IJPSR (2011), Vol. 2, Issue 8 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 April, 2011; received in revised form 14 July, 2011; accepted 24 July, 2011

MELATONIN - A REVIEW ON THE LESSER KNOWN POTENTIAL NUTRACEUTICAL

J. Jemima [1], P. Bhattacharjee*[2] and R. S. Singhal [1]

Department of Food Engineering and Technology[1], Institute of Chemical Technology, Matunga, Mumbai- 400 019, Maharashtra, India

Department of Food Technology and Biochemical Engineering [2], Jadavpur University, Kolkata- 700 032, West Bengal, India

Keywords:

Synthesis,
Role in mammals,
Plant based food sources of melatonin,
Analytical methods,
Bioavailability

Correspondence to Author:

Dr. Paramita Bhattacharjee

Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata – 700 032, India

ABSTRACT

Melatonin, a pineal hormone is known to regulate rhythmic behavior of mammals. Its ubiquitous presence, established recently has brought into focus several health-promoting benefits of this molecule. However, till date, melatonin has been less utilized from its nutraceutical and functional point of view. This paper reviews the chemistry, chemical synthesis, metabolism of melatonin; its sources including its bioavailability from the same, its role in mammals and in plants and the analytical techniques for quantitative analysis of the same. The nutraceutical potential of melatonin and its functional properties have been elaborated. Melatonin's function as a direct free radical scavenger endows it the potency to act as strong antioxidants- a property which needs to be further researched upon to establish its efficacy in food applications.

INTRODUCTION: It was observed that the extract from beef pineal gland could lighten frog's skin by reversing the darkening effect of melanocyte stimulating hormone and the compound responsible for this effect was named as 'melatonin' since it caused aggregation of melanin granules [1]. Later the chemical structure of melatonin was elucidated as N-acetyl-5-methoxy tryptamine. Melatonin is one of the major hormones secreted by pineal gland and is called active pineal factor. It is 100 times active as adrenaline and nor-adrenaline, 200 times active as triiodothyronine, and 5000 times as active as serotonin in preventing darkening of frog skin by contracting melanophores [1]. This discovery of melatonin led to further proliferation of research on pineal gland and its products. Pineal gland is an unpaired pine cone shaped organ with extensive vascularization and is reported to coordinate the psychophysiological functions in mammals.

The functions of pineal gland can be directly related and implicated to its hormone-melatonin, which faithfully reflects pineal activity [2]. The functions of melatonin and its basic physiology and pathophysiology in mammals are well studied and established. The recent discovery of melatonin in different structures of higher plants and also in lower phyla including algae and bacteria established the ubiquitous presence of this molecule and its role in co-coordinating certain basic events of life. Melatonin has strong immune-enhancing and antioxidative functions and plays significant role in neurological and physiological health. Melatonin with its nutraceutical potency could find possible application as antioxidant in foods. This paper reviews the chemistry, metabolism, sources of melatonin, its biosynthesis and its beneficial functions in the human system with special emphasis to its nutraceutical properties.

The analytical techniques for quantitative analysis and melatonin's bioavailability from exogenous sources have also been discussed here.

Structure and chemistry of Melatonin: Melatonin $C_{13}H_{16}N_2O_2$ (molecular weight: 232) or N-acetyl-5-methoxytryptamine, is an indoleamine, derived from the essential amino acid tryptophan [1]. It is a white powder having melting point of 117°C. It is an amphiphilic molecule with high lipid and water solubility which facilitates its movement across the cell membrane and various body fluids. This makes melatonin a prominent molecule in almost all tissues of mammals [3]. The indole ring of melatonin acts as the chromophore showing maximum absorbance at 223 nm, and the functional groups contribute to its fluorescence property. Its functional groups confer it the receptor specificity for its hormonal functions and aids in its oxidation chemistry.

Melatonin - Metabolism:

Biosynthesis of melatonin in mammals: The major source of melatonin in mammals is the pineal gland; the secondary sources include retina, gut, platelets, bone marrow and probably a few more; whose contribution is insignificant. Tryptophan taken up by the pinealocytes from the blood is converted to 5-hydroxytryptophan by tryptophan 5-hydroxylase which is then decarboxylated to serotonin by 5-hydroxytryptophan decarboxylase. Serotonin is acetylated by the rate limiting enzyme N-acetyl-transferase to N-acetylserotonin which further undergoes methylation reaction by the enzyme hydroxyindole-O-methyl transferase to form melatonin [4] **(fig. 1)**.

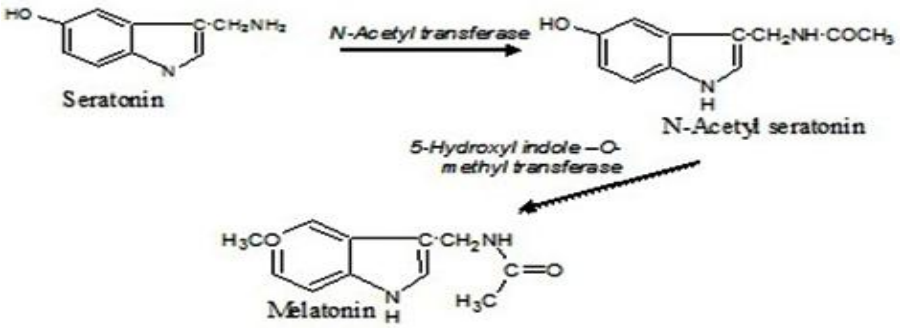

FIG. 1: BIOSYNTHESIS OF MELATONIN IN MAMMALS

Catabolism: The circulating plasma melatonin is hydrolyzed to 6-hydroxymelatonin by CYP450 monooxygenases in liver followed by conjugation to give 6-sulfatoxymelatonin or 6-hydroxymelatonin glucuronide **(fig. 2)** which is then excreted. Urine 6-sulfatoxymelatonin excretion closely parallels the plasma melatonin profile [4].

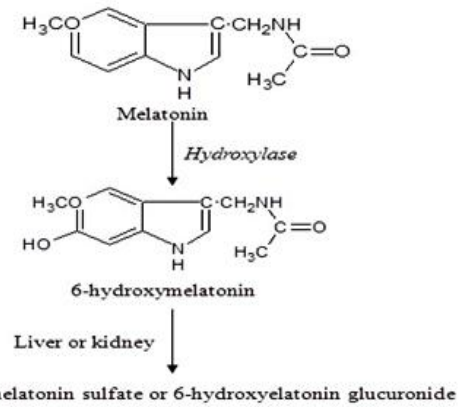

FIG. 2: CATABOLISM OF MELATONIN BY KYNURIC PATHWAY

About 1% melatonin is excreted unchanged in urine. 3-Hydroxyl melatonin in urine is considered as a biomarker for hydroxyl radical generation and form the basis of tumor detection. The tissue melatonin undergoes oxidative pyrrole ring cleavage resulting in N-acetyl- N-formyl-5-methoxykynuramine which is deformylated to N-acetyl-5-methoxykynuramine. This is known as the classical kynuric pathway of melatonin metabolism [3, 5].

Role of Melatonin in Humans: Melatonin is often associated with the treatment of sleep related disorders such as insomnia and jet lag since it acts as an endogenous synchronizer. Apart from regulating the biological clock in human, it plays a major role as an antioxidant by scavenging the free radicals, thereby enhancing the immunity and preventing cancer. The role of melatonin in humans is discussed in the following sections.

Circannual cycle: Circannual cycle denotes the occurrence of processes or functions in a living organism including plants, animals and human on a yearly basis. Events that display an annual rhythm can include life cycles such as those of mating behavior and growth patterns and some kinds of movements such as migration.

Melatonin is a well known chronobiological marker for the circannual rhythms. The fluctuations in the levels of melatonin and its duration of secretion in mammals are widely reported and it is found to regulate the seasonal changes in reproduction, metabolism and other behavioral and physiological functions. In healthy individuals, the nocturnal levels of melatonin are higher and its secretion is longer in winter than in summer. However, there is no variation in noon melatonin levels between summer and winter.

Other reported patterns of annual variation in the melatonin levels include its inverted pattern of melatonin secretion in young men wherein level of it is comparatively higher in the summer than in winter. In contrast, some investigators have reported no such fluctuations in melatonin levels. These contradictory results could have been owing to different methodological strategies, employed by different researches. The widespread use of artificial light in modern society shield humans from the seasonal changes in the day length which contributes further to fluctuations in levels of melatonin [6, 7].

The seasonal affective disorder (SAD) of winter type is characterized by recurrent depression during the short photoperiod and its remission during summer is directly associated with photoperiodic variation. It was hypothesized that the change in phase of melatonin secretion play a major role in the pathogenesis of SAD and melatonin is widely recommended for its therapy [8].

Thermoregulation of melatonin production: It was hypothesized that the effect of melatonin on sleep may be mediated by its thermoregulatory function as the nocturnal fall in the body temperature coincides with the peak in endogenous melatonin production. Biological night is a period in constant routine conditions that corresponds to the endogenous melatonin production and secretion in the blood stream. The beginning of this biological night is characterized by onset of the melatonin surge accompanying increase in sleep propensity as well as a decrease in core body temperature; the opposite occurs as the biological night and sleep ends. It was found that administration of melatonin during day time or in the evening increased sleep propensity, subjective sleepiness and fatigue while decreasing sleep latency. It was hypothesized that the effect of melatonin on sleep may be mediated by its thermoregulatory function as the nocturnal fall in the body temperature coincides with the peak in endogenous melatonin production [2, 8].

Melatonin is known to decrease the core body temperature and increase the distil skin temperature. Similar changes in core and skin temperature also occur at onset of sleep suggesting the role of melatonin in thermoregulation. Suppression of melatonin levels by exposure to bright light is associated with elevated core body temperature and this effect is reversed by administration of exogenous melatonin [9]. This variation in concentration of melatonin with temperature has been elaborated in the following section.

Circadian cycle: A circadian rhythm is a 24h cycle in the biochemical, physiological, or behavioral processes of living entities, including plants, animals, human, fungi and cyanobacteria. It is present in the sleeping and feeding patterns of animals including human beings. The patterns of core body temperature, brain wave activity, hormone production, cell regeneration and other biological activities mark the circadian cycle in human.

In addition, photoperiodism, the physiological reaction of any organism to the length of day or night, is vital to both plants and animals, and the circadian system enables the measurement and interpretation of day length by the biological system. In mammals, the rhythmic production of melatonin is controlled by light and it is regarded as an endogenous circadian cycle synchronizer. The oscillator for the melatonin synthesis is localized in suprachiasmatic nucleus (SCN) of the brain which receives visual information from retina through retino-hypothalamic tract.

This signal synchronizes the circadian activity of the biological clock - SCN, which in turn impacts the pineal gland through multi-synaptic pathway controlling the melatonin production. Melatonin feeds back to the SCN and acts on the pars tuberalis of the pituitary and other brain areas to modulate seasonal neuroendocrine functions [6, 10].

Melatonin levels are likely to be affected by physiological functions. Melatonin levels in plasma shows a great inter-subject heterogeneity and varies throughout the day and also with age, gender, temperature and season. Radioimmunoassays have indicated the melatonin production in healthy adults to range from 28.8 µg/day to 39.2 µg/night. The rate of secretion at night as estimated by GC-MS is about 4.6 µg/h in males and 2.8 µg/h in females.

In healthy humans, melatonin concentration rises from 5 pg/ml (detectable limit for RIA) during evening and plasma level reach a maximum around 03:00- 04:00 am and then start decreasing before habitual wake-up time. Melatonin synthesis in mammals is maximally sensitive to short wavelength light range of 450-480 nm. Light intensities ranging from 2000-2500 lux for 2h (02:00-04:00 am) completely suppress melatonin secretion [4, 6, 8, 9, 11].

The most effective wavelengths that inhibit the secretion of melatonin in mammals are in the range 460-470 nm, while the maximum production is during the dark period. Thus at any stage of the light/day cycle, serum melatonin concentration gives information about the time of the day [4, 6].

Administration of exogenous melatonin is reported to cause a phase shift in the circadian cycle. The size and the direction of shift can be controlled by the dose and time of administration of melatonin with phase delays being observed following the morning administration and phase advance being observed following the evening administration. **Figure 3** indicates the phase shift and its synchronization by treatment with melatonin. Timed administration of melatonin is widely recommended for the treatment of circadian rhythm disorders and is used in the readjustments of acute phase shifts [12].

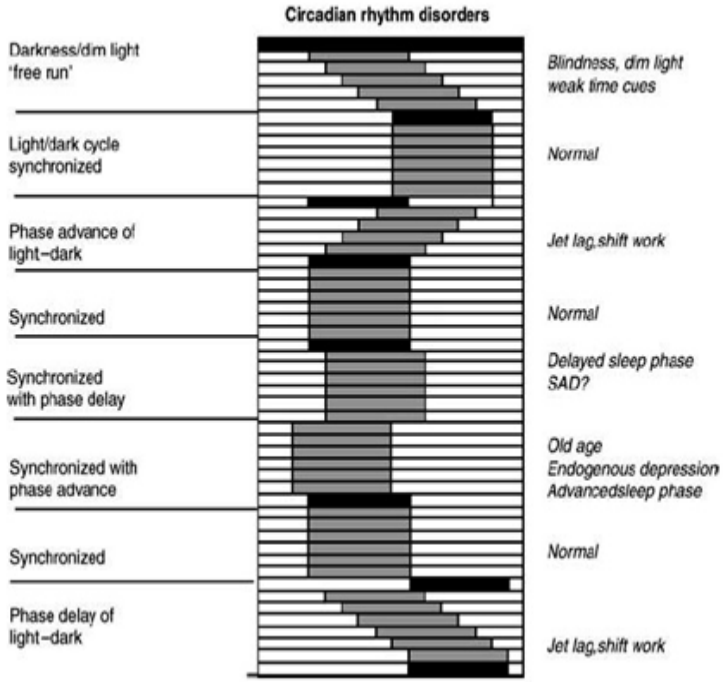

FIG. 3: TREATEMENT OF VARIOUS CIRCADIAN DISORDERS BY ALTERING MELATONIN LEVELS

Cardiovascular system: The diurnal variation in human cardiovascular function with lowered blood pressure, heart rate and higher cardiac output and higher peripheral vascular resistance at night when melatonin levels are high relative to day, is well known. It was also found that the risk of myocardial infractions and stroke are more in early morning, when the levels of melatonin fall significantly. In healthy individuals, coronary heart disease patients have significantly low melatonin levels. It was hypothesized that action of melatonin on SCN possibly affects the release of vasopressin which regulates the circadian and sympathetic functions of the cardiac tissues.

It was reported that in healthy individuals day time melatonin administration acutely reduces the pulsatility index of carotid artery, systolic and diastolic pressure and blood nor-epinephrine levels. Heart rate is also lowered following day time melatonin administration. A number of animal studies suggest pineal extracts to decrease the blood pressure, the effect probably being the presence of melatonin which acts as a hypotensive agent. In contradiction, certain studies provide evidences for vasoconstrictive effect of melatonin in rats and pigs. It has been suggested the vasorelaxant effects of melatonin is due to its effect on NE levels while its vasoconstrictive property is possibly due to suppression of prostaglandin and nitric oxide.

However, the mechanisms underlying these effects need to be clearly elucidated for its application in cardiovascular health [6, 8].

Reproductive function: The studies conducted on seasonal breeders have shown that the duration of melatonin secretion conveys the photoperiodic information that modulates the reproductive activity. However, the relationship between endogenous melatonin levels and reproductive physiology is still not known satisfactorily. It was found that after the maturation of pineal gland in humans, the rhythmic melatonin production reaches a maximum at the age of 3 to 6 years. Then the nocturnal melatonin levels drop by 80% progressively until the age of puberty. This fall in the melatonin secretion is linked with the appearance of sexual maturity and onset of puberty. Melatonin levels are significantly low in cases of precocious puberty and its level decreases tremendously in the treatment of delayed puberty [6].

It was also found that in male primary hypogonadic patients and in patients with gonadotropin releasing hormone deficiency, there is an increase in melatonin secretion. The sperm motility is greatly inhibited in normal semen following melatonin administration. During the ovarian cycle, it was hypothesized that melatonin regulates the secretion of steroidal hormones especially that of progesterone.

Administration of melatonin is reported to induce a decrease in luteinizing hormone secretion thus blocking ovulation, and to increase the luteal phase progesterone without affecting follicle stimulating hormone inhibiting estradiol. The transient elevated secretion of melatonin during and after menopause could well be related to the decrease in the estrogen secretion. Since high levels of plasma melatonin is often associated with lowered sex hormonal activity, its role as an oral contraceptive has been well studied and is found to have significant anovulatory effect when administered with synthetic progestin [8, 13].

Melatonin as antioxidant: Melatonin was discovered to be a direct free radical scavenger by and subsequently there has been a vast body of research documenting its potent and diverse antioxidant capabilities. **Figure 4** describes the different kinds of antioxidant protection offered by melatonin. Different mechanisms of protective action of melatonin against free radicals are reported to significantly reduce oxidative stress [11].

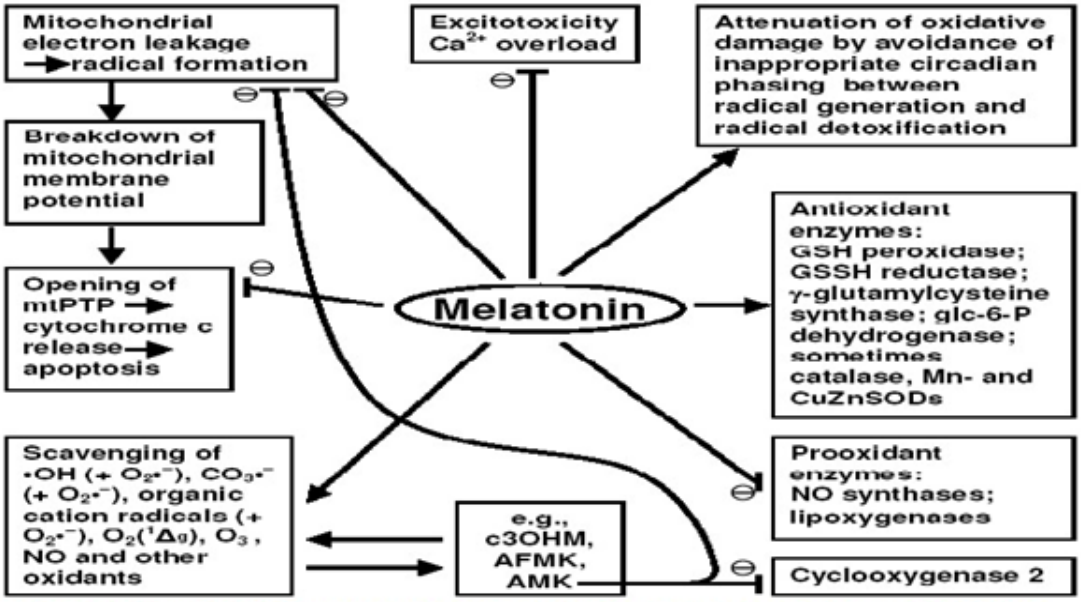

FIG. 4: OVERVIEW OF PLEITROPIC ACTIONS OF MELATONIN AND SOME OF ITS METABOLOITES IN ANTIOXIDATIVE PROTECTION

Melatonin functions as an antioxidant, acts as a direct free radical scavenger, lowers free radical generation and protects DNA from free radical damage. It is a stimulator of antioxidative enzymes, enhances efficiency of mitochondrial oxidative phosphorylation and reduces electron leakage and thereby augments the efficiency of other antioxidants [11]. The ability of melatonin to scavenge a number of reactive oxygen and nitrogen species and its amphiphilicity makes it a potent free radical scavenger in the biological system.

Melatonin as Direct Radical Scavenger: Melatonin is an endogenous free radical scavenger as it detoxifies a variety of free radicals and reactive oxygen intermediates such as hydroxyl radical, hydrogen peroxide, peroxynitrite anion, singlet oxygen and nitric oxide. Melatonin is five times more efficient than glutathione (GSH) in neutralizing hydroxyl radical and 15-fold more effective than exogenous scavenger mannitol and 2-fold more efficient in scavenging lipid peroxide radical than vitamin E. Therefore, melatonin could have potential role as food antioxidants [14].

The kynuric pathway of melatonin breakdown includes a series of radical scavengers which may be regarded as a scavenger cascade with a possible sequence of melatonin, cyclic 3-hydroxymelatonin, N-acetyl-N-formyl-5-methoxykynuramine (AFMK) and N-acetyl 5-methoxykynuramine (AMK). Melatonin can be alternately converted to AFMK directly by scavenging four free radicals. It was also reported that even higher numbers of free radicals can be eliminated during this conversion of melatonin to AFMK. The potent scavenger AFMK consumes further radicals to form AMK. AMK not only interacts with reactive oxygen but also with reactive nitrogen species and several other free radicals [3].

Melatonin activates Antioxidant Enzymes: Melatonin acts as a signaling molecule at the cellular level and up-regulates a number of antioxidant enzymes. This has been demonstrated for glutathione peroxidase and glutathione reductase. In some tissues Cu, Zn-and/or Mn-superoxide dismutases and rarely, catalase are also upregulated. Melatonin's free radical scavenging ability is well known and is widely documented, while the antioxidant enzyme activation by the indole-amines and the mechanisms underlying melatonin-regulated processes have not yet been clearly elucidated.

The activation of enzyme by melatonin could be a consequence of antioxidant enzyme m-RNA synthesis. Although there are studies that established melatonin as inducer for gene expression, no clear evidence exists till date [15]. One such mechanism of enzyme activation by melatonin is hypothesized that RORα, a nuclear transcription factor, acts as a prooxidant factor that down regulates a number of antioxidant enzymes. Application of melatonin to this pathway leads to the abolishment of the RORα effects, favoring the antioxidant mechanisms displayed by cells [15].

Melatonin and mitochondrial oxidative phosphorylation: Effect of melatonin on mitochondrial functions has been discovered recently and was found to be more important than any other protective actions described above. Mitochondrion in any cell is the main source of free radicals and is often implicated with aging. Like any other antioxidant, melatonin was reported to attenuate mitochondrial lipid peroxidation, prevent oxidation of protein and DNA modifications, involve in preservation of ultrastructure and confer resistance against toxins. In addition, melatonin affects redox-active compounds in mitochondria, in particular, to decrease reactive nitrogen and oxygen species and to restore normal levels of reduced glutathione and coenzyme Q_{10}.

It also protects the mitochondrial membrane and prevents its leakage by scavenging the free radicals that damage it. Mitochondria play a key role in apoptosis of cells, while melatonin in mitochondria has anti-apoptotic effects through interference with apoptotic signals originating in mitochondria. Studies have shown melatonin to significantly increase activation of complexes I and IV of the mitochondrial electron transport chain (ETC) and to reverse the effect of inhibitors on these complexes. These opposing processes lead to increased mitochondrial respiration rate and consequently ATP synthesis [16].

Effect of melatonin on electron transport chain has been widely studied and a new perspective for diminishing radical formation was proposed by Hardeland and Pandi-Perumal[3]. It is hypothesized that electron leakage is reduced by single-electron exchange reactions between melatonin and components of the electron transport chain. The melatonin which forms its cation radical during the process of electron donation in the respiratory chain acts as an alternate electron acceptor to molecular oxygen and thereby decreases the rate of oxy-radical formation.

Synergism with other antioxidants: An important aspect of the antioxidant action of melatonin is its interactions with classic antioxidants. This is of

profound significance from the nutritional point of view since melatonin spares the antioxidant vitamins for their biological functions. Melatonin is reported to potentiate the effects of ascorbate, Trolox, reduced GSH and NADH. These findings suggest that melatonin exhibits synergistic rather than additive effect with the same. Its interaction with other antioxidants is by the redox-based regeneration of antioxidants transiently consumed. This may, in fact, be of practical importance, since melatonin is also shown to prevent the decrease in hepatic ascorbate and α-tocopherol levels in vivo under conditions of long lasting experimental oxidative stress induced by a high cholesterol diet [3].

Immune enhancing: Melatonin is found to have immune-modulatory effect. The decrease in endogenous levels of melatonin has a pronounced effect on the spleen and thymus activity and in primary antibody response to T-dependent antigens. Melatonin is found to adverse these effects and also increases T-helper cell activity and IL-2 production and suppresses the 5-lipoxygenase gene expression in lymphocytes. It regulates the production of interleukin-1, inhibits the immunorepressive action of corticosteroids and has a stimulatory effect on the natural killer cells. Several lymphoid organs and lymphocytes are found to contain the receptors for melatonin suggesting its possible role as an immune modulator [6, 17].

In diseases that present rhythmic symptoms as seen in rheumatoid arthritis and nocturnal asthma, melatonin provides the functional link between the neuro-endocrine and immune haematopoietic system. The mechanism proposed for the immune modulating effect of melatonin is the long term resetting of circadian modulation of immune functions affecting hematopoiesis and thymocyte mitosis.

Melatonin has potentiating role in release, regulation and proliferation of T, B, NK cells, monocytes, thymocytes, cytokines, met-enkephalin and other immune-lipids and also in anti-apoptosis. In addition, the interaction between melatonin production and immune system are bidirectional since interleukins and cytokines affect its synthesis and release from the pineal gland.

The oncostatic property of melatonin is well studied and documented. It is found to slow down tumor formation and its progression. This is attributed to its potent antioxidative ability [6, 8].

Psychological and neurological functions: Melatonin plays a significant role in the neurological and psychological health in human and is vital in determining the mood of an individual. A number of studies report the altered melatonin levels with several psychiatric and neurological disorders. There are indications regarding the involvement of melatonin in the progression of senile dementia and Alzheimer disease as the levels of melatonin showed significant abnormality such as reduced levels and diminished total secretion in Alzheimer's patients as compared to their healthy elderly counterparts.

Long term potentiation (LTP), an electrophysiological process is linked to memory, is also influenced by melatonin[18]. Decreased melatonin levels are observed in ischemic stroke, acute cerebral hemorrhage and aneurysm rupture. Studies have shown low melatonin levels in depression as well as in dysrhythmia. There are indications of correlation between the emotional and cognitive retardation symptoms with reduced melatonin secretion. Fibromyalgia, which is characterized by achy pain, tenderness, and stiffness of muscles and neurological symptoms of depression, fatigue and sleep disturbances are well correlated to low melatonin levels [8, 19].

In treatment of epilepsy, melatonin plays a significant role. In high doses, it has anti-convulsing effect while at low doses it acts as an anti-depressant. Melatonin when effectively combined with valproate is found to have a supra-additive antiepileptic efficacy in animal models. The variations and anomaly in the melatonin levels in various neurological conditions and disease states are generally thought to be consequences of the existing disorder and this altered melatonin secretion in turn affect the endocrine system and consequently the course of disorder. The contribution and role of melatonin to psychological and neurological health is not yet clearly established, medical practitioners however, widely prescribe melatonin in treatment of various neurological disorders [6].

Other functions: Melatonin binds with Ca^{2+}-calmodulin in cells and antagonizes some of its effects. Since Ca^{2+}-calmodulin complex regulates many calcium-dependent cellular functions, it was suggested that melatonin binding may affect a broad range of biological processes as calmodulin is calcium signal transducer. Melatonin affects the cytoskeleton, causing either microtubule enlargement or de-polymerization- at physiological concentrations (10^{-9}M); it antagonizes the inhibition of tubulin polymerization (caused by calmodulin) and stimulates microtubule growth. At levels of 10^{-7} to 10^{-5}M, melatonin binds to tubulin and prevents its polymerization and thereby disrupts the mitotic apparatus [20]. Melatonin's effect on animal cell processes controlled by calmodulin needs further investigations to gain complete understanding of its effects.

Sources of Melatonin: Melatonin has wide applications in treatment of numerous sleep related disorders and psychological illnesses. It is either chemically synthesized or obtained from natural source for these pharmaceutical applications.

Chemical synthesis of melatonin: Melatonin is chemically synthesized by reducing 5-methoxy-indole-3-acetonitrile with sodium and ethanol, and then by acetylating the product with glacial acetic acid and acetic anhydride. It is also synthesized from 4-acetamidobutanal, which reacts with 4-methoxyphenylhydrazine to form intermediate hydrazone. This in the presence of an acid undergoes Fischer-indole reaction to give melatonin **(figure 5)** [21].

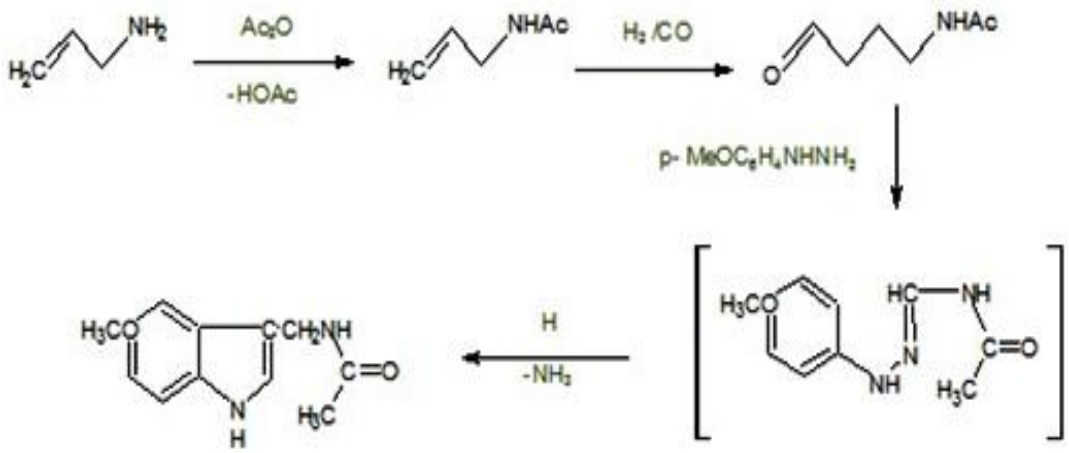

FIG. 5: CHEMICAL SYNTHESIS OF MELATONIN

Another commercial method of melatonin synthesis is by warming 5- methoxytryptamine, pyridine and acetic anhydride in the presence of nitrogen atmosphere and pouring the resulting reaction mixture in cold water to obtain a solid which is 85% pure melatonin [22]. The melatonin which is chemically synthesized is further purified by countercurrent distribution and silicic acid chromatography.

Melatonin from plant sources: Initially, the presence of melatonin was established only in the animal kingdom; later with appropriate extraction and detection methods, melatonin was detected almost in every organism. Melatonin was found outside the animal kingdom for the first time in the dinoflagellate algae, *Lingulodinium polyedrum*. Since then, melatonin is presently regarded as ubiquitous molecule, although its presence is not clearly established in some important taxas- particularly in archaea, mosses, ferns, gymnosperms, sponges, annelids, chelicerates and echinoderms [3]. As a ubiquitously distributed molecule, melatonin was first identified in edible plants by [24]. Since then, melatonin has been found in many plants and in different parts of plants - roots of Lupinus albus [25], seeds of sunflower, mustard and walnuts [26], fruit of tomato [27], skin of grapes [28] and rind of tart cherries [29] and it has been reported to be in extremely high levels in several medical herbs including feverfew and St. John's wort [30]. **Table 1** lists the melatonin content in various plants and plant-based foods.

TABLE 1: REPORTED LEVEL OF MELATONIN IN VARIOUS PLANTS AND IN FOODS

Plant / food source	Level of melatonin	Reference
Alfalfa	16 ng/g dry seed	Manchester et al., 2000
Almonds	39 ng/g dry seed	Manchester et al., 2000
Anise seed	7 ng/g dry seed	Manchester et al., 2000
Black mustard	129 ng/g dry seed	Manchester et al., 2000
Celery seeds	7 ng/g dry seed	Manchester et al., 2000
Fennel	28 ng/g dry seed	Manchester et al., 2000
Fenugreek	43 ng/g dry seed	Manchester et al., 2000
Flax seeds	12 ng/g dry seed	Manchester et al., 2000
Grape skin	0.03-0.97ng/g dry matter	Iriti, Rossoni, & Faoro, 2006
Green cardamom	15 ng/g dry seed	Manchester et al., 2000
Olive oil (virgin)	0.07-0.12ng/ml	Puerta et al., 2007
Olive oil (refined)	0.05-0.08ng/ml	Puerta et al., 2007
Poppy seeds	6 ng/g dry seed	Manchester et al., 2000
Sunflower seed oil (refined)	0.05 ng/ml	Puertaet al., 2007
Sunflower seeds	29 ng/g dry seed	Manchester et al., 2000
Tart cherries	2–13 ng/g fw	Burkhardt et al., 2001
Tomato fruit	0.01-0.02ng/g	Van-Tassel & O'Neill, 2001
White mustard	189 ng/g dry seed	Manchester et al., 2000
Wine	0.05-0.08 ng/mL	Garcia-Parrilla, Cantos, & Troncoso, 2009
Wolf berry	103 ng/g dry seed	Manchester et al., 2000

Melatonin from microbial sources: Melatonin is reportedly present in prokaryotes such as Moneras and Protista; in *Rhodospirillum rubrum* and in *Gonyaulax polyedra*. Melatonin and its structurally related compound, 5-methoxytryptamine are also reported in *Saccharomyces cerevisiae* in high concentrations. Depending on conditions, levels above 10-20 ng of melatonin per mg protein are known. Addition of exogenous tryptophan, serotonin or N-acetylserotonin is reported to elevate melatonin levels above 300 ng/mg proteins.

In the primary pathway of melatonin formation in yeasts, tryptophan is converted to serotonin via 5-hydroxytryptophan; serotonin is successively acetylated to N-acetyl serotonin which is finally converted to melatonin. The high levels of melatonin metabolites - 5-methoxytryptamine and 5-methoxytryptophol in yeasts however suggest that there exists a secondary pathway for melatonin formation in the same through acetylation of 5-methoxytryptamine.

Although melatonin is ubiquitously present in plants, animals and microbes, its role in these is not clearly established. Melatonin is found to be not necessarily associated with the transduction of photoperiodic signals in lower organisms. An exception to this is however found in the dinoflagllate, Lingulodinium polyedrum (syn. Gonyaulax polyedra) where melatonin is shown to oscillate in a circadian fashion (with a pronounced nocturnal peak) and down-regulates a diurnal peak enzyme - tryptophan hydroxylase. In yeasts, melatonin has antioxidative and radical scavenging function [3].

Role of Melatonin in plant: The mechanism of melatonin synthesis in plants is still not known, although there are evidences that suggest the presence of enzymatic machinery for synthesis of the same. In algae, melatonin is synthesized abundantly in the dark and shows a photoperiodicity but no evidence as yet exists for the same in higher plants. The role of melatonin in plants has not been established clearly, till date.

Melatonin's primary role in plants may be related to its antioxidant activity protecting the plant from environmental assaults. Of all the plant tissues, the germ tissues and seeds are reported to contain high levels of melatonin. These high levels found in seeds may be due to their protective role as antioxidants. Seeds being dormant and low-moisture systems are less enzymatically active and low molecular weight antioxidants such as melatonin within are at an advantage. Moreover, melatonin's amphiphilicity may also possibly favor its accumulation in oilseeds as well [26, 31].

As in mammals, melatonin is thought to play a significant role in regulating the photoperiodicity and rhythmic behavior in plants and algae. It was reported that some algal species show daily melatonin rhythms with nocturnal maxima. However, in higher plants, these photoperiodicity and melatonin levels could not be correlated, since application of melatonin did not show short day responses in higher plants. Melatonin synthesis in *Chenopodium rubrum* shows a daily rhythm as is in mammals and algae. However, several other species of plants with photoperiodically controlled flowering showed no promotion of

flowering after melatonin application. It was opined that melatonin could possibly affect calmodulin (calcium signal transducer) controlled processes in plants [20].

Melatonin is structurally quite similar to auxin (indole 3- acetic acid, IAA), both being indole derivatives. Hence it was hypothesized that melatonin could have auxinic activity in plants. This has been confirmed and melatonin is shown to mimic IAA and promote shoot growth in hypocotyls of monocots. Experiments on Lupins albus showed applied melatonin to stimulate the elongation growth of hypocotyls in a concentration range similar to that of auxin.

The levels of endogenous IAA and melatonin are high in the hypocotyl zone which has maximum growth [32]. This suggests that melatonin may have hormone-stimulatory role in plants. There could be a possibility that melatonin may get converted into auxin in the growing tissues and could be utilized further. Research has to be continued to confirm the auxinic activity of melatonin in plant tissues.

Analysis of Melatonin: The presence of melatonin in higher plants has led to newer perspectives and has broadened the use of vegetal melatonin as a natural component of food intake. In order to evaluate the contribution of melatonin from plants as a bioactive compound, it is necessary to device and validates suitable methods for melatonin analysis. The analysis of melatonin from plant tissues is difficulty due to its low detectable amount (picograms to micrograms per gram of tissue) in the same, compared to animal tissues.

Thus, any analytical method developed should be sensitive to very low levels of melatonin; secondly, amphiphilic character of melatonin makes it difficult to choose a solvent that allows complete recovery and thirdly, melatonin (being a potent antioxidant) reacts rapidly with other food constituents and pro-oxidants and gets oxidized, necessitating careful handling and storage of extracting samples. Thus, analytical methods to be used for melatonin estimation should take the above factors into consideration besides having appropriate sensitivity, reproducibility and specificity [31]. Melatonin is analyzed by various techniques which include chemiluminescence method, cyclic voltametery and immunological techniques such as enzyme immunoassay, radio- immunoassay and chromatographic techniques such as liquid chromatography, high pressure liquid chromatography, gas chromatography and gas chromatography-mass spectrometry (coupled with fluorescence or refractive index detector), to cite a few. The various conventional methods of melatonin analysis, their principle, application, advantages and disadvantages are discussed in the sections below.

Immunoassays: Radioimmunoassay (RIA) is a technique based on antigen-antibody interaction which is used to determine melatonin content in biological fluids such as saliva and serum. In this assay, a specific antibody (Ab) reacts with the corresponding antigen (*Ag) labeled with ^{125}. Upon addition of sample, an increase in the amount of the unlabeled antigen from the sample causes a corresponding decrease in fraction of (*Ag) that is bound to the antibody. Bound and free (*Ag) is separated by centrifugation.

The bound radioactivity is subsequently determined in a gamma counter and results are obtained using standard curves. In enzyme immunoassay (EIA), instead of a radioactive antigen, an enzyme is linked to the antibody. When the antibody bounds to the corresponding antigen from the sample, the antibody-bound enzyme is released and exhibits its activity in the sample solution which is quantified using a suitable substrate and correlated to the amount of melatonin in the sample. This method was first employed for melatonin estimation in olive oil and further employed to quantify melatonin in various tissues and foods such as grape skin, wine and in cow milk.

The major advantages of immunoassays are that they are less cumbersome and consume less time and less sample volume for analysis. The availability of commercial kits for RIA and EIA is an added advantage. The detection limit of RIA is 4pg/mg of tissue. However, a prior fractionation step by HPLC or GC is required for melatonin analysis by immunoassay since the presence of similar compounds cross-reacts with melatonin immunoassay antiserum leading to false positive findings and its overestimation [27, 33].

Liquid and gas chromatographic techniques: The lack of specificity between the antigen-antibody interactions in the presence of related compounds led to the development of liquid chromatography coupled with RIA (LC-RIA), where column chromatography was used to separate various fractions of the plant tissue and melatonin was quantified by radioimmunoassay [34]. The detection limit of LC-RIA is 15pg/mL. Gas chromatography coupled with mass spectroscopy has a lower detection limit of 1pg/mL.

It was reported an HPLC method with fluorometric detection with high sensitivity for detection of melatonin in a single pineal cell [35]. Detection limit of this method was 3 pg/mg pineal tissues. Detection conditions were set as excitation and emission wavelengths at 285 nm and 345 nm, respectively, gain of 1000 and attenuation of 4. For vegetable tissues, a C18 reverse-phase column HPLC coupled with fluorescence, electrochemical, colorimetric and mass detectors have been employed for melatonin detection. Mobile phase of either 0.1 mM potassium phosphate buffer (pH 4.5) with acetonitrile (20%) or a mixture (40:60, v/v) of methanol: 50 mM Na_2HPO_4/H_3PO_4, pH 4.5 is widely used in melatonin quantification in plant tissues [36].

Gas chromatography coupled with mass spectroscopy was used [27] for estimation of melatonin in tomato. These GC- MS methods have very low detection of 1 pg/mL, post automated solid phase extraction (for plasma samples) and liquid- liquid extraction (for saliva samples). LC or GC coupled with tandem mass spectrometry operated in positive electro-spray ionization mode (MS/ESI+) with direct flow injection has also been used recently for melatonin estimation.

The single ion product-ion transition has been monitored for melatonin (with scan range m/z of 233-174). Validation for biological samples showed that the analytical performance for this method has a recovery of 68.9-70.5%; excellent intra and inter-assay precision, 0.8-2% and 1.5-5.9%, respectively, and the limit of detection is 0.1 ng/mL and quantification is 0.5 ng/mL. Lower detection and quantification limits achieved with a triple quadrupole mass spectrometer in saliva samples were 1.05pg/mL and 3.0pg/mL, respectively [37].

Selecting a suitable solvent system for extraction of melatonin from the food matrix poses a problem in chromatographic techniques. Besides, the limit of detection and quantification varies greatly with different detectors.

Other techniques: Cyclic voltametery has been used to determine melatonin in pharmaceutical preparations. This method is based on the redox behavior of the molecule but the major drawback in this method is that an intermittent renewal of the electrode surface is required, making this method an expensive one [38].

A chemiluminescence method has been proposed for the determination of melatonin [39]. The assay is based on emission of ultra-weak chemiluminescence after the reaction of melatonin with H_2O_2 and acetonitrile, under alkaline conditions. The formation of singlet oxygen in the reaction implies that other compounds apart from melatonin such as phenolics and terpenes can interfere with the analysis. Therefore, it is not advisable to use this method with vegetable tissue wherein these compounds are in prominence.

Alternative methods of melatonin analysis include procedures based on molecular emission spectrofluorimetry and micellar electrokinetic chromatography[40]. Both methods have been proven to be suitable for rapid and reliable determination of melatonin in different pharmaceutical preparations, containing melatonin alone or melatonin in association with other active principles such as tryptophan and adenosine.

Melatonin has also been determined spectrophotometrically after reaction with $KMnO_4$ and formaldehyde, in acid medium. This method has been used to determine melatonin in grape skin extracts with little accuracy since other compounds naturally occurring in grapes have possibly reacted (Iriti and others 2006).

Bioavailability of exogenous Melatonin: Melatonin being an amphiphilic molecule is readily absorbed in the gastrointestinal tract when taken orally in pure form, and influences the blood plasma concentration. Feeding of chicks with melatonin-rich foodstuffs is reported to increase the serum melatonin level. Melatonin is a short lived molecule with an average life

of about 20 to 40 min, depending upon the conditions. Melatonin requires 45 min for its absorption in gastrointestinal tract and its detection in blood after oral administration (before its metabolism and elimination. Melatonin is released from the GI tract to the circulation in remarkable amounts upon chemical stimuli, particularly in response to tryptophan and other indole amines.

The oral consumption of melatonin rich food is reported to increase the serum melatonin concentration. An experiment conducted on rats to monitor its blood melatonin concentrations after consumption of walnuts showed serum melatonin concentration to increase significantly, which positively correlated with an increase in total antioxidant capacity. Therefore it could be concluded that the fluctuations in blood melatonin concentrations can be correlated with the ability of the blood to detoxify toxic free radicals and related reactants in mammals, including humans [11, 41].

CONCLUSION: Melatonin, a pineal gland hormone which regulates the circadian cycle, is an established potent antioxidant and is also a molecule of health, nutritional and pharmaceutical importance. As the production of pineal melatonin is known to decline progressively with age leading to several health complications, external supplementation of melatonin is a prerequisite for maintenance of good health. The natural sources of melatonin, its bioavailability and its contribution to health is well known; however, further studies are needed to establish plant based foods as primary sources of melatonin. The pronounced health benefits of melatonin could enable this molecule to find wide application as a functional food ingredient and as a nutraceutical.

REFERENCES:

1. Lerner AB, Case JD, Heinzelmann RV: Structure of melatonin. J. Am. Chem.Soc 1959; 81: 6084–6085.
2. Advisory Group on Ionizing radiation, Pineal gland and melatonin, Power frequency electromagnetic fields, melatonin and the risk of breast cancer, Great Britain: Health Protection Agency 2006; 31 – 60.
3. Hardeland R, Pandi-Perumal SR: Melatonin, a potent agent in antioxidative defense: actions as a natural food constituent, gastrointestinal factor, drug and prodrug. Nutr. Metab 2005; 2: 22 – 37.
4. Beyer CE, Steketee JD, Saphier D: Antioxidant properties of melatonin- an emerging mystery. Biochem. Pharmacol 1998; 56: 1265–1272.
5. Sliwinski T, Rozej W, Morawiec-Bajda A, Morawiec Z, Reiter R, Blasiak J: Protective action of melatonin against oxidative DNA damage: chemical inactivation versus base-excision repair. Mut. Res 2007; 634: 220– 227.
6. Macchi MM, Bruce JN: Human pineal physiology and functional significance of melatonin. Front. Neuroendocrin 2004; 25: 177–195.
7. Morera AL, Abreu P: Seasonality of psychopathology and circannual melatonin rhythm. J. Pineal Res 2006; 41: 279–283.
8. Claustrat B, Bruna J, Chazot G: The basic physiology and pathophysiology of melatonin. Sleep Med. Rev 2005; 9: 11–24.
9. Blask DE: Melatonin, sleep disturbance and cancer risk. Sleep Med. Rev 2009; 13: 257–264.
10. Stehle JH, Gall CV, Korf HW: Melatonin: a clock-output, a clock-input. J. Neuroendocrinol 2003; 15: 383–389.
11. Reiter RJ, Tan DX, Mayo JC, Sainz, RM, Leon J, Czarnocki Z: Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. Acta Biochim. Pol 2003; 50: 1129–1146.
12. Arendt J: Importance and relevance of melatonin to human biological rhythms. J. Neuroendocrinol 2003; 15: 427–431.
13. DerMarderosian A, Liberti L, Beutler JA, Grauds C, Tatro DS, Cirigliano M, DeSilva Jr. D: Melatonin In: DerMarderosian A, Liberti L, Beutler JA, Grauds C, Tatro DS, Cirigliano M, DeSilva Jr. D; editors, The Review of Natural Products, St. Louis, Facts and Comparisons Publishing Group, 2008; 400–403.
14. Poeggeler B, Thuermann S, Dose A, Schoenke M, Burkhardt S, Hardeland R: Melatonin's unique radical scavenging properties-role of its functional substituents as revealed by its comparison with its structural analogs. J. Pineal Res 2002; 33: 20-30.
15. Tomas-Zapico C, Coto-Montes A: A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. J Pineal Res 2005; 39: 99–104.
16. Martin M, Macias M, Escames G, Reiter, RJ, Agapito MT, Ortiz GG, Acuna-Castroviejo D: Melatonin-induced increased activity of the respiratory chain complexes I and IV can prevent mitochondrial damage induced by ruthenium red in vivo. J. Pineal Res 2000; 28: 242–248.
17. Haldar C, Ahmad R: Photoimmunomodulation and melatonin. J. Photoch. Photobio. B 2010; 98: 107–117.
18. Aher VD, Wahi AK: Melatonin: a novel biomaterial for immunotherapy. Int. J. Pharm. Sc. Res 2011; 2: 772-777.
19. Pappolla MA, Chyan YJ, Poeggeler B, Frangione B, Wilson G, Ghiso J, Reiter RJ: An assessment of the antioxidant and antiamyloidogenic properties of melatonin: implications for Alzheimer's disease, J. Neural Transm 2000; 107: 203–231.
20. Kolar J, Machackova I: Melatonin in higher plants: occurrence and possible functions. J Pineal Res 2005; 39: 333–341.
21. Verspui G, Elbertse G, Sheldon FA, Hacking MAPJ, Sheldon AR: Selective hydroformylation of N-allylacetamide in an inverted aqueous two- phase catalytic system enabling a short synthesis of melatonin. Chem. Commun 2000; 1363–1364.
22. He L, Li JJ, Zhang JJ, Su P, Zheng SL: Microwave assisted synthesis of melatonin. Synthetic commun 2003; 33: 741–747.
23. Hardeland R, Poeggeler B: Non-vertebrate melatonin. J. Pineal Res 2003; 34: 233–241.
24. Hattori A, Migitaka H, Masayake I, Itoh M, Yamamoto K, Ohtani-Kaneko R, Hara M, Suzuki T, Reiter RJ: Identification of melatonin in plant seed and its effects on plasma melatonin

levels and binding to melatonin receptors in vertebrates. Biochem Mol. Bio. Int 1995; 35: 627–634.
25. Hernandez-Ruiz J, Cano A, Arnao MB, Melatonin: a growth-stimulating compound present in lupin tissues. Planta 2004; 220: 140–144.
26. Manchester LC, Tan DX, Reiter RJ, Park W, Monis K, Qi W: High levels of melatonin in the seeds of edible plants: possible function in germ tissue protection. Life Sci 2000; 67: 3023–3029.
27. Van-Tassel DL, O'Neill SD: Putative regulatory molecules in plants: evaluating melatonin. J. Pineal Res 2001; 31: 1–7.
28. Iriti M, Rossoni M, Faoro F: Melatonin content in grape: myth orpanacea? J. Sci Food Agr 2006; 86: 1432–1438.
29. Burkhardt S, Tan DX; Manchester LC, Hardeland R, Reiter RJ: Detection and quantification of the antioxidant melatonin in Montmorency and Balaton tart cherries (Prunus cerasus). J. Agr. Food Chem 2001; 49: 4898–4902.
30. Murch SJ, Simmons CB, Saxena PK: Melatonin in fever few and other medicinal plants. Lancet 1997; 350: 1598–1599.
31. Garcia-Parrilla CM, Cantos, Troncoso AM: Analysis of melatonin in foods. J. Food Comp Anal 2009; 22: 177–183.
32. Chen Q, Wen-bo Q, Reiter RJ, Wei W, Wang BM: Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of Brassica juncea. J. Plant Physiol 2009; 166: 324-328.
33. Puerta DC, Carrascosa-Salmoral MP, Garcia-Luna PP, Lardone PJ, Herrera JL, Fernandez-Montesinos R, Guerrero JM, Pozo D: Melatonin is a phytochemical in olive oil. Food Chem 2007; 104: 609–612.
34. Simonin G, Bru L, Lelievre E, Jeanniot JP, Bromet N, Walther B, Boursier- Neyret C: Determination of melatonin in biological fluids in the presence of the melatonin agonist S20098: comparison of immunological techniques and GC–MS methods. J. Pharmaceut. Biomed 1999; 21: 591– 601.
35. Vitale AA, Ferrari CC, Aldana H, Affanni JM: Highly sensitive method for the determination of melatonin by normal-phase high-performance liquid chromatography with fluorometric detection. J. Chromatogr. B 1996; 681: 381–384.
36. Reiter RJ, Manchester LC, Tan DX: Melatonin in walnuts: influence on levels of melatonin and total antioxidant capacity of blood. Nutrition 2005; 21: 920–924.
37. Eriksson K, Ostin A, Levin JO: Quantification of melatonin in human saliva by liquid chromatography–tandem mass spectrometry using stable isotope dilution. J. Chromatogr. B 2003; 794: 115–123.
38. Radi A, Bekhiet GE: Voltametery of melatonin at carbon electrodes and determination in capsules. Bioelectrochem. Bioenerg 1998; 45: 275–279.
39. Lu J, Lau C, Lee MK, Kai M: Simple and convenient chemiluminescence method for the determination of melatonin. Anal Chim Acta 2002; 455: 193– 198.
40. Pucci V, Ferranti A, Mandrioli R, Raggi MA: Determination of melatonin in commercial preparations by micellar electrokinetic chromatography and spectrofluorimetry. Anal. Chim. Acta 2003; 488: 97–105.
41. Maldonado MD, Moreno H, Calvo JR: Melatonin present in beer contributes to increase the levels of melatonin and antioxidant capacity of the human serum. Clin. Nutr 2009; 28: 188–191.

****