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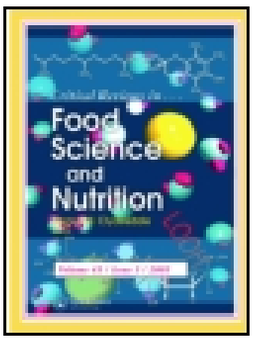

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Anti-inflammatory effects of Melatonin: a mechanistic review

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Abstract

N-acetyl-5-methoxy-tryptamine (melatonin) is a natural substance produced both by plants, as a secondary metabolite, and animals, by the pineal gland and other tissues. In humans, melatonin participates in numerous functions including the regulation of mood, sleep, reproduction, promotion of immunomodulation, antioxidant defense and as an anti-inflammatory agent. The anti-inflammatory activity of melatonin could yield

beneficial effects on intake, particularly against the chronic inflammation which underlies many chronic diseases. This review aims to provide an assessment of the literature data on the anti-inflammatory activity of melatonin, with a particular focus on the mechanisms responsible for this behavior. We can conclude that many *in vitro* studies and *in vivo* studies in experimental animal model systems show that melatonin exerts anti-inflammatory activity in a number of chronic diseases which affect different organs in different circumstances. Clinical trials, however, often fail to reach positive results and are thus far inconclusive. Thus, in the future, long-term well-designed investigations on melatonin-rich foods or melatonin food supplements could provide valuable information towards public health recommendations on melatonin, taking into account both the nature of the compound and the optimal dose, for protection from long-term inflammation linked to chronic diseases.

Keywords: chronic inflammation; pro-inflammatory markers; oxidative stress; nuclear factor kappa-B.

1. Introduction

Inflammation is a complex physiological event which can be subdivided into 1) acute inflammation, which is essential for tissue healing and is triggered by the immune system in response to biotic (bacterial, viral, and parasitic infections) and abiotic (radiation exposure, very high or low temperatures, or environmental pollutants) stresses, and 2) chronic inflammation (long-term inflammation), which is often linked with noncommunicable chronic diseases such as cardiovascular, neurodegenerative,

pulmonary, gastrointestinal and metabolic diseases, and certain types of cancer (Maione et al. 2016; He et al. 2015).

When inflammation becomes chronic, it causes pathological alterations of the signaling pathways (especially nuclear factor kappa-B (NF-κB) and signal transducer and activator of transcription 3 (STAT3)), which, in turn, induce an increase in oxidative stress resulting in the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The activation of NF-κB can be induced by both microbial products and pro-inflammatory cytokines, leading to the activation of RelA (p65)- or cRel (p50/p105)- containing complexes, and by lymphotoxin β, CD40 ligands, B cell activating factor, and receptor activator of NF-κB ligands, resulting in RelB/p52 complex activation. NF-κB pathway activation induces the transcription of genes involved in immunoregulation, inflammation, carcinogenesis and apoptosis, in immune cells (i.e macrophages, B cells, T cells, dendritic cells, and neutrophils) (Ruan and Chen 2012; Grivennikov and Karin 2010).

In addition to NF-κB, STAT3, a transcription factor belonging to the STAT protein family and encoded by the STAT3 gene, plays a crucial role in inflammation, being activated by many cytokines and growth factors. Similarly to NF-κB, the activation of STAT3 through its phosphorylation by receptor and nonreceptor protein tyrosine kinases at the tyrosine residue at 705, leads to its translocation into the nucleus and subsequent DNA binding, which results in the transcription of genes regulating inflammation, cell proliferation, and apoptosis, with the eventual production of pro-inflammatory cytokines and chemokines (Ahmad et al. 2017; Aggarwal et al. 2009; Amani et al. 2017).

A large body of evidence has highlighted a close association between chronic diseases and chronic inflammation, especially in cardiovascular and neurodegenerative pathologies, due to deleterious effects on cells in the brain, heart and arterial walls (Chung et al. 2017; Heneka et al. 2015; Bang 2014). Moreover, a growing body of evidence suggests that long-term inflammation is linked with the development of cancer and autoimmune diseases (Payne 2014). In fact, increases in pro-inflammatory biomarkers such as cytokines have been found in several forms of cancer, as well as in multiple sclerosis and rheumatoid arthritis (Holmdahl, Malmström, and Burkhardt 2014; Lalive et al. 2017; Axtell et al. 2010).

Diets rich in plant foods such as vegetables, fruits, legumes, and spices have been shown to delay the onset and development of chronic diseases through the suppression of chronic inflammation (Wu and Schauss 2012; Prasad, Sung, and Aggarwal 2012). The compounds responsible for the anti-inflammatory activity of certain foods belong to chemically diverse classes such as polyphenols, indigestible carbohydrates (fiber), or omega-3 fatty acids. Another plant-derived compound which has been found to possess anti-inflammatory activity is N-acetyl-5-methoxy-tryptamine (melatonin), a secondary metabolite found in various plants (Table 1) (Fernández-Mar et al. 2012; Garde-Cerdán et al. 2015). The role of melatonin in plant physiology has been reported by several authors (Ahmed 2000; Reiter et al. 2009; Hernández-Ruiz, Cano, and Arnao 2005; Arnao and Hernández-Ruiz 2015). Melatonin (Fig. 1) acts as an anti-stress agent against abiotic stressors (salinity, drought, acute temperature changes, intensive UV radiation, and toxic chemical agents) and biotic

stresses (microbial infections) (Arnao and Hernández-Ruiz 2015; Cooper et al. 2006). In addition, melatonin is involved in plant growth and photosynthesis, and has been applied to seeds as a biostimulator with the aim of improving seed germination, seedling/plant growth and crop production, especially under stress conditions (Janas and Posmyk 2013).

In 1958, Lerner et al. discovered melatonin in the pineal gland of cows (Lerner et al. 1958). Since then, many investigations have shown that melatonin is produced both there and in other extra-pineal sites such as the retina, the gut (Peuhkuri, Sihvola, and Korpela 2012), gastrointestinal tract, airway epithelium, pancreas, adrenal gland, thyroid gland, thymus, urogenital tract and placenta (Kvetnoy 1999; Wallasch 2014).

Melatonin is involved in the regulation of mood, regulates the sleep and wake cycles, and reproduction (Brzezinski et al. 1997). Exposure to light decreases its secretion, reducing the quality of sleep. Moreover, it is recognized as a scavenger of a number of reactive oxygen and reactive nitrogen species both *in vitro* and *in vivo* (Reiter et al. 2001). Additionally, Reiter *et al.* (2003) reported other antioxidant functions, including the stimulation of antioxidative enzymes, increasing the efficiency of mitochondrial oxidative phosphorylation and reducing electron leakage and synergistic effects with other antioxidants (Reiter et al. 2003). In newborn hypoxic-ischemic brain injury, melatonin has shown strong protective effects via the modulation of the MT1 receptor (Sinha et al. 2018). Additionally, it exhibited protective effects in fatty liver (Zhou et al. 2018). Many of its reported beneficial properties have been linked with its antioxidant effects, such as its inflammatory properties, rhythmicity, promotion of

immunomodulation and cytoprotective properties (Janas and Posmyk 2013; Bonnefont-Rousselot and Collin 2010; Zhang et al. 2014; Habtemariam 2017).

This paper aims to review the available literature regarding the anti-inflammatory activity of melatonin and its mechanisms of action. In addition, we provide a brief summary of the melatonin content of food, and its biosynthesis and bioavailability in mammals.

1.1 Melatonin food content

Several parts of plants are considered to be good sources of melatonin, including seeds, roots, leaves and fruits. Table 2 reports the concentrations of melatonin found in different food matrices. Variations in melatonin levels depend on the variety and cultivar of the plant, and are affected by different climatic conditions, horticultural practices, ripeness, processing and storage (Janas and Posmyk 2013; Chen et al. 2014; Feng et al. 2014). Stürtz *et al.* (2011) have reported considerable differences in the melatonin concentrations of different varieties and harvests of tomatoes and strawberries (Table 2) (Stürtz et al. 2011). In a study carried out by Riga *et al.* (2014), the melatonin content of different tomato and red pepper cultivars was determined against shade conditions (Riga et al. 2014). Melatonin content was found to increase (up to 135%) in tomatoes and to decrease in red pepper cultivars (to 64%) under shadier growth environments.

Melatonin can be found in foods which are highly consumed by humans, such as edible seeds (rice and sweet corn), however, these foods are not considered to be primary sources of melatonin, due to its instability under cooking methods, which may destroy the compound (Garcia-Parrilla, Cantos, and Troncoso 2009). Melatonin has also been found in fruit such as strawberries, kiwis, apples, pineapples and bananas (Table 2). While it is found in lower concentrations in these fruits than in edible seeds, the fact that these are generally consumed raw without thermal treatment, as well as their antioxidant content (i.e. ascorbic acid and carotenoids), may protect melatonin from degradation. Consequently, these fruits are a good source of melatonin, able to contribute positively to its intake as part of a normal diet (Garcia-Parrilla, Cantos, and Troncoso 2009). Melatonin has also been found in varying amounts in grapes and wine. The use of *Saccharomyces cerevesiae* for melatonin production has been revealed as a crucial part of the winemaking process (Fernández-Mar et al. 2012; Rodriguez-Naranjo et al. 2011a; Rodriguez-Naranjo et al. 2011b). Red wines present higher melatonin content than white wines (Iriti, Rossoni, and Faoro 2006). A possible explanation may be that melatonin occurs in the skin of red grapes, and is extracted during the winemaking process[41]. Several factors have been pointed out to influence the melatonin content of wine including environmental factors (climatic and edaphic conditions), cultivar, agricultural practices, and the winemaking process (Iriti, Varoni, and Vitalini 2010; Iriti and Varoni 2016). While a wide range of melatonin concentrations have been found in different studies, Iriti and Varoni made a survey of

published data of melatonin content in grapes and grape wine, and found that, in general, melatonin was found at a concentration near 1 ng/g in wine grape skin and 0.5 ng/mL in wine itself (Iriti and Varoni 2016; Mena et al. 2012). Rodriguez-Naranjo *et al.* (2011) reported the absence of melatonin in grapes and musts and concluded that melatonin was synthetised by yeast during alcoholic fermentation in wines. Also, Mena et al. (2012) observed that melatonin was absent in pomegranate juice but present in corresponding wines (0.54-5.50 ng/mL) (Mena et al. 2012). Melatonin has also been found in beer, where its concentration increases with the alcohol by volume, most likely due to the solubility of melatonin in alcohol (Iriti, Varoni, and Vitalini 2010; Maldonado, Moreno, and Calvo 2009; Maldonado, Reiter, and Pérez-San-Gregorio 2009). While this melatonin may be produced from tryptophan by *S. cerevisiae*, barley used in the brewing process would provide another potential source (Table 2). Melatonin was also found in olive oil. In fact, De la Puerta et al. (2007) reported higher levels in extra virgin olive oil than in refined olive or sunflower oil samples (De la Puerta et al. 2007).

Due to an increasing interest in finding new dietary sources of melatonin, new analytical methods and tools are being developed aiming at simply quantifying this indoleamine in complex food matrices. Garcia-Parrilla *et al.* (2009) and Stege *et al.* (2010) have enumerated the following difficulties in the analysis of melatonin in foods: 1) melatonin in foods can be found at a wide range of concentrations (from μg/g to pg/g), 2) it is difficult to select the best solvent for analysis due to its amphipatic properties, 3) melatonin requires careful handling due to its unstable nature, as it easily reacts with other food components due to its antioxidant properties (Garcia-

Parrilla, Cantos, and Troncoso 2009; Stege et al. 2010). Moreover, it should ideally be protected from light to avoid oxidation, and a fast extraction procedure is thus advisable (Stege et al. 2010; Cutando et al. 2007).

Extraction methods and analytical techniques have also been held partially responsible for the different quantites of melatonin measured from the same food matrices (Janas and Posmyk 2013). Table 3 reports several of the most common methods used to determine melatonin content. These methods include immunological techniques such as radioimmunoassay (RIA) (McIntyre et al. 1987) and enzyme-immunoassay (EIA) (De la Puerta et al. 2007; Iriti, Rossoni, and Faoro 2006) spectrophotometry (Iriti, Rossoni, and Faoro 2006), chemiluminescence (Lu et al., 2002), electrophoresis (Stege et al. 2010) and chromatography (High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC)). Different detectors have been coupled to HPLC to detect melatonin, including fluorescence (Vitale et al. 1996), electrochemical (Reiter, Manchester, and Tan 2005) and mass spectrometry detection (Karim, Tolbert, and Cao 2006). HPLC combines several advantages such as short analysis time, simplicity, high selectivity and sensitivity (Garcia-Parrilla, Cantos, and Troncoso 2009). Recently melatonin levels have also been analyzed using Ultra High Performance Liquid Chromatography (UHPLC) couples with diode array detection (DAD) or mass spectrometry (MS) detectors (Cerezo et al. 2016).

1.2 Biosynthesis

Melatonin is mainly synthesized and secreted by the pineal gland at night under normal light/dark conditions. It is produced from an amino acid precursor, L-tryptophan, in a cascade of several enzyme-based reactions. L-tryptophan originates 5-hydroxytryptophan, which, in turn, originates serotonin (5-hydroxytryptamine). This molecule originates N-acetylserotonin which is finally transformed to melatonin by deacetylation. N-acetyltransferase (NAT) is considered a rate-limiting factor for melatonin synthesis. Another pathway for melatonin synthesis is the O-methylation of serotonin to originate 5-metoxytryptamine and its subsequent conversion into melatonin mediated by NAT (Fernández-Mar et al. 2012; Yu and Reiter 1992).

Both synthesis and secretion of melatonin follows a circadian rhythm with maximum levels during nocturnal darkness. Environmental light also controls melatonin synthesis. In fact, this is the main environmental factor influencing melatonin levels (Peuhkuri, Sihvola, and Korpela 2012). Therefore, secretion of melatonin by the pineal gland can impart both daily and seasonal information to the organism (Reiter 1993). In addition, the influence of melatonin on food intake and behavioral rhythms (feeding and locomotor activities) was also reported in fish with varying results depending on the species (Rubio, Sanchez-Vazquez, and Madrid 2004; López-Olmeda, Madrid, and Sánchez-Vázquez 2006).

The production of melatonin varies from individual to individual and is affected by other factors such as age (it decreases with age), disrupted light/dark cycles, night work and being overweight (Peuhkuri, Sihvola, and Korpela 2012). Garfinkel *et al.* (1995) have recommended controlled-release melatonin replacement therapy to elderly people with insomnia, after concluding that melatonin deficiency may have an important role in the high frequency of insomnia among these individuals (Garfinkel et al. 1995).

Nutritional factors can also affect melatonin production to a lesser extent (Peuhkuri, Sihvola, and Korpela 2012). The influence of diet is linked to certain vitamins and minerals, which act as co-factors and activators of the melatonin synthesis cascade. Therefore, a deficiency in certain nutrients may restrict endogenous melatonin synthesis. Moreover, the bioavailability of melatonin in plant-based foods may influence its daytime levels and could also explain the health benefits of diets rich in vegetables, fruits and grain products (Peuhkuri, Sihvola, and Korpela 2012).

After its synthesis, melatonin is released into the blood and starts to circulate. Therefore, plasma concentration of melatonin reflects its pineal production. Melatonin can also be measured in saliva and urine (Benloucif et al. 2008; Nowak et al. 1987). The endogenous production of melatonin overnight has been estimated at between 10 and 80 mg, while the corresponding daytime production is significantly less (Peuhkuri, Sihvola, and Korpela 2012).

1.3 Bioavailability

In addition to the melatonin intake received as a normal part of the diet, it can be obtained from food supplements and functional foods (Janas and Posmyk 2013), where melatonin is used as a bioactive ingredient. Melatonin is metabolized very rapidly (its half-life in humans varies between 10-60 min following exogenous administration). It is mainly metabolized in the liver and excreted as part of urine. 6-sulphatoxymelatonin (6-SMT) is a urinary metabolite that reflects the concentration of melatonin in plasma and is thus used as a biomarker to evaluate its status (Peuhkuri, Sihvola, and Korpela 2012). Most of 6-SMT (50-80%) appears in samples taken overnight (Peuhkuri, Sihvola, and Korpela 2012). Di *et al.* (1997) reported a study on the bioavailability of melatonin. They concluded that this varies widely and the biological effects of oral melatonin are unlikely to be fully understood as long as its bioavailability retains its current variability (Di et al. 1997). An important point of interest is the fact that melatonin is able to cross physiological barriers and is found in all cell compartments (Iriti, Varoni, and Vitalini 2010).

In a study carried out on healthy volunteers, melatonin levels and total antioxidant levels increased 45 min after drinking beer (Maldonado, Moreno, and Calvo 2009). Another study reported that in rats fed with walnuts containing melatonin, the concentration of this indoleamine increased more than 3 times, as did serum antioxidant capacity (Reiter, Manchester, and Tan 2005). Johns and collaborators

(2013) have reported a study in which 30 healthy volunteers consumed tropical fruits with moderate melatonin content (Sae-Teaw et al. 2013). The consumption of banana, orange and pineapple increased urinary 6-SMT but no relationship was found between the melatonin content of the fruit and urinary 6-SMT. Rubio et al. (2004) carried out a study with sea bass (*Dicentrarchus labrax* L.) and concluded that orally administered melatonin affected the food consumed and the pattern of macronutrients selected (Johns et al. 2013). These findings suggest that dietary melatonin is absorbed and enters the circulation, influencing physiological processes via receptor or non-receptor-mediated processes (Feng et al. 2014).

Melatonin has also improved the sleep quality of the elderly after controlled-release melatonin replacement therapy (Garfinkel et al. 1995). In fact, the results of similar studies have led the European Food Safety Authority to accept health claims relating to melatonin alleviating feelings of jet lag, reducing of sleep onset latency, and improving sleep quality (Özer and kirmaci 2010; Keijzer et al. 2014). This dose should be between 0.5 and 5 mg. This has also increased the attractiveness of finding a method to control the dose of melatonin in food supplements. Cerezo et al. (2016) have validated an UHPLC-DAD method with reliable results. Seventeen supplements marketed in Spain and the US were analyzed and 4 showed significant deviations compared to their label (from 60% to 20%) (Cerezo et al. 2016).

2. Anti-inflammatory effects of melatonin

In 2010, Mahmood and colleagues first showed the anti-inflammatory effects of melatonin in a dose-dependent manner in a rat model of chronic inflammation, induced by formalin. The study, which lasted seven days, was conducted on 54 Sprague-Dawley rats subdivided into nine groups, these were treated with a saline solution, piroxicam (an anti-inflammatory non-steroidal drug, at a dose of 5.0 mg/kg), dexamethasone (an anti-inflammatory steroidal drug, at a dose of 1.0 mg/kg) and melatonin (at the doses of 0.25, 0.5, 1.0, 2.0, 5.0, 10.0 mg/kg), respectively. These treatments were administered intraperitoneally 30 minutes before the induction of inflammation. The results obtained showed that all treated groups registered a significant inhibition in inflammation markers (paw thickness), except for melatonin administered at 0.25 mg/kg. Melatonin at 5.0 mg/kg, induced an inhibition percentage in paw thickness comparable to that registered in the piroxicam 5.0 mg/kg group (Mahmood , Jumma, and Hussain 2010).

2.1 Nervous system

Neuroinflammation is recognized as being strictly implicated in neurological disorders (Wee 2010). Many research articles have reported the anti-inflammatory effects of melatonin, and linked these with an improvement in neurological function. The role of chronic inflammation in Multiple Sclerosis (MS) is well known; in fact, the initial pathogenesis of this disease has a strong inflammatory-demyelinating component (Brück and Stadelmann 2003). Farhadi et al. showed that in addition to increasing the

levels of pro-inflammatory cytokines, patients with MS presented a decrease in serum levels of melatonin (Farhadi, Oryan, and Nabiuni 2014). Kang JC et al. showed that supplementation with exogenous melatonin during the inflammatory-demyelinating process could be useful for the improvement of the myelin status of nerve fibers (Kang et al. 2009). The beneficial effects of melatonin in the treatment of experimental autoimmune encephalomyelitis (EAE) have been inferred using an experimental rat model of demyelinating disorder, which showcased its ability to induce the production of natural killer cells, interleukin (IL)-4, and reduce the expression of NF-kB and Intracellular Adhesion Molecule 1 (ICAM-1). ICAM-1 is a glycoprotein, expressed on endothelial cells and on cells of the immune system, which facilitates the mobility of leukocytes into the central nervous system (Anderson and Rodriguez 2011). This phenomenon represents a major event in the pathogenesis of MS, with ICAM-1 levels increasing in the early stages of inflammatory demyelinating diseases. In a study by Kang JC et al., 18 Lewis rats with EAE were divided into two groups, one treated with 5 mg/kg/day of a melatonin solution (melatonin plus ethanol 5% w/v) and one treated with 5 mg/kg/day of a placebo solution (water plus ethanol 5% w/v). At the end of the 14 day treatment period, a section of spinal cord was extracted and analyzed using immunohistochemistry. The analysis results highlight a significant inhibition in ICAM-1 production in the group treated with melatonin. This finding could explain the observed improvement in paralysis in the rat group treated with melatonin and suggests a possible mechanism for this molecule (Kang et al. 2001). In contrast with this study by Kang et al., the beneficial effect of melatonin in the treatment of inflammatory demyelinating diseases was not confirmed by Roostaei et al. (2015) in a

double-blind, randomized clinical trial. In this study, 26 patients affected by relapsing-remitting MS (RRMS) and treated once a week with interferon beta were divided into two groups. For the duration of the study (12 months), patients from group one received 3 mg/day of melatonin and patients from group two received a placebo. In this study, no beneficial effects were registered in the melatonin treated group compared to the placebo group, considering both primary and secondary outcomes represented by number of relapses, changes on the Extended Disability Status Scale, number of brain lesions, and changes in fatigue, depression and performance abilities. The authors hypothesized that these insignificant results could be due to inadequate statistical power and design of the subsequent study (Roostaei et al. 2015). Inflammatory processes, including caspase-mediated cell death, in combination with oxidative stress processes, are major factors in the pathogenesis of another neurodegenerative disease, amyotrophic lateral sclerosis (ASL). A research article published in 2006 studied the effects of high-dose enteral melatonin as a neuroprotective agent in the pathogenesis of ALS, considering both a mouse model of ALS (mutant-human copper-zinc superoxide dismutase mice – mSOD1G93A) and patients affected by ALS. In the part of this study conducted on 120 experimental animals, 60 mSOD1G93A mice were treated with 0.5 mg/mL of melatonin in drinking water, and a control of 60 mice received 0.5 mg/mL of ethanol in drinking water, each for a period of 20 weeks. It has been estimated that the mean daily melatonin uptake in the treated group was 88.3 ± 2.1 mg/kg. The treatment delayed disease progression by 25%. In the clinical trial, 31 patients affected by ALS were enrolled. Melatonin was administered rectally at a daily dose of 300 mg for a treatment period of 24 months, so

as to bypass primary metabolism of the molecules and to avoid swallowing. Results of the study conducted on humans do not show clinical efficacy of melatonin in ALS patients, but this study could be considered to be evidence of the safety of melatonin at a high dosage (Weishaupt et al. 2006). More recently, in 2013, a research article found evidence that melatonin exerts a protective effect against neuronal death, using a mSOD1G93A mouse model of ALS. Muscle strength, and coordination and motor deficits were evaluated, three times/week, in 30 mSOD1G93A mice treated with melatonin at a dose of 30 mg/kg/die (n = 15) or vehicle-control (n = 15) for a treatment period of 78 days. Subsequently the spinal cord was removed and prepared for immunohistochemical analyses. The melatonin treated group showed an improvement in motor-neuron loss and spinal-cord atrophy. Authors ascribed these protective effects of melatonin on its ability to inhibit caspase-mediated cell death. Here, as in MS, ALS mice showed a decrease in melatonin and melatonin receptor 1A in spinal cord tissue (Zhang et al. 2013). A subarachnoid hemorrhage (SAH) is a particular subtype of stroke, which usually follows an ischemic event. The immediate injury following SAH is defined as an Early Brain Injury (EBI). Numerous studies showed that inflammation is strictly implicated in the development of EBI, including the overexpression of different pro-inflammatory cytokines. Moreover, vascular endothelial growth factor (VEGF) and matrix metallopeptidases (MMPs) are involved in blood brain barrier (BBB) disruption, an event occurring prior to EBI. An interesting study showed that melatonin supplementation in a rat SAH model attenuates EBI after SAH, underlining the strong action of melatonin in the regulation of pro-inflammatory cytokines. The study was conducted on 90 rats divided into three groups, of which rats

from group one and two were subjected to SAH induction. Group one was treated intraperitoneally with a single 150 mg/kg dose of melatonin two hours after induction of SAH, while group two received 5 mL/kg of vehicle. Group three did not receive either melatonin or vehicle. Melatonin-treated rats (group one) exhibited a significant reduction in levels of IL-1β, IL-6, tumor necrosis factor alpha (TNF-α), VEGF and MMPs in tissue samples of the left basal cerebral cortex, compared with vehicle-treated rats (group two). Furthermore, before the culling of experimental animals at 24 hours after SAH induction, group one showed reduced neurological deficits (Chen et al. 2014).

Chronic inflammation, together with oxidative stress, promotes the initiation and propagation of neuropathic pain (NP). Some scientific evidence suggests that melatonin is able to improve pain in experimental animal models. El-Shenawy et al. showed that melatonin, administered both systemically (0.5 – 1.0 mg/kg) and topically (20 – 40 μg per paw) in rats, exerts anti-nociceptive and anti-inflammatory effects. Melatonin was administered 30 minutes before the induction of acute inflammation, through a sub-plantar injection of carrageenan (El-Shenawy et al. 2002). A more recent study reported the beneficial effects of melatonin in the treatment of rats affected by inflammatory pain induced by intradermal injection of complete Freund's adjuvant (CEA) in rat paw. Melatonin or vehicle were administered intraperitoneally at doses of 60 mg/kg and 50 mg/kg. The results showed a reduction in spinal cord brain-derived neurotrophic factor (BDNF) concentration in the melatonin-treated group following three days treatment, compared to the vehicle-treated group. In fact, BDNF is involved in the transmission of physiological and pathological pain (Laste et al. 2015). Unfortunately, despite the positive evidence in rats reported by Laste et al., that same

year Andresen et al. published the results of a clinical trial on the analgesic effects of a single dose of melatonin in a validated human inflammatory model. In this case, no significant differences were registered between the treated group (10 – 100 mg melatonin) and the control group (Andresen et al. 2015). A more recent study evaluated a combination of melatonin with extracorporeal shock wave (ECSW) therapy, as an enhancer of its pain attenuating effects. This treatment was originally used for calculi, but is used nowadays also used in the treatment of musculoskeletal pain. ECSW alone possesses anti-inflammatory, anti-oxidative and anti-apoptotic effects. The combined therapy using ECSW and melatonin was found to be more effective in the suppression of pro-inflammatory cytokines and oxidative markers (Chen et al. 2017).

2.2 Gastro-intestinal system

Many research articles have found that melatonin could be involved in inflammation-related gastro-intestinal diseases. A large body of evidence suggests the beneficial effects of melatonin on colitis. In rat models of colitis induced by a pre-treatment of 2,4,6,-trinitrobenzene sulfonic acid (TNBS), melatonin was found to exert positive effects by inhibiting NF-kB activation (melatonin treatment at a dose of 10 mg/kg/day for 30 days), and reducing myeloperoxidase activity (melatonin treatment at a dose of 2 mg/kg/day for 21 days, and melatonin treatment at a dose of 10 mg/kg/day for 15 days) (Marquez et al. 2006), as well as reducing malondialdehyde and caspase-3 levels (melatonin treatment at a dose of 10 mg/kg/day for 15 days) (Marquez et al. 2006), nitric oxide, colonic prostaglandin E2 (PGE2), nitric oxide synthase (NOS), and cyclooxygenase-2 (COX-2) levels (melatonin treatment at a dose of 5-10 mg/kg/day for

28 days) (Dong et al. 2003), and increasing glutathione levels. In a rat model of colitis induced by intracolonic injection of dinitrobenzene sulphonic acid, melatonin showed anti-inflammatory effects by reducing metalloproteinase-9 and -2, TNF-α, cyclooxygenase-2 and nitric oxide synthase (melatonin treatment at a dose of 15 mg/kg/day for 4 days) (Necefli et al. 2006). Similar results have been obtained from a rat model of colitis induced by acetic acid. In particular, the inhibition of NF-kB expression, and reduction in serum concentration of lipid peroxide, was achieved following a melatonin treatment at a dose of 10 mg/kg/day for 4 weeks (Sayyed et al. 2013). A confirmation of the beneficial effects of melatonin on colitis, linked to its anti-inflammatory action, was provided by a clinical trial conducted on 64 patients affected by left-sided ulcerative colitis. They received mesalazine (2 g/day) and melatonin (5mg/day) (treated group) or placebo (control group) for 1 year. The authors showed that melatonin supplementation reduced C-Reactive Protein (CRP) concentrations in blood levels and helped patients in remission (Chojnacki et al. 2011). Melatonin showed positive effects related to its anti-inflammatory effects on a rat model of pancreatitis. Carrasaco et al. demonstrated a reduction in pro-inflammatory cytokines IL-1β and TNF-α, and an increase in anti-inflammatory cytokine IL-4 levels in serum, following the administration of a single dose of 25 mg/kg melatonin in a rat model of caerulein-induced acute panceratitis (Carrasco et al. 2013). Liang et al. confirmed the positive effects of melatonin in pancreatitis, using a L-arginine-induced rat model of acute necrotizing pancreatitis. Here too, the authors observed a decrease in pro-inflammatory cytokine TNF-α in rats treated with melatonin (50 μg/kg for 3 days) (Liang et al. 2014).

A recent research article on a cadmium induced mouse model of liver injury reported hepato-protective effects from intraperitoneal supplementation of melatonin (10 mg/kg/day for 3 days). The authors observed a strong inhibition of the expression of thioredoxin-interacting protein (TXINP), induced by melatonin. This enzyme plays a key role in the pathogenesis of acute liver failure, by activating a NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome. Thus, through the down regulation of TXINP, melatonin exerts protective effects against cadmium-induced liver inflammation and hepatocyte death (Cao et al. 2017). Similarly, Liu and colleagues most recently (2018) reported melatonin ameliorated adipose inflammation in an animal model, due to upstream regulation of α-ketoglutarate and diverting adipose-derived exosomes to macrophages (Liu 2018).

2.3 Cardiovascular system

According to current estimates, 17.7 million people die worldwide each year from cardiovascular diseases (CVDs), of which 14.16 million die from heart attacks or stroke (http://www.who.int/cardiovascular_diseases/world-heart-day-2017/en/).

Myocardial infarction, and consequent ischemia reperfusion injuries, are involved in several pathophysiological mechanisms, such as left ventricular remodeling, vascular refraction, fibrosis, generation of oxygen byproducts and aggregation of platelets, inflammatory response and oxidative stress, resulting in death of cardiomyocytes (Dwaich et al. 2016). Of the various therapeutic options for this pathology, stem cell-based therapies show much promise (Ghaeli et al. 2015). In the past decade, mesenchymal stem cell (MSC) therapy has been employed in the treatment of

myocardial infarction both in *in vitro* and *in vivo* studies, with promising results. MSCs are interesting, due to their multipotency, self-renewing activity and multidirectional differentiation. However, there are limitations to the efficacy of this therapy. More than 80-90% of grafted cells die within 72 hours. This low survival rate and apoptosis are triggered by inflammation and oxidative stress, which occur in the micro-environment of ischemic sites in the infarcted heart (Han et al., 2016) (Zhu et al. 2015). Therefore, there is great interest in finding an efficient approach to protect transplanted MSCs from oxidative stress and inflammation, inhibiting the apoptotic process. Recent reports in scientific literature have found that melatonin exhibits cyto-protective effects against ischemic injury in various organs, such as brain, liver, kidney and heart (Han et al. 2016).

Zhu and colleagues observed that pre-treating MSCs with 5 μM melatonin in cell culture for an incubation time of 24 hours prior to transplantation in a rat model of myocardial infarction, enhanced the survival of the engrafted MSCs and promoted their therapeutic activity. As shown in *in vitro* studies, melatonin pre-treatment increased the expression of antioxidant enzymes (catalase, superoxide dismutase), protecting MSCs from H_2O_2 trigged apoptosis, and increasing synthesis of mitogenic factors (Zhu et al. 2015). Afterwards, Han et al. found that, following the induction of infarction in a mouse model, intraperitoneal administration of 20 mg/kg/day of melatonin for 28 successive days post-transplantation of MSCs was more effective than pre-treatment of MSCs before transplantation, underlining the role of melatonin in activation of SIRT1 signaling, which leads to enhanced survival of MSCs. SIRT1 regulates the acetylation levels of p53, NF-kB, and FoxO1, respectively involved in

apoptosis, inflammation and oxidative stress (Han et al. 2016). This mechanism of action of melatonin, through SIRT1, was previously assessed in a rat model of myocardial infarction, without MSC therapy, in which rats received 10 mg/kg/day of melatonin with intraperitoneal injection 7 days prior to the myocardial ischemia/reperfusion operation, and 15 mg/kg 15 minutes before reperfusion (Yu et al. 2014).

Clinical trials aimed to evaluate the effect of melatonin on myocardial ischemia-reperfusion injury, confirming its positive effect on the outcomes of both coronary artery bypass grafting surgery (Dwaich et al. 2016) and primary percutaneous coronary intervention (Ghaeli et al. 2015).

2.4 Musculoskeletal system

Chronic inflammation can cause many pathologies affecting the musculoskeletal system, such as osteoarthritis, rheumatoid arthritis, osteoporosis, and low back pain.

In the scientific literature there is some evidence of the positive effects exerted by melatonin in musculoskeletal diseases.

Osteoarthritis, a joint disease characterized by loss of joint cartilage leading to loss of function in the knees and hips, affects about 10% of men and 18% of women, above 60 years of age. Another common joint disease is rheumatoid arthritis, which affects 0.3-1.0 of the worldwide population (Woolf and Pfleger 2003). It has recently been shown that melatonin stimulates the destruction/regeneration process of cartilage, modulating the expression of circadian clock genes, such as brain and muscle aryl hydrocarbon receptor nuclear translocator like 1, cryptochrome and differentially

expressed in chondrocytes 2. The inhibition of these genes is due to the over-production of pro-inflammatory cytokines, such as TNF-α, NF-kB, IL-1β. It has been observed that melatonin, through its anti-inflammatory effect, exerts a protective effect on cartilage both in *in vivo* and in *in vitro* studies. This finding has been confirmed in osteoarthritis models, but results are conflicting for rheumatoid arthritis (Jahnaban-Esfahlan et al. 2017). Clinical studies are therefore needed to clarify the protective effects of melatonin on joint diseases.

Among musculoskeletal diseases, osteoporosis is a major risk factor for fractures of the hip, vertebrae, and distal forearm, which account for 8.9 million fracture events every year (Johnell and Kanis 2006). Amstrup and colleagues investigated the effect of melatonin supplementation in post-menopausal women with osteopenia, as a population characterized by a hightened risk of development of osteoporosis. A one-year double-blind randomized placebo-controlled clinical trial was conducted on 72 women (56-73 years old), supplemented with 1 mg/day, 3 mg/day, or placebo. Bone mineral density was measured every 3 months. Results collected from melatonin treated groups showed an increase in bone mineral density at femoral neck and spine levels, in a dose-dependent manner (Amstrup et al. 2015).

2.5 Beneficial effect in cancer therapy

Radiation therapy and chemotherapy, the most relevant treatment options in cancer therapy, in spite of their documented efficacy, have many side effects, which are closely related to inflammation. Melatonin, due to its anti-inflammatory property, has been considered as a protective agent in cancer therapy in several studies using both

in vitro and *in vivo* models (Najafi et al. 2017a). Melatonin has been investigated for use in a protective therapy against the damage induced by abdominopelvic and total body radiation, due to its known anti-inflammatory properties. Intestinal cells have a high proliferation rate and are thus highly radiosensitive. Following total body or abdominopelvic irradiation, acute abdominal inflammation occurs, with the loss of intestinal epithelial cells. In a study on a rat model by Guney et al., total body and abdominopelvic irradiation caused an increase in thiobarbituric acid reactive substances (TBARSs), which are indicators of lipid peroxidation, due to oxidative stress and inflammatory conditions in the small intestine. Melatonin, administered intraperitoneally before (10 mg/kg), immediately after (2.0 mg/kg) and 24 hours after (10 mg/kg) irradiation, exerted positive effects on oxidative damage, decreasing TBARs levels (Guney et al. 2007).

Nowadays, it is well known that bone tissue is damaged by exposure to radiation, as happens for example in radiotherapy, causing bone necrosis, sclerosis, loss of bone mass and bone fractures. It has been observed that pelvic fractures occur in 13% of women within 5 years following radiotherapy. Amifostine, the first approved radioprotective drug, is usually administered before and after radiation therapy to protect bone tissue. Because of the free radical scavenging and anti-inflammatory activities of melatonin, and its positive effect on bone tissue, melatonin has recently been studied for use as a bone protective agent in radiotherapy. Cakir et al. published a research article on the protective effects of a single dose of melatonin in the prevention of bone deterioration in 40 Sprague Dawley rats exposed to radiation, in comparison with amifostine. Results show that a single dose of 25 mg/kg melatonin

increases the bone mineral density and biochemical properties of diaphyseal femur bone (organ-level mechanical properties, bone ultimate strength, deformation, stiffness and energy absorption capacity) after irradiation in a similar manner to amifostine, in comparison with placebo (Cakir et al 2016).

As shown in gastro-intestinal (Guney et al. 2007) and musculoskeletal (Cakir et al 2016) systems, melatonin, due to its anti-inflammatory activity, shows protective effects on heart injury caused by radiation therapy in a rat model. Rats developed severe myocardial fibrosis, vascular damage, vasculitis and myocyte necrosis following radiation therapy. Rats were treated by intraperitoneal injection of a melatonin dose of 50 mg/kg, 15 minutes prior to exposure to radiation. Results showed that melatonin exerted preventive effects on the development of vasculitis and decreased the severity of fibrosis and necrosis (Guerses et al. 2014).

Radiation therapy has a relevant impact on immune cells, especially T and B lymphocytes, which leads to the immune system dysfunction. This side effect may induce to moderate intensity of radiation therapy. More recently, several good reviews have been published on the role of melatonin as immunomodulatory agent in radiotherapy. For instance, the review by Najafi et al. reports that, besides the protective effect of melatonin on various damages induced by radiation therapy, several studies have been elucidated the inhibitory effect of melatonin in tumor and metastasis growth, trough its activity on tumor tissues in the apoptosis induction, inhibition of angiogenesis, stimulation of natural killer cells anti-tumor function, and reduction of T regulatory cells involved in radiotherapy resistance (Najafi et al. 2017b).

2.1 Molecular targets underlying anti-inflammatory effects of melatonin

In recent years, melatonin has been studied in different model systems so as to unravel the mechanisms of action underlying its anti-inflammatory effects. A study on chronically hypoxic rats has shown that melatonin reduced translocation of NF-κB, thus decreasing the synthesis of pro-inflammatory cytokines such as TNFα, interleukins and inflammatory mediators such as COX-2 and iNOS (Musicki et al. 2018; Frey, Ushio–Fukai, and Malik 2009). Melatonin also promoted the phosphorylation form of eNOS (ser 1177) in chronic hypoxia by activating the protein kinase B (Akt) pathway. Thus, it increased the levels of NO and decreased the symptoms of pulmonary hypertension (Pinheiro, Tanus-Santos, and Castro 2017). Melatonin is also secreted by the retina. In a rat model of streptozotocin- (STZ-) induced diabetic retinophaty (DR) the production of melatonin was found to be reduced. Melatonin treatment decreased apoptosis in diabetic rats via the PI3K/Akt/Nrf2 signaling pathway, which inhibited NF-κB thus inhibiting the synthesis of pro-inflammatory mediators and cytokines such as TNF-α, IL-1β and iNOS (Foster and Samman 2010). By activating the phosphatidylinositol 3-kinase (PI3K)/Akt/nuclear factor erythroid 2 (Nrf2) signaling pathway, melatonin increases GSH levels and has antioxidant effects on the diabetic retina (Jiang et al. 2012). Previous studies showed that 5-lipoxygenase (5-LOX) and Phospholipase A2 (PLA2) mRNA were decreased in rat pineal gland following melatonin treatments both *in vitro* and *in vivo* (Leon-Blanco et al. 2003).

Melatonin is involved in the modulation of colonic ion transport and inhibiting PGE2-induced cAMP in colonic mucosa (Mrnka et al. 2008). Another observation indicated that melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), inhibit the expression of COX-2, iNOS and decrease NO levels in LPS-stimulated RAW 264.7 macrophages and in glioma cells in a dose-dependent manner, while having no effect on COX-1 levels, constitutive isoform (Silva et al. 2003). Due to its effect on COX-2, melatonin pre-treatment induces apoptosis in LPS-treated cells (Mayo et al. 2005; Steinhilber et al. 1995). Besides the direct effects of melatonin and its metabolites on ROS and RNS, it can also stimulate antioxidative enzymes such as superoxide dismutase and glutathione peroxidase, playing an important role as an endogenous and exogenous antioxidant. Melatonin can down regulate the expression of 5-LOX in B lymphocytes by binding to retinoid orphan nuclear hormone receptors; RZRαn (Wiesenberg et al. 1995). Melatonin reduces the expression of metalloproteinase and TNFα in a mouse model of traumatic spinal cord injury (Genovese et al. 2007) and rat colitis (Dong et al. 2003). One study has shown that melatonin and polyunsaturated fatty acids have synergistic anti-tumor activities on a mammary gland adenocarcinoma model via inhibition of COX and LOX activities (Pardini 2016).

3. Conclusion and future prospects

Growing evidence suggests that melatonin exerts anti-inflammatory activity in a number of chronic diseases which affect different organs in different circumstances. Literature data largely concerns *in vitro* studies and *in vivo* studies in experimental animal model systems. Clinical trials often fail to reach positive results. The scientific evidence obtained from clinical trials is thus far inconclusive, as is the case for other investigations on the activity of food components (i.e. polyphenols), likely due to weaknesses in experimental design (i.e. the duration of the treatment, the doses used, and the speed of compound metabolism). In the future, long-term, well-designed investigations on melatonin-rich foods or melatonin food supplements would provide valuable information towards establishing public health recommendations on melatonin, taking into account both the nature of the compound and its optimal dose, for protection from long-term inflammation linked to chronic diseases. In addition, while melatonin is generally found at low levels in the diet, this can provide a significant contribution under certain dietary habits. In the future more comparative studies, with reliable methods, should be carried out in order to evaluate the dietary intake of melatonin.

Author contributions

MD, HK, SMN and JX contributed to the conception of the manuscript. AB, SFN, AS, ARD, SS and ASS drafted the manuscript. Finally, MD, HK and SMN critically revised the manuscript and gave the final approval.

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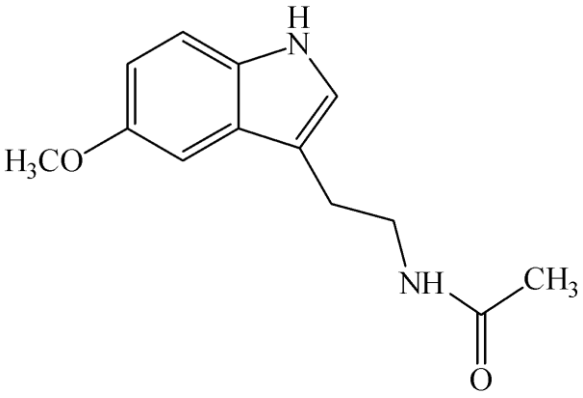

Fig. 1. Chemical structure of melatonin.

Table 1. Some physical properties of melatonin (CAS 73-31-4).

Physical Properties	**Value**
Molecular weight	232.2814 g/mol
Melting Point	117 °C
Log P (octanol-water)	1.650
Atmospheric OH Rate Constant	2.12×10^{-10} cm^3/molecule-sec at 25 °C

Table 2. Melatonin levels in some food matrices.

Sample	**Concentration**	**References**
Alfalfa (*Medicago sativa* L.) (seed)	16 ng/g dw	(Manchester et al. 2000)
Almond (*Prunus amygdaloides* Schltr.) (seed)	39 ng/g dw	(Manchester et al. 2000)
Anise (*Pimpinela anisum* L.) (seed)	7 ng/g dw	(Manchester et al. 2000)
Apple (*Malus domestica* Borkh.)	48 pg/g	(Hattori et al. 1995)
Asparagus (*Asparagus officinalis* L.)	9.5 pg/g	(Hattori et al. 1995)
Banana (*Musa sapientum* Linn.)	0.47 ng/g	(Dubbels et al. 1995)
	8.9 pg/g wet fruit	(Sae-Teaw et al. 2013)
Barley (*Hordeum vulgare* L.)	378 pg/g	(Hattori et al. 1995)
	82 pg/g	(Hernández-Ruiz, Cano, and Arnao 2005)
Beer	51.8-170 pg/mL	(Maldonado, Moreno, and Calvo 2009)
	94.5 pg/mL	(Kocadağlı, Yılmaz, and Gökmen 2014)
Black mustard (*Brassica nigra* (L.) K.Koch) (seed)	129 ng/g dw	(Manchester et al. 2000)
Black olive (*Olea europaea* L. -	5.3 pg/g	(Kocadağlı, Yılmaz, and Gökmen 2014)

naturally fermented)		
Bread (crumb)	342 pg/g	(Kocadağlı, Yılmaz, and Gökmen 2014)
Bread (crust)	138 pg/g	(Kocadağlı, Yılmaz, and Gökmen 2014)
Cabbage (*Brassica oleracea* L.)	107 pg/g	(Hattori et al. 1995)
Cacao powder (*Theobroma cacao* L.)	7.2 pg/g	(Kocadağlı, Yılmaz, and Gökmen 2014)
Carrot (*Daucus carota* L.*)*	55 pg/g	(Hattori et al. 1995)
Celery (*Apium graveolens* L.) (seed)	7 ng/g dw	(Manchester et al. 2000)
Cherry (*Prunus cerasus* L.) (Montmorency and Balaton varieties)	2.06-13.46 ng/g	(Burkhardt et al. 2001)
Cherry products (*Prunus cerasus*) (Montmorency and Balaton varieties)- frozen and dried cherries	nd- 12.3 ng/g dw	(Kirakosyan et al. 2009)
Coriander (*Coriandrum sativum* L.) (seed)	7 ng/g dw	(Manchester et al. 2000)
Cow milk	1.8 pg/mL - 10.5 pg/mL	(Kollmann et al. 2008)
Cucumber (*Cucumis sativus* L.)	25 pg/g	(Hattori et al. 1995)
Fennel (*Foeniculum vulgare* Mill.) (seed)	28 ng/g dw	(Manchester et al. 2000)

Fenugreek (*Trigonella foenum-graecum* L.) (seed)	43 ng/g dw	(Manchester et al. 2000)
Flax (*Linum usitatissimum* L.) (seed)	12 ng/g dw	(Manchester et al. 2000)
Ginger (*Zingiber officinale* Roscoe)	584 pg/g	(Hattori et al. 1995)
Goji berry (*Lycium barbarum* L.)	103 ng/g dry seed	(Manchester et al. 2000)
Grape skin (*Vitis vinifera* L.) (cv. Malbec, cv. Cabernet Sauvignon, cv. Chardonnay)	0.6- 1.2 ng/g	(Stege et al. 2010)
Green cardamom (*Elettaria cardamomum* (L.) Maton)	15 ng/g dry seed	(Manchester et al. 2000)
Green coffee (*Coffea* L. spp.)	39 pg/g	(Kocadağlı, Yılmaz, and Gökmen 2014)
Indian spinach (*Basella alba* L.)	39 pg/g	(Hattori et al. 1995)
Kiwi fruit (*Actinidia chinensis* Planch.)	24 pg/g	(Hattori et al. 1995)
Mango (*Mangifera indica* L.)	699 pg/g wet fruit	(Sae-Teaw et al. 2013)
Milk thistle (*Silybum marianum* (L.) Gaertn)	2 ng/g dry seed	(Manchester et al. 2000)
Oat (*Avena sativa* L.)	1796 pg/g	(Hattori et al. 1995)
	91 pg/g	(Hernández-Ruiz, Cano, and Arnao 2005)

Olive (*Olea europaea* L.) oil	50-119 pg/mL	(De la Puerta et al. 2007)
Onion (*Allium cepa* L.)	32 pg/g	(Hattori et al. 1995)
Orange (*Citrus reticulata* Blanco)	150 pg/g wet fruit	(Sae-Teaw et al. 2013)
Papaya *(Carica papaya* L.)	241 pg/g wet fruit	(Johns et al. 2013)
Pepper (*Capsicum annuum* L.)	31-93 ng/g dw	(Riga et al. 2014)
Pineapple (*Ananas comosus* (L.) Merr.)	36 pg/g	(Hattori et al. 1995)
	302 pg/g wet fruit	(Sae-Teaw et al. 2013)
Pomegranate (*Punica granatum* L.) wine	0.54-5.50 ng/mL	(Mena et al .2012)
Poppy (*Papaver somniferum* L.)	6 ng/g dry seed	(Manchester et al. 2000)
Probiotic yogurt	127 pg/g	(Kocadağlı, Yılmaz, and Gökmen 2014)
Rice (*Oryza sativa* L.)	1006 pg/g	(Hattori et al. 1995)
Salvia (*Salvia officinalis* L.) dried herb	2.8-4 ng/g	(Stege et al. 2010)
Strawberry (*Fragaria* L. spp.)	12 pg/g	(Hattori et al. 1995)
Strawberry (*Fragaria* L. spp.) (varieties: Camarosa, Candonga, Festival, and Primoris)	1.38 - 11.26 ng/g	(Stürtz et al. 2011)

Sunflower (*Helianthus annuus* L.)	29 ng/g dry seed	(Manchester et al. 2000)
Sweet cherry (*Prunus avium* L.)	0-0.224 ng/g	(González-Gómez et al. 2009)
Sweet corn (*Zea mays* L.)	1366 pg/g	(Hattori et al. 1995)
Tomato (*Lycopersicon esculentum*)	32 pg/g	(Oishi et al. 1996)
Tomato (*Lycopersicon esculentum* Mill.) (varieties: Bond, Borsalina, Catalina, Gordal, Lucinda, Marbone, Myriade, Pitenza, Santonio, Perlino, Platero)	4.11 - 114.5 ng/g fw	(Stürtz et al. 2011)
Tomato (*Lycopersicon lycopersicum* Mill.)	7.5-250 ng/g dw	(Riga et al. 2014)
Tomato (*Lycopersicon lycopersicum* Mill.)	28.9 pg/g	(Kocadağlı, Yılmaz, and Gökmen 2014)
Walnut (*Juglans regia* L.)	3.5 ng/g	(Reiter, Manchester, and Tan 2005)
	138 pg/g	(Kocadağlı, Yılmaz, and Gökmen 2014)
Welsh onion (*Allium fistulosum* L.)	86 pg/g	(Hattori et al. 1995)
Wheat (*Triticum* spp)	125 pg/g	(Hernández-Ruiz, Cano, and Arnao 2005)
White mustard *(Sinapis alba* L.) (seed)	189 ng/g dw	(Manchester et al. 2000)

Wine (*Vitis vinifera* L.) (cv. Malbec, cv. Cabernet Sauvignon, cv. Chardonnay)	0.16-0.32 ng/mL	(Stege et al. 2010)
Wine (*Vitis vinifera* L.) (*Palomino Fino* (white); red: *Cabernet Sauvignon, Merlot, Syrah, Tempranillo, Tintilla de Rota*)	74.13-423.0 ng/mL	(Rodriguez-Naranjo et al. 2011)

dw- dry weight; fw- fresh weight.

Table 3. Some analytical methods used to determine melatonin.

Methods	**Limit of detection**	**References**
RIA	1 pg/tube	(Hattori et al. 1995)
ELISA	3.0 pg/mL	(De la Puerta et al. 2007)
		(Iriti, Rossoni, and Faoro 2006)
		(Maldonado, Moreno, and Calvo 2009)
Chemiluminescence		(Iriti, Rossoni, and Faoro 2006)
	100 nM	(Lu et al. 2002)
CEC with immobilized c-MWNT capillary electrochromatography with immobilized carboxylic multi-walled carbon nanotubes	0.01 ng/mL	(Stege et al. 2010)
CZE	0.03 ng/mL	(Stege et al. 2010)
HPLC-ECD		(Iriti, Rossoni, and Faoro 2006)
		(Hernández-Ruiz, Cano, and Arnao 2005)

HPLC-Fl	2.2 µg/L	(Stürtz et al. 2011)
	0.5 ng/mL juice	(Johnson et al. 2013)
		(Iriti, Rossoni, and Faoro 2006)
	3 pg/mg pineal tissue	(Vitale et al. 1996)
HPLC-MS	4.3 ng/mL	(González-Gómez et al. 2009)
LC-MS/MS	5 pg/mL	(Cao et al. 2009)
	0.13 ng/mL	(Van Tassel et al. 2001)
		(Mena et al .2012)
	confirmation only	(Cerezo et al. 2016)
UHPLC-MS/MS		(Riga et al. 2014)
UHPLC -DAD	0.03 mg/L	(Cerezo et al. 2016)
GC-MS		(Van Tassel et al. 2001)

CEC- capillary electrophoresis; CZE- capillary zone electrophoresis ECD- electrochemical; ELISA- enzyme-linked immunosorbent assay; DAD- Diode array detection; Fl- Fluorescence; GC- Gas Chromatography; RIA- radioimmunoassay; HPLC- High Performance Liquid Chromatography; UHPLC- Ultra High Pressure Liquid Chromatography; MS- Mass spectrometry.