity [32]. Melatonin efficiently interacts with various reactive oxygen and reactive nitrogen species (receptor independent actions) and it also upregulates antioxidant enzymes and down-regulates pro-oxidant enzymes (receptor-dependent actions) [31]. The lipophylic nature of melatonin allows its entry into the cells and subcellular compartments and to cross morphophysiologic barriers. These findings implicate the protective effects of melatonin in cardiac diseases induced by oxidative stress. Its direct free radical scavenging activity [33] and its regulation of gene transcription [34] for antioxidative enzymes has been reported in the literature [35]. The antioxidant properties of melatonin have been extensively studied and summarized its use as a cell protector and as a potential disease-preventing agent [36–39]. Further, melatonin has been proven to be an efficient oxidant scavenger of a variety of radical and non-radical reactants [40]. In addition to this, it has been shown that this neurohormone is able to increase the activity of glutathione peroxidase in rat brain cortex as well as the gene expression for some antioxidant enzymes [41].

3. Melatonin as an oncostatic

Several preclinical and clinical studies have reported the beneficial effects of melatonin against a wide range of tumour [42,43]. Most of these studies focus on hormone-dependant cancers related to disorder of endocrine system included breast, prostate, uterus, cervical uterine, mammary tumours [44], tumour growth, osteosarcoma [45]. Impaired secretion of melatonin has been reported in patients suffering from breast, endometrial, or colorectal cancer [46]. Further increased incidence of breast and colorectal cancer observed in nurses and other night-shift workers suggests a possible correlation between the reduced melatonin secretion and their increased light exposure at night [47,48].

Melatonin exhibits the oncostatic action by its two main properties firstly by *protective effect* such as reversible cellular injury [49] through neurohormone regulation and secondly by *antiproliferative effects*. Moderation of cellular cGMP and cAMP ratios regulates cellular metabolic processes and thus control the production of antioxidants in the cell. Melatonin deficiency results in uncontrolled cAMP synthesis, leading to unregulated oxidative processes and subsequent free radical damage [50]. Alterations of the intracellular redox state play a key role in the effects of high concentrations of melatonin in cancer cells, reducing conditions being associated with a decrease in cell proliferation and oxidative conditions with apoptosis [51]. Melatonin mediates suppression of cAMP levels through melatonergic receptor and thus inhibits uptake of mitogenic substances involves on growth of tumour [52]. It has been demonstrated in various carcinoma cell lines that melatonin increases the activity of glutathione-S transferase enzyme (GST) implicating the role of GST gene on 11q 13 chromosome in cancer [53]. This is supported by the study where melatonin was reported to bound to DNA, chromatin or heterochromatin [10].

Melatonin shows antigonadotropic and antioestrogenic actions in different study module, *in vitro* and *in vivo* on hormone-dependent tumours and cancer cell line [44] as well as antagonised the action of prolactin on human breast cancer cell (HBC) [54]. It selectively neutralised the effects of estrogens on the breast and the local biosynthesis of estrogens from androgens [55]. It has reported that melatonin modulates the enzymes involved in the local synthesis of estrogens by regulating the estrogen receptor expression and transactivation. Further, it has been reported that melatonin activates the immune system [56]. Recently it has been reported

that there is increased expression of melatonin receptor in gastric adenocarcinoma tissues, in consistent with other studies reported high expression in marginal tissues in breast and colon cancer indicates a refractory mechanism and defending role of melatonin in GI system [57].

Secondly the oncostatic property such as antiproliferatlve effects on MCF-7 cell growth in culture [58], modulation of cell cycle and induction of apoptosis, inhibition of telomerase activity [56], and antiangiogenesis. Melatonin exerts an anti-angiogenic activity in HepG2 cells by interfering with the transcriptional activation of vascular endothelial growth factors (VEGF), via hypoxia-inducible factor 1-alpha (Hif1a), and signal transducer and activator of transcription 3 (STAT3) [59].

Melatonin is a broad-scale epigenetic modulator of gene expression [60] and stimulation of cell differentiation [56]. It shows apoptosis of Ehrlich ascites carcinoma (EAC) cells and phase delay of the cell cycle [53]. Gonzalez et al. demonstrated that melatonin shows oncostatic effect in MCF-7 human breast cancer cells by melatonin receptor 1 (MTR1) thus confirms MTR1 as a main mediator in the melatonin signalling pathway in breast cancer [61]. Further, melatonin inhibits aromatase activity and expression by regulating the gene expression of specific aromatase promoter regions [62].

Melatonin plays an important role in different stages of cancer (tumour growth and metastasis) through different pathways and it may have therapeutic significance [63]. Melatonin was used as an adjunct to the routine chemotherapy of osteosarcoma, it helps to improve the prognosis of this diseases [45]. Melatonin decrease bone resorption and have a bone protecting effect [64]. The future holds much promise for melatonin as a therapeutic treatment. High expression levels of MT1-mRNA together with low OPG-mRNA were found in both osteosarcoma cell lines, while in normal human osteoblasts and bone marrow stromal cells, high OPG-mRNA levels were associated with low MT1-mRNA levels. These data on the abundant expression of MT1-mRNA in human bone tumors and osteosarcoma cells lines suggest an important role for MT1 in bone pathology [65]. Recently, one study investigated the effect of melatonin on proliferation of human osteosarcoma cell line MG-63 wherein they reported that melatonin significantly inhibit human osteosarcoma cell proliferation in a dose-dependent and time-dependent manner and this inhibition involves the downregulation of cyclin D1, CDK4, cyclin B1 and CDK1 [66].

4. Melatonin as a hypnotic

The first clinical evidence for the involvement of melatonin in sleep was observed by Lerner [1]. Melatonin was intravenously administered at a dose of 200 mg to two volunteers who became sleepy. Subsequently Lerner and his collaborators treated 5 patients with hyper-pigmentation using prolonged ingestion of 1 g melatonin daily where they observed that all patients became drowsy [67]. More than 15 studies showed that melatonin promoting sleep in healthy volunteers [68]. Thus melatonin as hypnotic analogues is made over counter for healthy individuals who want to improve their sleep.

Melatonin, being described as chronobiotic molecule [69] has also been used successfully for treatment of sleep problems related to perturbations of the circadian time keeping system like those caused by jet lag and shift-work disorder [68]. An irregular work schedules have been associated with a loss of daily rhythms and increasingly fragmented sleep [70,71]. Previous studies showed the beneficial effect of melatonin and its hypnotic analogues on sleep disorder in shift worker to rebalance the sleep-wake cycle and promotion of sleep [72]. In addition to this, several studies reported that melatonin is used to counteract jet lag, associated with travel results due to changes in time zone in both adult and children [73,74].

Metabolic syndrome (MS) patients exhibit sleep/wake disturbances and other circadian abnormalities, and these may be associated with more rapid weight increase and development of diabetes and atherosclerotic disease. Melatonin and its hypnotic analogues (ramelteon, agomelatine, tasimelteon) showed beneficial effects on sleep disturbances associated with various diseases include neurological, psychiatric, cardiovascular and metabolic diseases [75]. Being an effective chronobiotic agent, melatonin is able to change the phase and amplitude of circadian rhythms. It has also significant cytoprotective properties preventing a number of MS sequelae in animal models of diabetes and obesity. Further, melatonin showed therapeutic beneficial in treatment of sleep disorder associated with obesity [76]. Sleep disturbances are a frequent problem in cancer patients; melatonin can attenuate insomnia and improve quality of life [77]. In diabetes, sleep disturbance is common and melatonin and its analogues exerted beneficial effects. Other studies evaluated the efficacy of melatonin in promoting better sleep in persons with primary insomnia. Meta-analysis study reported that melatonin decreases sleep onset latency, increases total sleep time and improves overall sleep quality [78]. Moreover melatonin and its hypnotic analogues replacement therapy have been shown to be beneficial in treating elderly insomniacs [68,79]. Further, the British Association of Psychopharmacology recommended melatonin as the first choice treatment of insomnia in elderly insomniac patients.

Hypnotic analogues of melatonin include ramelteon, tasimelteon and a chlorinated derivative of melatonin TIK-301 are approved by the FDA for treatment of insomnia during 2005–2010. All the hypnotic analogues act on MT_1/MT_2 melatonin receptor [80]. The FDA granted tasimelteon and TIK-301 orphan drugs designation status for blind individuals without light perception with non-24-hour sleep-wake disorder between 2004 and 2010. The FDA granted orphan drugs to use as a treatment for circadian rhythm sleep disorder in blind individuals without light perception [77]. Melatonin hypnotic analogues (MHAs) have limited potential for abuse, cognitive and functional impairment due to the fact that they are devoid of affinity for benzodiazepine, dopamine, and opiate, ion channels and receptor transporters. MHAs showed safety more than triazolam and benzodiazepine derivatives. Because MHAs such as ramelteon caused no significant effect on these problematic adverse events at up to 20 times the recommended dose (8 mg daily at bedtime) [81]. There were no apparent next-day cognitive or motor effects or evidence of rebound insomnia or withdrawal effects following treatment discontinuation. MHTA were considered for patients with sleep-onset insomnia, particularly those who are treatment

naïve, who have a history of substance abuse, who are older adults susceptible to the effects of benzodiazepine and non-benzodiazepine hypnotics, and who require minimal interference with the arousal response [82]. Melatonin agonists are potentially efficacious and safe drugs in the treatment of comorbid insomnia with add-on positive effects in a variety of neurological, psychiatric, cardiovascular and metabolic disorders [75].

In contrary to this, a meta-analysis, undertaken by Brzezinski et al. [83] using 17 different studies involving 284 subjects, most of whom were older, concluded that melatonin is effective in increasing sleep efficiency and reducing sleep onset time. Based on this meta-analysis, the use of melatonin in the treatment of insomnia, particularly in aged individuals with nocturnal melatonin deficiency, was proposed.

Melatonin affects sleep and the sleep-wakefulness cycle by acting on receptors in the hypothalamic suprachiasmatic nuclei (SCN) [84]. It shows increased non-rapid eye movement (NREM) sleep in rat [85]. In diurnal species, suppression of electrical activity in the SCN is suggested as the possible mechanism by which melatonin regulates sleep [86]. Further, these effects are thought to be mediated through MT_1 because knockout mice for MT_1 receptor gene are devoid of sleep [87]. The suppression of neuronal activity by melatonin is one of the possible mechanisms by which this hormone contributes to the regulation of sleep [68]. The high density of melatonin receptors in the hypothalamic suprachiasmatic nuclei (SCN) [84] suggested that melatonin affects sleep and the sleep-wakefulness cycle by acting on these receptors. Local injection of pharmacological amounts of melatonin (1–50 μg) in the medial preoptic area of the rat hypothalamus during daytime increased non-rapid eye movement (NREM) sleeps [85]. Melatonin receptors are members of the G protein-coupled receptor (GPCR) family. M_1 is a Gi/o protein-coupled receptor linked, in part, to pertussis-toxin sensitive G proteins that mediate inhibition of cAMP in both recombinant expression systems and native tissues. M_2 is a Gi/o protein-coupled receptor capable of inhibiting cAMP and cGMP production in recombinant systems and stimulating PKC activity in a native tissue, the SCN [88]. Three genes for melatonin receptors have been cloned. The MT_1 (or Mel1a or MTNR1A) and MT_2 (or Mel1b or MTNR1B) receptor subtypes are present in humans and other mammals. Gene polymorphisms reveal that numerous mutations are associated with diseases and disorders. The phylogenetic analysis of receptor genes indicates that *GPR50* is an out-group to all other melatonin receptor sequences [66]. Genetic polymorphisms have been reported for melatonin receptors in human. Seven mutations were found in the MT_1 receptor, with two that resulted in amino acid changes: R54W in the first cytoplasmic loop and A157V in the fourth transmembrane domain. Two mutations were also reported for the hMT2: G24E in the N-terminal domain and L66F in the first cytoplasmic loop. However, neither shows altered MT_1 MT_2 receptor binding characteristics [89]. The effect of these mutations in melatonin receptor function has not been reported.

Melatonin has been shown to induce sleep by altering the functions of the GABAA-benzodiazepine receptor complex by increasing both amplitude and frequency of GABAergic and enhances GABAergic inhibitory transmission in cultured rat hippocampal neurons [90].

5. Melatonin as antidepressant

The master biological clock resides in the suprachiasmatic nuclei of the hypothalamus, a small bilaterally paired structure just above the optic chiasm and plays key role in orchestrating the circadian rhythms of multiple biological processes. Increasing evidence points to a role of the biological clock in the development of depression as it drives 24h-rhythms in physiology and behaviour, and aligns endogenous rhythms to the external solar day in a close temporal relationship. Disturbance of sleep and circadian rhythms is a prominent feature of depression, and therefore, regarded as central for understanding the pathophysiology and treatment of depression. Though contradictory, disrupted melatonin secretion is regarded as a link between circadian rhythm and major depression. In 2011, Quera et al. reported that antidepressants with intrinsic chronobiotic properties could offer a novel approach to treatment of depression [91]. Melatonin and melatonin agonists have been reported to possess chronobiotic effects, which mean that they are able to readjust the circadian system. It was suggested that seasonal affective disorders and mood disturbances caused by circadian malfunction could be treated by manipulating the circadian system using chronobiotic drugs, chronotherapy or bright light therapy. In major depressive disorder, melatonin per se showed no antidepressant action but novel melatoninergic compounds have demonstrated antidepressant properties. Abnormal melatonin rhythms have been noted in several diseases including depression in shift workers [92].

In 2013, in a preclinical study on mice, Haridas et al. evaluated possible beneficial action of chronic night-time melatonin treatment against chronic mild stress-induced behavioural impairments. It was reported that chronic melatonin administration during night-time significantly ameliorated stress-induced behavioural disturbances, especially the cognitive dysfunction and depressive phenotypes, and suggested that melatonin mitigates chronic mild stress-induced behavioural changes, including the cognitive dysfunctions and reaffirm its potential role as an antidepressant [93].

The circadian rhythm hypothesis of bipolar disorder suggested a role for melatonin in regulating mood and hence, melatonergic antidepressant, agomelatine as well as type I (acute) or II (cases of bipolar depression) have been investigated as novel therapies. In a study involving 28 bipolar disorder type –II (BD-II) patients who received open label agomelatine (25 mg/bedtime) for 6 consecutive weeks as an adjunct to treatment with lithium or valproate, followed by an optional treatment extension of 30 weeks, the intent to treat analysis results showed that 18 of the 28 subjects (64%) responded to medication after 6 weeks (primary study endpoint), while 24 of the 28 subjects (86%) showed response by 36 weeks. The study concluded that agomelatine, 25 mg/day, was an effective and well-tolerated adjunct to valproate/lithium for acute depression in BD-II. However, authors suggested the need to confirm the findings in double blind, controlled clinical trials [94].

Recently in a systematic review and meta-analysis reported that there was no clear evidence of a therapeutic- or prophylactic effect of melatonin against depression or

depressive symptoms although some studies were positive [95]. In MELODY trial, 6 mg oral melatonin was investigated on depressive symptoms, anxiety, sleep, cognitive function and fatigue in patients with breast cancer in a three month time period post-surgery. Melatonin had an effect on reducing the risk of developing depressive symptoms and also increased sleep efficiency perioperatively and total sleep time post-operatively [96]. A randomized, double-blind, placebo-controlled trial melatonin significantly reduced the risk of depressive symptoms in women with breast cancer during a three-month period post-surgery [97].

An ideal antidepressant should not only reduce sleep onset difficulties, but also provide daytime alertness and freshness. Melatonin is free of adverse effects such as a 'hangover' and has no abuse potential; in addition, it has been shown to improve sleep patterns in patients with insomnia associated with depression [98]. Melatonin exerts its antidepressant effect through antagonism of the 5HT receptor. It acts through 2 G-protein coupled receptors, MT1 (MEL1a) and MT_2 (MEL1b). The most advanced is the novel melatonin agonist agomelatine, which combines joint MT_1 and MT_2 agonism with 5-HT_{2C} receptor antagonism. Adding a chronobiotic effect to the inhibition of 5-HT_{2C} receptors may explain the rapid impact of agomelatine on depression, since studies showed that agomelatine had an early impact on sleep quality and alertness at awakening. Agomelatine was approved for the treatment of major depressive disorder in adults by the European Medicines Agency in February 2009. It is an analogue of melatonin and features a unique pharmacodynamic profile with agonism on both types of melatonergic receptors (MT1/MT2) and antagonism at serotonergic 5-HT_{2C} receptors. It has been referred to as an effective antidepressant agent in the acute phase of major depressive disorder due to its statistically significant advantage over placebo based on the results of two recent comprehensive meta-analyses of published and unpublished clinical trials [99]. It lacks affinity for histaminergic, adrenergic or dopaminergic receptors. However, despite its efficacy agomelatine was reported to have the potential to exhibit severe hepatotoxic reactions as a new safety concern and is currently poorly understood [99]. Hence, for now it was suggested that it cannot be deemed as antidepressant agent of first choice.

Recently, it has been demonstrated that MT_2 receptors are involved in the pathophysiology and pharmacology of sleep disorders, anxiety, depression, Alzheimer disease and pain. Further selective MT_2 receptor agonists have shown hypnotic and anxiolytic effect. However, the development of selective MT_2 receptor ligands and their preclinical evaluation revealed the role of MT_2 receptor in regulating brain functions. Further, the MT_2 receptors offer great potential for drug discovery [100].

6. Melatonin as antiepileptic

Treatment for epileptic complications continue to pose challenge because of the poor adherence and drug interactions associated with multi drug prescriptions and also for the fear of worsening seizures by the individual medications for complications.

Melatonin, an endogenous hormone secreted by pineal gland has a prominent role in epilepsy. The ability of melatonin to cross the blood–brain barrier gives hope that it could be useful in the treatment of seizures. Melatonin was reported to modulate the electrical activity of neurons by reducing glutamatergic and enhancing GABA-ergic neurotransmission. The hormone and its metabolites act as free radical scavengers and antioxidants. The results from a recent study have shown that melatonin inhibits neurotransmitter release through the blocking of voltage-sensitive Ca^{2+} channels suggesting a possible mechanism for the antiepileptic effect of melatonin [101].

High doses of melatonin (20 mg/kg) given to mice undergoing electroconvulsive stimulation resulted in a strong long-term memory deficit, although electroconvulsive stimulation can also be linked to memory deficit. However, melatonin is well tolerated in doses up to 800 mg/kg in mice [102]. Later, a vast majority of experimental and clinical data have indicated anticonvulsant properties of the hormone. Melatonin inhibited audiogenic and electrical seizures, as well as reduced convulsions induced by pentylenetetrazole, pilocarpine, L-cysteine and kainate [103]. In 2011, a study by Lima et al. investigated the effects of treatment with melatonin and N-acetylserotonin on the pilocarpine-induced epilepsy model. They concluded that melatonin exerted an important neuroprotective effect by attenuating status epilepticus (SE)-induced post lesion and promoting a decrease in the number of seizures in epileptic rats. The authors suggested, for the first time, that melatonin could be used co-therapeutically in treatment of patients exhibiting SE to minimize associated injuries in these situations [104]. A recent study confirmed that long-term melatonin treatment after SE has a potential to attenuate seizure activity and neuronal loss, it was unable to restore epilepsy-associated behavioural abnormalities in spontaneously hypertensive rats [105]. Melatonin exhibited an additive effect with another anticonvulsant drug in decreasing pentylenetetrazole-induced seizure threshold in mice, probably through melatonin $MT_{1/2}$ receptors [106]. In 2013, Tchekalarova et al. examined the effect of melatonin treatment (10 mg/kg/day, diluted in drinking water, 8 weeks) during epileptogenesis on the consequences of a kainate-induced status epilepticus in rats. They reported that melatonin increased the latency in the appearance of spontaneous recurrent seizures and decreased their frequency only during the treatment period. The behavioural alterations associated with hyperactivity, depression-like behavior during the light phase, and deficits in hippocampus-dependent working memory were positively affected by melatonin treatment in rats with epilepsy. Melatonin reduced the neuronal damage in the CA1 area of the hippocampus and piriform cortex and recovered the decrease of hippocampal 5-HT level in rats with epilepsy. Tchekalarova et al. concluded that long-term melatonin treatment after SE did not suppress the development of epileptogenesis but have a potential in reducing some of the deleterious alterations that develop during the chronic epileptic state in a diurnal phase-dependent mode [107]. In 2013, a preclinical study reported that melatonin significantly potentiated the anticonvulsant efficacy of phenobarbital, but did not exert anticonvulsant effects on its own. The data from this study provided additional evidence for the further

examination of melatonin as an adjunct therapy in neonatal/pediatric epilepsy [108]. Melatonin reduces seizure latency and frequency, and improves electroencephalography (EEG) tracing [109]. In a study of 14 patients of the age of 2–19 years, complete disappearance of seizures was observed with melatonin therapy. Such therapy is known to reduce seizure latency in young children as well as adults with different kinds of epilepsy [110]. Subsequently, many studies were conducted to examine the role of melatonin in epilepsy and have revealed that, as an adjunct, melatonin helps to improve seizure management, although the effects of melatonin alone have yet to be explored [111]. In a study on 23 children with intractable epilepsy and 14 children with controlled seizures, use of melatonin in children with intractable seizures was associated with improvement of both many sleep-related phenomena and the severity of seizures [112]. In 2011, another clinical study, reported that melatonin was a good regulator of the sleep-wake cycle for paediatric patients suffering from severe epilepsy and suggested it to be a better control of convulsive episodes [113]. In 2012, Goldberg-Stern, in a pilot study, examined the effect of melatonin on seizures, sleep quality, and behavior in 10 patients aged 9–32 years with intractable epilepsy. The study concluded that melatonin could be effective and safe for decreasing daytime seizure frequency in patients with intractable epilepsy [114].

Another important consideration is that melatonin administration reduces the seizure threshold and might act as a proconvulsant [110] and some study have shown no anticonvulsant effect with melatonin. In 2009, a study reported that serum melatonin levels decreased in children with epilepsy or complex febrile seizure (FS), and supplement of exogenous melatonin might be a promising treatment for epilepsy and febrile seizures in children [115]. However, recently, Mahyar et al. determined the relationship between serum melatonin levels and FS and epilepsy in children. They revealed that there was no association between serum melatonin level and simple or complex FS and epilepsy. Also, they reported that melatonin played no significant role in these convulsive disorders [116]. Because of the increasing evidence favouring anticonvulsant activities of melatonin, large randomised double-blind trials are warranted to establish melatonin as a novel anticonvulsant drug.

7. Melatonin as immunomodulator and anti-inflammatory

Some clinical studies reported that melatonin could be capable of protecting gastrointestinal mucosa against damage through stimulating the immune system and fostering microcirculation and epithelial regeneration [117]. Melatonin has been shown to be involved in the regulation of both cellular and humoral immunity. Melatonin not only stimulates the production of natural killer cells, monocytes and leukocytes, but also alters the balance of T helper (Th)-1 and Th-2 cells mainly towards Th-1 responses and increases the production of relevant cytokines such as interleukin (IL)-2, IL-6, IL-12 and interferon-gamma. The regulatory function of melatonin on immune mechanisms is seasonally dependent [118].

Furthermore, it has been reported that melatonin certainly plays an important role in regulation of epithelial functions as well as significant anti-inflammatory and anti-apoptotic effects. The probable mechanism of melatonin could be attributed due to its ability to reduce bacterial translocation and its anti-apoptotic effect and therefore it can reduce the extent of mucosal damage. This finding suggests that melatonin exert a beneficial role in human inflammatory bowel disease (IBD) through reduction of the tumor necrosis factor-α (TNF-α) levels [119]. In addition to this, melatonin also can decrease free radical levels by stimulating the activities of enzymes involved in antioxidative defence [120].

However, in an experimental model of colitis it was shown that short-term administration of melatonin is protective while in the long term it negatively influences evolution of inflammatory colitis. Therefore, the immuno-stimulatory effect of melatonin in some situations when given chronically, such as during inflammatory bowel disease could have negative consequences [121].

Inflammation is an essential response to tissue injuries induced by physical, chemical or biological insults. The nervous and endocrine systems can interact with the immune system in order to modulate its function. Melatonin is able to modulate immune functions through its neuro-endocrine action; where its primary role is a regulator of circadian rhythms in a hormone-like fashion by affecting target cells [122].

Furthermore, melatonin can modulating other functions depending on the photoperiod including regulation of photoperiodic oscillations of the immune/inflammatory response; where leukocytes possess melatonin specific receptors including both MT1/MT2 and nuclear RZR/ROR alpha receptor. This provides the molecular basis for the sensitivity of leukocytes to melatonin, and hence it is considered as pleiotropic molecule that can regulate inflammation by different properties [123].

Melatonin exerts a concentration-dependent effect on the immune system. Indeed, increasing concentrations of melatonin induce T cell proliferation in a dose-dependent way. Whereas in some experimental studies reported that exogenous melatonin administration was found to increase the proliferative response of rat lymphocytes, increases the number of nature killer cells [124].

8. Melatonin as chronobiotic molecule

As per the International Classification of Sleep disorders, delayed sleep phase disorder (DSPD) is characterised by difficulty in falling asleep at their desired bedtime and an inability to wake spontaneously at the planned time in the morning. Delayed sleep phase disorder was first defined it as disorder and described as long sleep onset latencies and late sleep onset times caused by a delay of the major sleep period.

In 2005, Arendt and Skene [125] reviewed the effects of melatonin especially as a chronobiotic molecule wherein they agreed upon that melatonin is secreted at night in humans and it is thereby associated with sleep and other night time events. The period of melatonin secretion has been described as 'biological night'. Its main function in mammals is to

'transduce' information about the length of the night, for the organisation of day-length dependent changes, such as reproductive competence. Exogenous melatonin has acute sleepiness-inducing and temperature-lowering effects during 'biological daytime', and when suitably timed it will shift the phase of the human circadian clock to earlier or later times. The shifts induced are sufficient to synchronise to 24 h most blind subjects suffering from non-24 h sleep-wake disorder, with consequent benefits for sleep. Recently one study reported the successful use of melatonin as chronobiotic molecule in other sleep disorders associated with abnormal timing of the circadian system: jetlag, shiftwork, delayed sleep phase syndrome, some sleep problems of the elderly. Regulation of pineal melatonin biosynthesis is largely explained by control mechanisms acting on arylalkylamine N-acetyltransferase, at the levels of gene expression and/or enzyme stability influenced by phosphorylation and interaction with 14-3-3 proteins [126]. Further, it has been suggested that nuclear orphan receptors (NOR) can be regulated by the melatonin circadian rhythm in the pars tuberalis (PT) and could be the link between the physiological action of melatonin and the core of the molecular circadian clock in this tissue [127].

There is ample of evidence that DSPD arises from a delayed endogenous circadian rhythm [128]. DSPD and sleep onset insomniacs have altered sleep parameters, melatonin and core body temperature as compared to control groups [129]. If the core body temperature and melatonin circadian rhythms were phase delayed, then the "wake maintenance zone" would be delayed also. Although circadian rhythm phase delay is seen as the major contributor to DSPD, there are some important behavioural and cognitive factors that should be addressed to improve treatment effectiveness.

The ability of exogenous melatonin to phase advance circadian rhythms suggests that it will be effective in the treatment of instrinsic DSPS. Further, exogenous melatonin administration also is capable of shifting the circadian rhythm to a more desired time [130]. In the evening phase, melatonin advances circadian rhythm when combined with optimal time of administration and greater doses: (3–5 mg) 4–8 h prior to the onset of endogenous melatonin and smaller doses (0.3–0.5 mg) 3 h before the beginning of melatonin production. In the first placebo controlled crossover study, melatonin (5 mg fast release) was given at 22:00 h (5 h before sleep onset) to eight men with DSPS [131]. Melatonin has advanced the sleep onset and wake time ($P < 0.01$) as compared to placebo. Moreover, the beneficial effects of melatonin have been demonstrated in 25 DSPS patients when melatonin (5 mg) was given earlier (5 h before melatonin onset ≈7 h before sleep onset) [132]. However the lower doses (0.3–1.0 mg) of melatonin have also been assessed at 1, 3 and 5 h before the sleep onset. In line with this previous study showed that the addition of evening melatonin administration to morning bright light therapy produced a significantly greater phase advance than the morning bright light alone, suggesting that the two therapies are additive [133]. The magnitude of phase shift is affected by the timing, duration of light stimulus, brightness and the wavelength of light. The human phase response curve to light suggests that a phase advance of the circadian rhythm is achieved when the light stimulus is presented immediately after the normal circadian time (CT) [134].

Although exogenous melatonin appears to be safe with short-term use (less than 3 months), there is little information available on its long-term administration. However it is judicious to use much lower doses of melatonin because typical doses (3–5 mg) in many studies can elevate melatonin concentrations well above normal physiological plasma levels. Fortunately, the chronobiotic effects of low doses (0.3–0.5 mg) appear to be sufficient without requiring excessive supraphysiological levels. The adverse side effects associated with melatonin administration include headache, dizziness, nausea, and drowsiness [135]. The effect of exogenous melatonin on sleep was the object of a recent meta-analysis report, which states: "this meta analysis supports the hypothesis that melatonin decreases sleep onset latensy, increases sleep efficiency and total sleep duration [136]. In spite of the heterogeneity of the data the present meta analysis does land statistical support to the notion that melatonin preparations can improve sleep quality with regard to sleep onset latency, sleep efficiency and sleep duration."

9. Melatonin in cardiovascular disease

Many of deleterious agents and pathomechanisms such as ischemia/reperfusion, arterial and coronary atherosclerosis, hypertension, and cardiotoxic drugs can producing or/and processing free radicals harmful impact on cardiac physiology; thus may cause of disability and mortality [137].

Melatonin attenuates molecular and cellular damages resulting from cardiac ischemia/reperfusion in which destructive free radicals are involved. Anti-inflammatory and antioxidant properties of melatonin are also involved in the protection against a chronic vascular disease, atherosclerosis. The administration of melatonin, as a result of its antioxidant features, has been reported to reduce hypertension and cardiotoxicity induced by clinically used drugs and so melatonin's antioxidant effects are in fact due to its metabolites which are generated when melatonin interacts with free radicals [32].

Previous studies suggest that melatonin acts at the mitochondrial level which is responsible for its cell protective effect [138]. In line with this, Petrosillo et al. reported that melatonin protects against mitochondrial dysfunction associated with cardiac ischemia–reperfusion, by preventing alterations to several parameters involved in mitochondrial bioenergetics [139]. Furthermore, the same authors demonstrated that melatonin treatment significantly improves the functional recovery of Langendorff hearts on reperfusion, reduces the infarct size and decreases necrotic damage. All these effects appear to be due, at least in part, to the inhibition of MPTP opening via prevention of cardiolipin peroxidation.

10. Melatonin in bone disease

Melatonin plays a regulatory role in many physiological processes including bone metabolism [140]. Melatonin may affect bone metabolism through bone anabolic as well as antiresorptive effects. Bones are structures under a continuous process of remodelating by the coupled activity of cells with

resorptive functions (osteoclasts) and cells responsible for the formation of new bone (osteoblasts). Nocturnal plasma melatonin levels significantly decline after the age of 50 in both genders [141]. Since the time course of the reduction of melatonin production and the progression of bone deterioration run in parallel shows possible role of melatonin in bone metabolism.

It can prevent the premature destruction of bone and promote its recovery through mechanisms of melatonin-related receptors and receptors of independent action [142]. One study reported the role of melatonin and RANKL/RANK/OPG system (receptor activator of nuclear factor-kappa B ligand/receptor activator of nuclear factor-kappa B/osteoprotegerin) on the regulation of bone turnover [143]. Melatonin regulates the bone metabolism both by its direct influence on bone by acting on osteoclast as well as on osteoblast and indirectly by down-regulating RANK-mediated osteoclast formation and activation [144]. This mechanism brought a discussion about the clinical use for melatonin in bone-grafting procedures, in reversing bone loss due to osteopenia and osteoporosis, and in managing periodontal disease. Recently, an *in vitro* study demonstrates that melatonin promotes differentiation and mineralization of cultured osteoblast cells (MC3T3-E1 cells) under hypoxic conditions [145]. Thus this study concludes that melatonin promotes osteoblastic differentiation of MC3T3-E1 cells under hypoxic conditions via the p38 Mapk and Prkd1 signaling pathways.

A recently published study investigated the protective effects of melatonin as a direct therapeutic tool against chlorhexidine-induced bone cell damage [146]. Wherein they reported that melatonin protects osteoblasts, thereby implicating melatonin as a promising drug in periodontitis and peri-implantitis treatment. Although the study focused on the morphological and pathological changes without obvious efforts to prove it by suggesting a molecular mechanisms, supporting cell morphogenesis and growth, reducing ROS and superoxide generation, shifting the percentage of CHX-damaged cells from necrotic/late to early apoptotic events, and modulating metabolic activity in osteoblasts. In agreement with this another *in vitro* study investigated the effect of 5-methoxytryptophol on bone cell function and observed that melatonin needs higher dose to produce better osteoblastic differentiation effects but this high dose could be toxic [147].

More promising result was obtained when focused on bone sialoprotein (BSP), a mineralized connective tissue-specific protein expressed in the early stage of cementum and bone mineralisation where melatonin induce BSP transcription via the CRE1 and CRE2 elements in the human BSP gene promoter [148].

As a treatment of age related bone loss, there is a primary evidence of that dietary melatonin supplementation is able to exert beneficial effects against age-related bone loss in old rats, improving the microstructure and biomechanical properties of aged bones [149]. A new prospect of using melatonin as a bone therapy had been investigated, where the investigators tested a melatonin graft on a rabbit's tibia instead of procaine bone graft to accelerate the bone formation. The study also revealed that melatonin has proven to regenerate the width and length of cortical bone in tibiae rabbits more quickly than collagenized porcine bone. Melatonin acts as a bone stimulator compared with porcine bone and control sites [150].

Moreover, the potential beneficial effects of melatonin on rabbit tibial bone repair have been investigated wherein they reported that this effect could be associated with angiogenesis and increase in bone density [151]. Similar report was observed by another investigators where they observe that the administration of melatonin may be beneficial in suppressing the effects of free oxygen radicals and regulating antioxidant enzyme activity in the fracture healing process [152]. However, the fracture healing ability of melatonin was investigated by Histing et al., where they observed that melatonin protects the bone from fracture evidenced by radiological, biomechanical, histomorphometrical, and protein biochemical analyses at 2 and 5 wk after fracture. The study concludes that as the bone resorption is an essential requisite for adequate remodeling during fracture healing, melatonin impairs fracture healing by suppressing bone resorption through down-regulation of RANKL-mediated osteoclast activation [153]. Interestingly, as a bone protective therapy against osteoporosis, melatonin was investigated on the perimenopausal women where it was observed that melatonin supplementation was well tolerated; improved physical symptoms associated with perimenopause, and may restore imbalances in bone remodeling to prevent bone loss. But further investigation is warranted to justify this effect [154].

11. Melatonin as neuromodulatory molecule

It has been demonstrated that melatonin may modulate the function of various types of neurons in the central nervous system (CNS) by modifying the activity of ligand- and voltage-gated ion channels [155,156]. These actions of melatonin on central neurons are mediated by distinct intracellular pathways via activation of different subtypes of melatonin receptors. Exogenous administration of melatonin elevates both brain Bcl-2 and BDNF levels. Melatonin acts through the mirochondrial pathway and blocks the spill of cytochrome c to the cytosol and thus prevents activation of caspases, increasing cellular content of Bcl-2 in old rats and thus reduces apoptosis. Further melatonin regulates the complex Bax/Bcl-2 and antagonises apoptosis through the activation of Mitogen activated protein kinase/Extracellular signal-regulated kinases (MAPK/ERK pathway) and inhibition of stress kinases JNK and p38 MAPK in neuronal cells [157]. Melatonin and its activated receptors have been linked to the regulation of neurotrophic factors, including BDNF. Both G-proteins-mediated signalling and other pathways such as extracellular signal-regulated kinase (ERK) may contribute to melatonin action on BDNF [158].

Further, melatonin exerts beneficial effects on cholinergic neurotransmission in the brain by increasing the activities of choline acetyl transferase (ChAT) enzyme in the frontal cortex and hippocampus [158]. In addition to this, melatonin directly detoxifies free radicals and thus can enhance brain Ach activity as a result of the presence of an electron rich aromatic indole ring as an electron-donor.

12. Fate of melatonin

In all vertebrates, the indolamine melatonin is secreted from the pineal gland during the hours of darkness and acts as a hormonal message of the photoperiod. Melatonin is the final product of tryptophan metabolic pathway. The activity of tryptophan hydroxylase (TPH) determines the quantity of serotonin in the gland. Light-dark cycle and clock-driven rhythms affects the expression of TPH mRNA, peak levels of both occur at night [159] coincident with maximal melatonin generation. The low level of precursor is not the rate limiting in melatonin biosynthesis since the amount of serotonin in the pineal gland is reportedly exceptionally high [160].

The biosynthesis of melatonin from serotonin is generally considered to be coupled with arylalkylamine N-acetyltransferase (AANAT) activity [161]. Moreover the rhythm of pineal AANAT runs parallel with that of pineal and blood melatonin level, which are usually high at night [162,163]. The circadian rhythm in the anterior hypothalamus regulates the 24 h oscillations in AANAT activity and melatonin production [9]. The elevated production of melatonin at night is regulated primarily through β1-adrenergic receptors by post-ganglionic sympathetic nerve ending [164]. However, it has been also reported that pineal gland is also innervated by parasympathetic nervous system but it has negligible importance in melatonin production [165].

Norepinephrine (NE) release from sympathetic nerve endings is the primary stimulus for nocturnal melatonin synthesis in pineal gland. Moreover it is expected that some stressful stimuli that augment release of NE from other sites, elevating circulating catecholamine, would reach at pinealocyte stimulates daytime melatonin synthesis. However this does not occur because the sympathetic nerve endings in the pineal gland acts as an effective sink and thereby sequester excess circulating NE [166].

In mammalian species, melatonin disappears from the circulation as a result of 6-hydroxylation in the liver, followed by excretion in a sulfatoxy-conjugated form. The half-life of melatonin in plasma is about 10 min, due to a rapid hydroxylation to 6-hydroxymelatonin in the liver. In the apparent absence of storage or regulated secretion of the hormone, melatonin levels in blood directly mirror the rate of melatonin production.

13. Melatonin: receptors and signal transduction

The alteration of cellular physiology by melatonin is mediated via membrane receptors. In addition to this some receptor independent action of melatonin is mediated by its ability to scavenge free radicals and related reactants. MT1 and MT2 are the two major membrane bound G protein coupled receptors for melatonin. They differ in affinity for their natural ligand, melatonin. To date, the MT3 receptor has been found in the liver, kidney, brain, oocyte and ovary [167–169] and is seemingly identical to the cystosolic enzyme, quinone reductase 2 (QR2) [170]. Melatonin exerts its action by generating a variety of different second messenger after its binding to membrane receptors depending on the specific cell and on the species. A major means by which the MT1 and MT2 receptors regulate intracellular processes is via an inhibition of adenylate cyclase, a reduction in cAMP and modulation of protein kinase A (PKA) activity; this action involves a pertussis toxin sensitive Gi protein [171]. Further it has been reported that activated MT1 receptor inhibit cAMP response-element-binding protein (CREB) phosphorylation and formation of immediate early gene products such as c-fos and jun B [172].

MT1 and MT2 receptors, upon stimulation activates phospholipase C-β and thereby increases the production and release of inositol-(1,4,5)-triphosphate (IP3) and 1,2-diacylglycerol [173]. Stimulation of MT1 is also accompanied by an elevation of the phosphorylation of mitogen-activated protein kinase MEK1/2 as well as extracellular signal-regulated kinase [174]. Furthermore, activation of the MT1 receptor also increases potassium conductance by activating Kir3 (GIRK) inward rectifier potassium channels [175] and potentiates prostaglandin $F_2\alpha$ and ATP mediated stimulation of the activity of protein kinase C (PKC) [176].

Direct free radical scavenging activity of melatonin relates to its non-receptor mediated actions. Melatonin protects lipids, proteins and nuclear DNA from oxidative damage suggests that its intracellular distribution is wide. Electron donation is the principal mechanism by which melatonin detoxifies the free radicals [15]. In addition, melatonin has been reported to neutralize the most toxic oxidizing agents, hydroxyl radical (•OH) and the peroxynitrite anion (ONOO−), generated within the cells. Moreover, melatonin reportedly scavenges singlet oxygen ($1O_2$), superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), nitric oxide (NO•) and hypochlorous acid (HClO). Besides this several metabolites of melatonin with high free radical scavenging activity has been reported to be formed after its interaction with radicals [40,177,178].

14. Conclusion

The practical applicability of melatonin at clinically relevant concentrations remains unconfirmed in as much as most of the effects described have not been demonstrated. Moreover, as a result of pleiotropic nature it may also have side-effects. For instance, an immunoenhancing substance may not be beneficial in patients afflicted by an autoimmune disease. On the other hand, pure preparations of melatonin have usually been remarkably well tolerated. Therefore it is an important matter of future research to investigate the clinical efficacy and safety of melatonin in detail, under different pathological situations.

In context of this, the present review summarizes all up to date research of the probable therapeutic functions of melatonin. Furthermore, its actions as immunoregulatory, cardioprotectice, hypnotic, bone protective, antiepileptic, antidepressant, oncostatic, antioxidant, neuromodulatory and chronobiotic molecule have been well explained in literature. Obviously, while tremendous advances have been made in establishing melatonin as a beneficial component of optimal

health, a great deal still remains to be learned about the mechanisms involved.

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