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Review Article

Protection of Metal Toxicity by Melatonin - Recent Advances

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Abstract

Melatonin (N-acetyl-5-methoxytrptamine), a main product of pineal gland not only regulates the circadian rhythms in photoperiodic species but has the ability to remove reactive oxygen species including singlet oxygen, superoxide anion radical, hydroxyl radical, hydrogen peroxide and lipid peroxides. Its widespread sub cellular distribution enables it to interact with toxic molecules thereby reducing the oxidative damage to the cell. Melatonin also acts as antioxidant, chelating agent, anti-apoptotic agent and as an anti-aging molecule. These qualities of melatonin have been found to ameliorate the toxicity of heavy metals in man and animals. Melatonin prevents adverse effects of lead on immune system, nervous system and anti-oxidative enzymes. It does contribute in the prevention of lead induced genotoxicity. Melatonin alleviates cadmium induced cellular and endoplasmic reticulum stress, unfolded protein response (UPR), germ cell apoptosis and neurotoxic effects. Its protective effects against mercurial toxicity include myocardial toxicity, renal toxicity, neurotoxicity, thyrotoxicity and reproductive toxicity. Protective effects of melatonin on arsenic toxicity are manifested through anti-oxidative mechanisms. It induces autophagy and mitochondrial biogenesis. Melatonin has been found to be protective against chromium, copper and aluminium by way of exhibiting pleiotropic, anti-inflammatory, antioxidative, anti-lipidic and therapeutic effects. Melatonin mediates both extrinsic and intrinsic pathways of apoptotic cell death. It exhibits anti-metastatic effects through NFkB. These pleiotropic functions make this molecule an inevitable melatonin.

Keywords: *Melatonin; Heavy Metals; Apoptosis; Metastasis; Antioxidant*

Introduction

Environmental pollution particularly from hazardous heavy metals and minerals is an important social and public health problem. Some elements like- iron, zinc, copper, cobalt, chromium and manganese are essential for life in small quantities but may be toxic at higher concentrations. Others like lead, mercury, cadmium and arsenic have no beneficial role and are positively toxic. Environmental pollution from hazardous metals and minerals can arise from natural as well as anthropogenic sources. Natural sources are - seepage from rocks into water, volcanic activity and forest fires etc. Anthropogenic sources include industrial activities, commercial activities, and consumerism. Apart from industries, roadways and automobiles too contribute substantially to the environmental load of heavy metals in the form of particulate matter.

Heavy metals are known to cause a variety of ecotoxicological and health problems. The mechanisms of their toxicity have been persistently investigated [1-6]. A variety of therapeutic agents have also been employed to protect against heavy metal toxicity. These include antagonistic elements, chelating agents, essential amino acids, macronutrients and synthetic antioxidants. Several chelating agents are regularly used in hospitals as antidotes for occupational poisoning by metals, for chronic metal intoxication arising from therapy or household contamination or to hasten the excretion of radioactive elements. These include dimercaprol or British anti lewisite (BAL), D-penicillamine, calcium, EDTA, deferoxamine, 2-mercaptoethylamine and sodium diethyldithiocarbamate. Therapeutic use of antioxidants started after the realization that most of the toxic metals manifest their toxicity causing oxidative stress [7]. Hormones have sparingly been used to treat disorders caused by heavy metals [8]. However, emerging experimental evidence suggest that melatonin (MT) has been used to treat metal toxicity in man and animals. The aim of the present review is to document this information to help further investigations on the therapeutic management of heavy metal toxicity.

Structure and function of melatonin

Melatonin (N-acetyl-5-methoxytryptamine), a main product of pineal gland functions as a time giver (zeitgeber) in the regulation of circadian rhythms [9] and in synchronizing the reproductive cycle with the appropriate season of the year in photoperiodic species [10]. In non-photoperiodic species such as humans, the actions of melatonin are restricted to other functions of the circadian clock i.e. consolidation of sleep and regulation of the circadian rhythm or core body temperature [11]. Since 1993, melatonin has been recognized as a free radical scavenger with the ability to remove reactive oxygen species (ROS) including singlet oxygen, superoxide anion radical, hydroxyl radical, hydrogen peroxide and lipid peroxides [12-15]. Melatonin's ability to scavenge ROS has special relevance as it crosses all morpho-physiological barriers due to its distinct physical and chemical properties [16-19]. They allow its penetration in cell membranes and nucleus [20,21]. Its widespread subcellular distribution enables it to interact with toxic molecules, thereby reducing the oxidative damage to biomolecules in both aqueous and lipid environments of the cell. Melatonin also acts as an indirect antioxidant through activation of major antioxidant enzymes including superoxide dismutase, catalase and glutathione peroxidase [22-25].

Figure

An antioxidative protection by melatonin against oxidative damage has been reported against acetaminophen [26], carbon tetrachloride [27], methotrexate [28], streptozotocin [29] and gentamicin [30]. Cytoprotective effects of melatonin against necrosis and apoptosis induced by ischemia/reperfusion (I/R) injury in rat liver have also been studied [31]. The serum aminotransferase activity and lipid peroxidation levels were increased markedly by hepatic I/R which were suppressed significantly by melatonin. They hypothesized that the activation of caspase-3, caused apoptosis in I/R rats. The melatonin treated rats showed a few apoptotic cells and DNA fragmentation than did the I/R rats.

This information clearly demonstrates that melatonin expresses protective effects against several xenobiotics. Present review summarizes its protective effects observed against metal toxicity viz; lead, cadmium, mercury, chromium, arsenic, copper and aluminium etc.

Lead (Pb)

Lead is a ubiquitous element. Human exposure to lead occurs through several sources including air, water and food. Industrial and workplace exposure to lead are also known. Lead inhibits heme biosynthesis. Prophylactic effects of melatonin on these mechanisms were studied by El-Missiry [32]). It is one of the earliest studies made on the protective effects of melatonin against lead toxicity. The study showed that pretreatment with melatonin (30 mg/kg body weight) intra-gastrically prevented the suppressive effects of lead on heme synthesizing and antioxidants enzymes. Lipid- peroxidation was also normalized. Another study [33] reported protective effects of melatonin on immune toxicity of lead. They showed that within the lead and melatonin treated group, the relative thymus weights were significantly increased when compared with lead treatment. Hemagglutination (HA) tire, plaque-forming cell response to sheep red blood cell (SRBC) and secondary IgG antibody response to BSA were significantly enhanced in the lead and melatonin treated mice in comparison to the lead treatment alone. Splenic CD4(+) cells were significantly increased by MLT treatment when compared with lead treatment alone. Similarly splenic T and B cells were also increased by MLT treatment.

Lead is an established neurotoxin [34]. Several studies attribute its neurotoxicity to oxidative stress [35,36]. El-sokkary., *et al.* [37] showed that melatonin (100 mg/kg) given together with lead acetate (100 mg/kg) for 21 consecutive days prevented lipid peroxidation and restored levels and SOD activity. They concluded that melatonin may be useful in combating free radical induced neurotoxicity caused by lead. Melatonin was found to reduce oxidative stress induced by maternal lead exposure in rats [38]. Melatonin mediated therapeutic aspects were also reviewed [39]. By the time larger interest developed in the antioxidant properties of melatonin. Flora., *et al.* [40] worked on the therapeutic efficacy of melatonin. They showed that MLT provided significant protection to lead induced disturbed antioxidant defense. They suggested that combined therapy with an antioxidant moiety and a thiol chelating agent n-acetyl cysteine (NAC) might be a better choice for treating plumbism. The effects of melatonin on lead induced hematotoxicity were examined using rat blood and bone marrow [41]. The changes recorded in peripheral blood parameters and in bone marrow poly chromatic erythroid, lymphocytes and neutrophils were significantly ameliorated by melatonin. Another study investigated the potential protective effect of melatonin against the hepatic and renal toxicity of lead in male rats [42]. They studied levels of lipid peroxidation (LPO) products, superoxide dismutase-(SOD) activity, total glutathione (GSH) and histopathological changes in the liver and kidney. Melatonin treatment attenuated increase in LPO and restored the activity of SOD and levels of GSH. Morphological damage to liver and kidney was also reduced.

It was suggested that melatonin may be useful in combating free radical induced damage caused by lead toxicity. It has been reported that lead poisoning is characterized by the accumulation of δ-aminolevulinic (ALA) together with its increased urinary excretion. ALA is also able to cause DNA damage. Onuki., *et al.* [43] showed that melatonin treatment was able to inhibit DNA damage. Suresh., *et al.* [44] studied the protective effects of melatonin during exposures to low levels of Pb in human SH - SYSY neuroblastoma cell cultures. Lead decreased levels of glutathione (GSH) in a concentration dependent manner. Exposure of cells to Pb for 48 hr resulted in an eightfold increase in caspase - 3 activity and prostaglandin E - 2 level. Pretreatment with melatonin (10 mM) blocked the effects of Pbon GSH and caspase - 3 activity and reduced the level of PGE_2. The study suggested neuroprotective effect of melatonin in Pb induced neuroblastoma cell culture. Melatonin was reported to reduce lead induced genotoxicity [45]. N-acetylcysteine and melatonin were able to reduce significantly ($p < 0.05$) the lead and ALA induced sister chromatid exchange frequencies in human lymphocytes *in vitro.* Lead has been found to change the behavior and learning abilities. A study made in China showed that melatonin administration for a prolonged period to the lead exposed rats exacerbated LTP impairment, learning and memory defect induced by lead [46].

There has been a surge during recent years to study the protective effects of melatonin on lead toxicity. Martinez., *et al.* [47] made an interesting observation that subacute intraperitoneal administration of long 10 mg/Kg/day of lead for 15 days induced toxic levels of lead in the blood and caused renal toxicity. Melatonin co-treatment decreased lead induced oxidative stress and toxic effects on kidneys without altering the lead induced reduction in renal nitric oxides. Another study suggested that melatonin directly affects lead levels in organisms exposed to subacute lead intoxication [48]. Electronic density functional calculations showed that a lead/melatonin complex is energetically feasible. Further melatonin co-treatment increased the MT_2 mRNA expression. However, potential effects of MT_2 on tissue distribution and excretion of lead could not be established. In nut shell, melatonin prevents adverse effects of lead on immune system, nervous system and antioxidative enzymes. Further, it prevents from lead induced genotoxicity and plumbism.

Cadmium (Cd)

Industrial exposure is the most prevalent cause of cadmium toxicity. It has been implicated as a possible cause of lung cancer and kidney dysfunction. It has also been suggested that Cd may play a role in the pathogenesis of hypertension and cardiovascular diseases. Cd is also treated as a direct enzyme poison. Endocrine regulation of Cd toxicity has not attracted many studies, however, available literature shows that it affects several hormones [8].

It was Kim and coworkers [49] who reported that melatonin ameliorate Cd induced hepato-toxicity. Cd (1 mg/kg) with melatonin (10 mg/kg b.w., ip) was administered to SD rats daily for 15 days. Hepatic GSH concentration decreased by Cd alone was restored by melatonin treatment. Further, Cd induced histopathological changes were also reversed. In following years, significant interest emerged in studying the protective effects of melatonin on Cd toxicity. A report from National Institute of Toxicological Research [50] suggested that immunotoxicity induced by Cd was prevented by melatonin. Thymus, spleen and liver weight were restored to normal values. Further, hemagglutinatin (HA) titre, primarily IGM antibody response to SRBC and secondary IgG antibody response to BSA was significantly increased in Cd plus melatonin treated mice. A study made in hamsters [51] examined the protective effects of melatonin against CD induced lipid

peroxidation in hamster brain, heart, kidney, testes, lung and liver. They concluded that LPO induced by Cd in these organs is reduced by administering melatonin. An interesting study was made in a photosensitive bank vole (*Clethrionomys glareolus*) in Poland [52]. These workers reported that melatonin co-treatment brought about a significant increase in the hepatic (61%), renal (79%) and intestinal (77%) Cd concentration as compared to those treated with cadmium alone. This data showed that (i) subchronic melatonin injection has similar effect on tissue accumulation and toxicity of cadmium to that produced by a short photoperiod, (ii) melatonin decreases synthesis of melatonin. A comparative study in the protective effects of curcumin, resveratrol and melatonin against Cd induced oxidative damage in mice was made by Eybl., *et al* [53]. Their results demonstrated that curcumin, resveratrol and melatonin pretreatments effectively protect against Cd induced LPO and ameliorate the adverse effects of Cd on antioxidant status without any reduction in tissue Cd burden. Gene expression studies [54] confirmed protective effects of melatonin on Cd induced changes on redox balance in rat hypothalamus and anterior pituitary. Chwelatiuk and coworkers [55] showed that co-administration of melatonin and cadmium reduces Cd accumulation in liver and kidney. Hepatic and renal metallothionein levels followed the pattern of Cd accumulation. There are reports showing protective effects of melatonin when used with other antioxidants.

Konar., *et al.* [56] studied the effects of selenium and vitamin E together with melatonin against oxidative stress caused by cadmium in rats. Metallothioneins (MTs) are intracellular proteins that protect against Cd toxicity. Effect of melatonin on metallothionein expression in three lines of human tumor cells (MCF-7, MDA-MB 231 and He La cells) were also studied [57]. Their observation on several MT isoforms (MT-2A, MT 1X, MT -IF and MT-1 E) showed that melatonin increases Cd induced expression of MT-2A which is considered to protect against Cd toxicity. These results show that melatonin has oncostatic properties. Most of the workers have focused their studies on antioxidative effects of melatonin. Kara., *et al.* [58] studied the effects of selenium together with vitamin E on cadmium induced oxidative damage in rat liver and kidneys. A similar study was made in the liver of rat [59]. In addition to MDA, GSH and SOD, they investigated morphological changes using both light and electron microscopical methods. Melatonin restored histopathological changes caused by cadmium viz: cytoplasmic vacuolization, necrosis, destructed cristae of mitochondria, severe glycogen depletion and accumulation of collagen fibers. In a novel study [60], it was shown that cadmium disrupted the circadian expression of clock and redox enzyme genes in rat medial basal hypothalamus (MBH). This disruption in genes was prevented by melatonin. Another study [61] showed that melatonin modulated Cd- induced changes in biogenic amines in rat hypothalamus. Norepinephrine (NE), dopamine (DA), serotonin (%-HT), 3-4-dihydroxyphenyl acetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) were quantified by HPLC. It was shown that anterior hypothalamus was not sensitive to exogenously administered melatonin, whereas melatonin decreased the content of other amines in the mediobasal hypothalamus. The authors concluded that neurotoxic effects of cadmium can be prevented by melatonin. Ji., *et al.* [62] investigated the effects of melatonin on Cd evoked germ cell apoptosis in testes of mice using TUNEL assay. They showed that unfolded protein response (UPR) pathway is activated by Cd. Melatonin completely inhibited Cd induced ER stress and UPR in testes. In addition, melatonin attenuated Cd induced hemeoxygenase (HO)-1 expression and protein nitration in testes. Overall results suggest that melatonin alleviates Cd-induced cellular stress and germ cell apoptosis in testes. Recent investigations made in China observed that all Cd induced mitochondrial oxidative injuries were efficiently attenuated by melatonin pretreatment [63]. They showed that sirtulin-1 (SIRT-1) plays an essential role in the ability of melatonin to stimulate PGC-1 alpha (a key enzyme in mitochondrial biogenesis) and improve mitochondrial biogenesis and function through melatonin receptors. Another report from China by Pi., *et al.* [64] suggested that melatonin exerts a hepatoprotective effect on mitochondrial derived superoxide anion stimulated autophagic cell death that is dependent on SIRT-3/SOD 2 pathway.

Mercury (Hg)

Mercury is a cumulative poison. Elemental mercury is used in thermometers, barometers**,** diffusion pumps, mercury vapor lamps, electrical switches, dental fillings, paints, batteries, catalysts and the manufacture of chlorine. Mercury salts are used as medicine, paint pigments, explosive detonators and in the manufacture of paper. Organic mercury compounds are used as fungicides for seed treatment and in the manufacture of certain types of plastic. Target organs for its toxicity include liver, kidney and nervous system.

Earliest reports on the protective effects of melatonin on methylmercury induced mortality in mice are from Kim and coworkers [65] who reported 100% survival rate in treated group in comparison to 60% survival rate in MMc intoxicated mice. They hypothesized that this effect might be due to antioxidative effect of melatonin. Sener [66] compared the protective effects of melatonin and N-acetyl cysteine

against mercury induced oxidative damage in liver, kidney, lung and brain. They concluded that melatonin protects against mercury (II) induced renal tubular damage and mitochondrial morphometry in the kidney of mercurial chloride treated rats were studied [67]. These workers showed reduction in the tubular expression of stress proteins and nitric oxide synthase (iNOS). These markers exhibited significant recovery caused by melatonin against mercury toxicity. An *in vitro* study made on rat epididymal sperm also exhibited antioxidative potential of melatonin against mercury induced intoxication [68]. They assayed superoxide dismutase, glutathione peroxidase, glutathione reductase, TBARS and H_2O_2 and found that co-incubation of sperms with mercury and melatonin significantly inhibited oxidative damage in sperms caused by mercury. The same laboratory studied antioxidative effects of melatonin on thyrotoxicity of mercury in rats electing antioxidant enzymes as denominators of toxicity. They registered a protective effect against endocrine toxicity of mercury [69]. In subsequent years, protective effects of melatonin on mercury induced myocardial and genetic toxicity were studied [70]. Jindal., *et al.* [71] exposed Wistar rats to methyl mercury (0.5 mg/kg/day), mercuric chloride (3.7 uM/L) through drinking water. This treatment was followed by subacute treatment with melatonin (4 ug/ml/day). After one month, ventricular and diastolic pressure and lipid peroxide, GSH and SOD were determined. The results of this study indicated that altered basoreflex mechanisms caused by mercury led to impairment of cardiovascular functions. Antioxidant defense caused by melatonin led to improved myocardial function. Purohit and Rao [72] and Patel and Rao [73] reported mitigative effects of melatonin, α-tocopherol and curcumin on mercury induced genotoxicity. They examined human lymphocytes for cell type proliferative index (CCPI), proliferation replicative index (PRI) and sister chromatid exchange (SCE). They demonstrated that combined effects of melatonin and α-tocopherol expressed better protection than melatonin alone. The antimutagenic activity of these compounds on mercury induced genotoxicity was in order: melatonin>curcumin> andrographolide.

Arsenic (As)

Arsenic is a poison of kings and king of poisons. WHO has treated it as a global environmental health problem. Several countries including India, Bangladesh, Taiwan, Chile, Hungary, Argentina, and USA have been identified as hot spots of arsenic poisoning. Arsenic poisoning is characterized by perforation of the nasal septum, changes in skin pigmentation, keratosis and peripheral neuritis. Epidemiological evidence suggests excessive risk of lung cancer amongst workers exposed to arsenic.

Chelating agents like BAL have been used to treat dermatitis [74]. Several antioxidants i.e. ascorbic acid, GSH, selenium have also been used to treat arseniasis. However, endocrine modulation of arsenic toxicity is poorly known.

Influences of thyroid hormones were studied by Allen and Rana [75,76]. These experiments concluded that arsenic toxicity can be modulated by thyroid hormones.

A few investigators have studied the protective effects of melatonin on arsenic toxicity. Pal and Chatterjee [77] based on their observations on lipid peroxidation, GSH, free hydroxyl radical production; glutathione reductase and catalase showed that melatonin supplementation (10 mg/kg body weight/ip) reversed arsenic mediated changes. It was suggested that melatonin acts as a protective agent against arsenic induced cellular oxidative stress. *In vitro* studies on anti-genotoxic potential of melatonin in arsenic treated human blood were also made [78]. The frequency of SCE/cell, SCE/chromosome and primary DNA damage reduced significantly ($p < 0.001$) with a marked increase in CCPI upon addition of melatonin. Another study showed that melatonin ameliorated testicular injury mediated by arsenic [79]. The number of apoptotic germ cells was increased while the number of proliferating nuclear cell antigen (PCNA) positive germ cell was decreased in testes after arsenic administration. Melatonin treatment (25 mg/kg/day intraperitoneally) counteracted these defect. Further increased malondialdehyde, decreased activity of superoxide dismutase, catalase and glutathione peroxidase were also restored. They suggested that melatonin plays a protective role against arsenic induced testicular apoptosis. A recent report showed that melatonin protects against arsenic induced neurotoxicity [80]. It inhibits arsenic induced autophagy and autolysosome formation. It also ameliorated the arsenic induced reduction in growth associated protein 43 and discontinuous neuritis of rat primary cultured neurons. Melatonin also prevented As induced decreases in cytochrome-c oxidase levels. Overall results showed that melatonin exerted a neuroprotective effect by autophagy and enhancing mitochondrial biogenesis.

Other toxic elements

Although several laboratories concentrated on the protective effects of melatonin on the toxicity of lead, cadmium, mercury and arsenic, very few studies have been made on other environmentally significant metals viz: chromium, copper, manganese, aluminium etc. Available information is summarized in following paragraphs.

Chromium (Cr)

It is an established carcinogen [1]. Several attempts have been made to modulate its toxicity using antioxidants, chelating agents and other therapeutic agents. However, a study made in Japan by Susa., *et al.* [81] showed that incubation of primary cultures of rat hepatocytes with $K_2Cr_2O_7$ and melatonin resulted in a significant decrease in cellular levels of DNA single strand breaks caused by $K_2Cr_2O_7$. They attributed these changes to an increase in cellular levels of vitamin E and C as well as catalase activity and/or to the direct scavenging of toxic hydroxyl radicals in cells. In another study, the ability of Cr(III) to reduce oxidative DNA damage was examined by the formation of 8-hydroxyguanosine (8-OHdG) in calf thymus DNA [82]. Melatonin, ascorbate and vitamin E significantly inhibited the formation of 8-OHdG in a concentration dependent manner. The results showed that melatonin was 60 and 70 fold more effective than ascorbate or vitamin E in reducing oxidative DNA damage in their *in vitro* model.

Copper (Cu)

A few studies are available on melatonin interaction with copper. Copper is an essential trace element. However, it has also been implicated in various neurodegenerative disorders such as Wilsons' and Alzheimer's diseases. It was shown that melatonin protected against copper mediated lipid peroxidation in liver homogenates [83]. Further, melatonin's protection against free radical damage provided evidence for neuroprotective role of melatonin. A recent study [84] has put forward a new hypothesis. They have suggested that metabolites of melatonin i.e. cyclic 3-hydroxymelatonin (3 OHM), N(1)-acetyl-N(2)-formyl-5 methoxykynuramine (AFMK) and N(91)-acetyl-5-methoxykynuramine (AMK) are capable of chelating copper ions and form stable complexes. Two different mechanisms were suggested i.e. the direct chelation mechanism (DCM) and coupled deprotonation chelation mechanisms (CDCM). It was proposed that under physiological conditions, CDCM might be the main chelation route for Cu(II). Melatonin, AFMK and 3OHM prevented first step of the Haber-Weiss reaction consequently turning off the OH production via the Fenton reaction. 3OHM was identified as most efficient chelating agent amongst three.

Aluminium (Al)

Melatonin interaction with sodium, potassium, calcium, lithium and aluminium were studied by Lack., *et al* [85]. An electrochemical technique called adsorptive cathode stripping voltammetry (AdCSV) was employed to study metal chelation interaction. The trend of metal melatonin interaction was K+> Li+>Na+>Al3+. Aluminium and melatonin interaction was considered important in the aetiology of Alzheimer's disease. Another study showed protective effects of melatonin against neurotoxicity of Al [86]. Since kidneys are target organs for aluminium accumulation and toxicity, role of melatonin against aluminium induced renal toxicity was also studied [87]. These workers showed protective effects of melatonin on Al induced oxidative stress in the kidney of Wistar rats. Gene expression studies on Cu-Zn SOD, Mn-SOD, GPX and catalase by RT-PCR were also made [88]. The results showed that Al exposure promotes oxidative stress in rat hippocampus with an increase in Al concentration. They reported that Al acts as a pro-oxidant while melatonin exerts antioxidant action by increasing mRNA levels of the antioxidant enzymes.

Melatonin was found to exhibit pleiotropic, anti-inflammatory, antioxidant, anti-lipidic, therapeutic effects with regard to the control and prevention of aluminium intoxication.

Molecular pharmacology of melatonin

Above discussion leads to a conclusion that melatonin is an ubiquitous, pleiotropic molecule that exters efficient protection against oxidative/nitrosative damage by a variety of mechanisms. Most important is its chelating property that significantly contributes in reducing metal toxicity. Further, interaction of a few drugs viz: diazepam, tamoxifen and acetaminophen has also been studied [89] using radioimmunoassay techniques. These drugs do not impair the metabolic conversion of melatonin to 6-sulphatoxymelatonin. Still there are questions that need to be addressed. Is there any application of melatonin in cancer treatment? Melatonin has been established as direct free radical scavenger and an indirect antioxidant since it stimulates antioxidant enzymes and suppresses pro-oxidant enzymes (Figure 1). Further, there are reports implicating anti-apoptotic function of melatonin in normal cells. Nonetheless, melatonin has been found to

protect against the toxicity of carcinogenic compounds viz: benzene [90]. Ameliorating effects of melatonin on carbon tetrachloride [91], alcohol [92], endosulfan [93], ferric nitroacetate [94] toxicity have also been reported. All these reports focus mainly on its antioxidative properties.

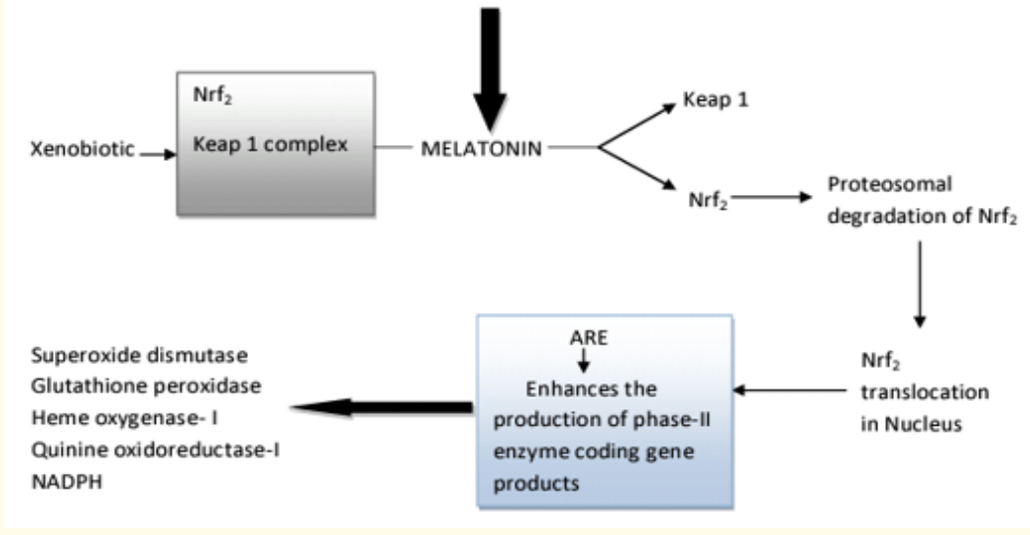

Figure 1: *Antioxidative protection by melatonin. In the first step melatonin dissociates Nrf2 from Keap 1 repressor. Nrf2 is translocated into the nucleus. It upregulates the antioxidant response element and increases the production of Phase II enzymes. (Adopted from Tuli., et al. 2015).*

Melatonin and metallothionein interaction does form an area of further investigations. However, only a few reports discuss this issue. Bovine pineal gland and retina continually synthesize metallothionein and other low molecular weight zinc binding proteins [95,96]. The presence of metallothionein I-II expressing system in the pineal gland is plausibly related to the antiaging effects of melatonin.

Melatonin and apoptosis

Melatonin is known to mediate both extrinsic and intrinsic pathways of apoptotic cell death. RAMOS-1 human leukaemic cells when treated with MLT resulted in the release of mitochondrial cytochrome-C followed by down regulation of Bcl-2 gene product which indicated the activation of apoptotic pathways [97]. In addition to the induction of apoptosis, MLT was found to arrest cell cycle by modulating the expression of cell cycle regulatory cyclins and cyclin dependent kinases (CDKs) [98]. More recently Wei., *et al.* [99] studied the dose dependent apoptotic effect of MLT in colorectal (LoVo) cancer cells. They reported that MLT not only stimulates dephosphorylation and nuclear import of histone deacetylase 4 (HDAC 4) but also decreases H3 acetylation, which resulted in the down regulation of Bcl-2 expression.

Melatonin and metastasis

In 7th colloquium of the European Pineal Society, several reports were presented about anti-metastatic effects of MLT. Qin [100] demonstrated the up-regulatory effects of MLT on tissue inhibitors of metalloproteinases (TIMP1 and TIMP2) via NF-kB translocation. Further, MLT can directly inactivate MMP activity by interacting with its catalytic site. Similarly, NFkB mediated anti-metastatic effect of MLT was studied in HepG2 cells via down regulation of TIMP1 and MMP-9 [101].

Melatonin and inflammation

Melatonin is known to have anti-inflammatory action in many neurological diseases including Parkinson, multiple sclerosis, strokes and brain ischemia/reperfusion. The NFkB pathway is considered as one of the important inflammatory mediator's pathways which have been found to be inhibited in the presence of MLT and its metabolites. In immunocompetent cells, MLT was found to modulate immune system by upregulating the production of certain cytokines. Experimental and clinical data revealed that MLT reduced the release of adhesion molecules and proinflammatory cytokines (IL-6, IL-8 and TNF) [102].

Organ protective functions

MLT has significant effect on apoptotic and necrotic cell death. MLT prevented the inhibition of caspase-3 [103]. It prevents the loss of mitochondrial membrane potential resulting in the inhibition of cytochrome- C release further inhibiting mitochondrial mediated cell death. A study reported by Molpeceres., *et al.* [104] suggested that MLT prevents ROS mediated anti-ageing effects by decreasing the cytoplasmic cytochrome-C concentration and modulating the Bcl-2/Bax ratio. Besides, MLT is known to possess a variety of other therapeutic effects (Figure 2).

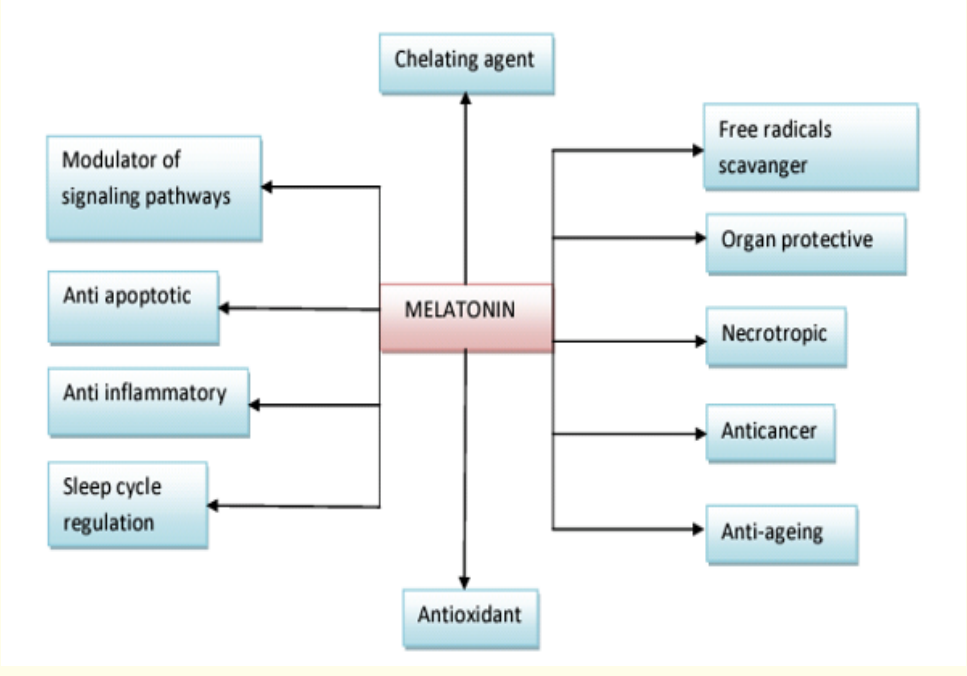

Figure 2: *Pleiotropic-function of melatonin.*

Melatonin as an antioxidant

The most powerful property of melatonin is its antioxidant action. It plays a major role in recycling the enzymes needed for glutathione synthesis [105,106]. It increases the efficiency of electron transport chain in the inner mitochondrial membrane [107]. Studies cited above have aptly shown that MLT attenuates the generation of free radicals by several heavy metals. It should stabilize the microsomal membrane to prevent oxidative damage. It inhibits the formation of 8-hydroxy-2- deoxyguanosine and thus prevents DNA damage. The expressions of mitochondrial free radical scavenging gene products were found to be increased by MLT [25]. Protective effects of MLT against various diseases related to oxidative stress such as rheumatoid arthritis, hypertension, type-II diabetes and infertility in females have also been reported.

Conclusion

The above discussion enumerates several *in vitro* and *in vivo* studies showing that MLT plays an important role in detoxification of xenobiotic and endobiotic compounds through pleiotropic mechanisms. The structure of MLT shows that it has two N and two O atoms. Therefore, it can make di-, tri-, and tetra-dentate ligands with transition metals enhancing its bioactivity. Quantitative structure -activity relationship (QSAR), might be helpful in delineating unknown targets of MLT. The use of advance drug delivery technology and sophisticated tools of nanomedicine may further find its therapeutic role not only against heavy metal toxicity but many other dreadful diseases including rheumatoid arthritis, hypertension, diabetes and cancer.

Conflict of Interest

The author has no conflict of interest.

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