MELATONIN – A "MAGIC BIOMOLECULE"

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(Recieved on 15.07.2007)

Abstract

Melatonin is the pineal hormone and its activity is associated with the two known receptors-ML1 and ML2. Melatonin is an indoleamine and has found potent role in the biological regulation of circadian rhythms, sleep-mood disorders, cancer, neurodegenerative disorders and aging. Melatonin passively diffuses into bloodstream showing its maximum effectiveness. Melatonin preserves mitochondrial homeostasis, increases gene expression for antioxidant enzymes and enhances quality of life. It has proved to be a powerful antioxidant and its efficacy is particularly exhibited in neurodegenerative disorders like Alzheimer's, Parkinson's disease whose pathogenesis is associated with the cytotoxic effect of oxygen free radicals. Therapeutic trials with melatonin have been effective in slowing down the progression of some neurodegenerative disorders. Studies suggest that melatonin may have a clinical potential for the treatment of neurodegenerative disorders and many other incurable ailments.

Keywords: Pineal, Melatonin, Antioxidant, Neurodegenerative disorders, Therapeutic trials.

Introduction

French philosopher and scientist Rene Descartes has declared in the seventeenth century that the pineal gland is "the seat of soul". Though first identified by 'Aron Lerner 'in 1958 and now the object of over 20,000 peer reviewed scientific studies, scientists are still discovering daily new and vital roles for this 'small but mighty indoleamine'.The pea-sized gland, pineal in the brain produces melatonin, a hormone secreted most profusely as the darkness falls and is the smallest hormone secreted in humans, in terms of volume having powerful effects. Melatonin, a derivative of an essential amino acid tryptophan, was first identified in bovine pineal tissue and subsequently it has been portrayed exclusively as a hormone. It is released every night as part of our time dependent biorhythms to help induce sleep and recuperation from fatigue. This hormone is postulated to mediate photoperiodicity, is light sensitive and subject to oxidation. It is not only a powerful hormone, synthesized in the pineal gland, secreted into the cerebrospinal fluid and the circulation, influenced by the light and dark but it is also a ubiquitous nutrient food, being found in many common plants from bananas to morello cherries and in most of the cereals. Melatonin is an unusually controversial biomolecule. This neurologically and endocrinologically active substance can reset the biological clock, fix jet lag, ward off depressive and panic disorders, fight common cold, cancers and even aging. Melatonin is potent antioxidant. It has also been described as the pacemaker of the aging clock in humans. It fortifies the immune system and stabilizes the nervous system. Melatonin plays an important role in proliferation of neuronal cell. It is recently called as a wonder drug useful for treating everything from AIDS to Alzheimer's. The reasons for this non-ending interest in melatonin is due to our modern age of jet travel, telecommunications, advanced techno times, electronic gadget-age and commercialization but it's darker counterpart as direct and indirect effects on the biotic life totally unknown.

What is the pineal gland?

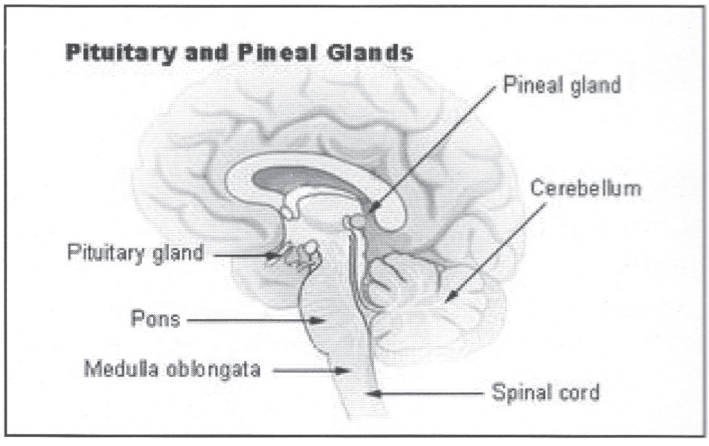


Fig.1 Longitudinal section of human brain to show the location of pineal gland.

The pineal gland is a tiny and mighty gland, crucial to the healthy functioning of our physical, mental, emotional and spiritual body. Scientists consider pineal to be the master gland, "the regulator of the regulators" which ensures proper biorhythms of the hormonal and cellular systems (1,2). The pineal gland or *epiphysis* synthesizes and secretes melatonin, a structurally simple hormone that communicates information about environmental lighting to various parts of the body, which induces sleep, regulates the circadian rhythms (3-4). Ultimately, melatonin has the ability to entrain biological rhythms and has important effects on reproductive function of many animals. The light-transducing ability of the pineal gland has led some to call the pineal the "third eye". As one grows older, balanced flow of the vital fluids is disturbed, the pineal gland starts to calcify or harden and the melatonin production decreases and too much of serotonin is released (5).

Anatomy of the Pineal Gland

The pineal gland is a small organ shaped like a pine cone (hence its name). It is located on the midline, attached to the posterior end of the roof of the third ventricle in the brain. The pineal varies in size among species; in humans it is roughly 1 cm in length, whereas in dogs it is only about 1 mm long. To observe the pineal, reflect the cerebral hemispheres laterally and look for a small grayish bump in front of the cerebellum.

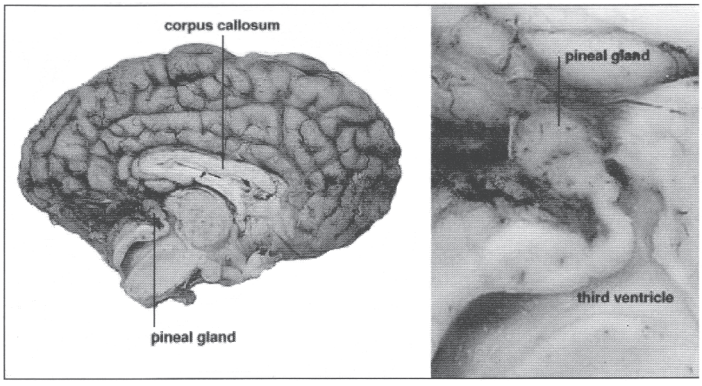


Fig. 2. The images show the pineal gland of a horse in relation to the brain.

Histologically, the pineal is composed of "pinealocytes" and glial cells. In older animals, the pineal often contains calcium deposits- "brain sand" and the Melatonin concentration decrease, as one grows older (6-8).

The retinal transmission of information about light-dark exposure to the pineal gland

Synthesis and secretion of melatonin is dramatically affected by light exposure to the eyes. The fundamental pattern observed is that serum concentrations of melatonin are low during the daylight hours, and increase to a peak during the dark (9). Light exposure to the retina is first relayed to the suprachiasmatic nucleus of the hypothalamus, (10) an area of the brain well known to coordinate biological clock signals(1,11).Fibers from the hypothalamus descend to the spinal cord and ultimately project to the superior cervical ganglia, from which post-ganglionic neurons ascend back to the pineal gland(12). Thus, the pineal is similar to the adrenal medulla in the sense that it transduces signals from the sympathetic nervous system into a hormonal signal (13).

Melatonin: Synthesis, Secretion and Receptors

The pineal produces melatonin from serotonin. Serotonin is a neurotransmitter and is most highly concentrated in the pineal. The precursor to melatonin is serotonin, that itself is derived from the amino acid tryptophan. Within the pineal gland, serotonin is acetylated and then methylated to yield melatonin (14). 6-sulphatoxy-melatonin (aMT6s) is the major metabolite of melatonin. Human body converts melatonin into aMT6s and excretes it in urine (15). It is naturally synthesized from the amino acid tryptophan (derived from serotonin) by the enzyme 5-hyroxyindole-methyltransferases (16). Circulating melatonin is mostly 6-hydroxylated molecule and excreted as 6-sulfatoxymelatonin. Pyrrole-ring cleavage is of higher importance in other tissues, especially the brain. The product, N1-acetyl-N2-formyl-5-methoxykynuramine, is formed by enzymatic, pseudoenzymatic, photocatalytic, and numerous free-radical reactions. Additional metabolites result from hydroxylation and nitrosation. The secondary metabolite, N1-acetyl-5-methoxykynuramine, supports mitochondrial function and down regulates cyclooxygenase2 levels (17). The duration of melatonin secretion each day is directly proportional to the length of the night. Sufficiently bright light suppresses melatonin. Normally, production of melatonin is inhibited by light and permitted by darkness. Abnormal melatonin production is seen when rhythms are disordered ;(18-19) for example as in shift work, jetlag, blindness, old age and poor sleep or some metabolic disorders. Two melatonin receptors have been identified from mammals designated Mel1A and Mel1B that are differentially expressed in different tissues (20) and probably participate in implementing differing biologic effects. These are G protein-coupled cell surface receptors (21). The highest density of receptors has been found in the suprachiasmatic nucleus of the hypothalamus, the anterior pituitary (predominantly pars tuberalis) and the retina (22). Receptors are also found in several other areas of the brain (23-24). The membrane melatonin receptors also controls reproductive phenomenon in mammals (25).

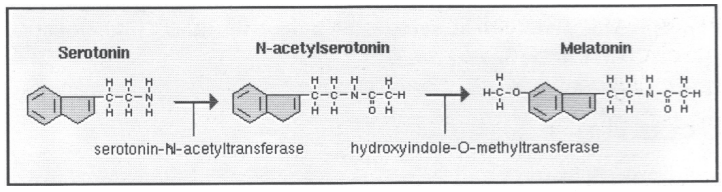


Fig.3 Melatonin synthesis from serotonin in the pineal gland.

Melatonin chemistry

Empiric formula- C13 H16 N2 O2

Molecular weight – 232.28

Melting point-116.11c.

Bioavailability- 30 to 50 %

Elimination- 32 to 40 minutes (half life)

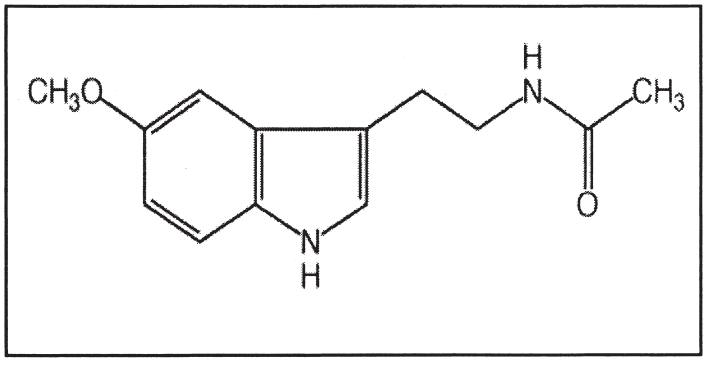
Metabolism- liver

Excretion- urine

Indicated for- jetlag, insomnia, sleeps disorders etc.

Structure of melatonin

5-methoxy-n acetyl tryptamine



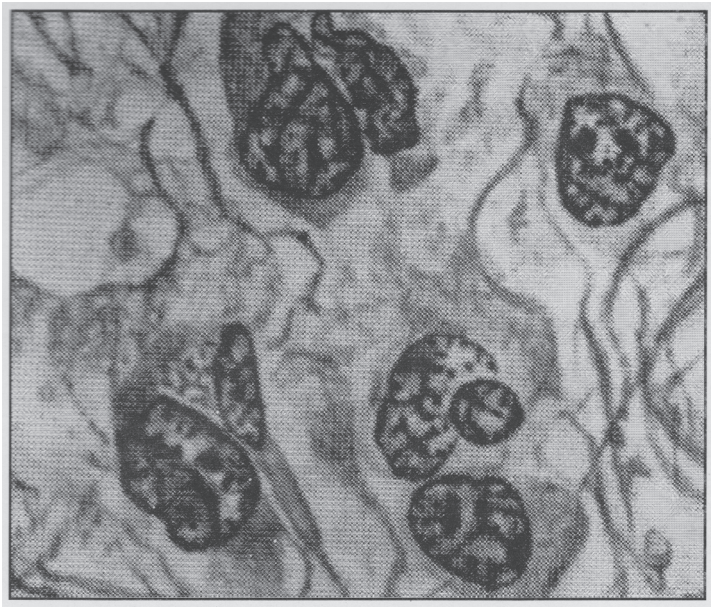


Fig. 4 The histological section of pineal to show pinealocytes that secrete melatonin.

Occurrence

Melatonin is found in all living creatures from algae to humans, at levels that vary in a diurnal cycle. In animals melatonin is most highly concentrated in the brain (nervous tissue) produced by pinealocytes and also by retina (26) and gastrointestinal tract. It distributes in all tissues and cellular compartments being especially high in the nucleus, mitochondria and bile (27). Tissues producing melatonin are retina, human ovary, lens, human bone marrow (28) etc. Melatonin, is produced by bacteria, protozoa, plants, fungi, invertebrates, and various extra pineal sites of vertebrates, including gut, skin, Harderian gland, and leukocytes (29).In plants various plants from banana to Morella cherries and even in rice and all our cereal crops synthesize melatonin. Melatonin has been identified in a large number of plant taxa (30). Among angiosperms, melatonin has been found in more than 30 species belonging to 19 different families and in both monocotyledons and dicotyledons (31). Within plant tissues, melatonin has been found in roots, stems, leaves, fruit, and seeds. Most of the plants that have been analyzed are edible. (30, 32) Melatonin is present in the earliest life forms and is found in all organisms including bacteria, algae, fungi, plants, insects, and vertebrates including humans. As melatonin is also ingested in foodstuffs such as vegetables, fruits, rice, wheat and herbal medicines, from the nutritional point of view, melatonin can also be classified as a vitamin. It seems likely that melatonin initially evolved as an antioxidant, becoming a vitamin in the food chain, and in multicellular organisms (32, 33).

Role and Effects

When the sun goes down, darkness falls, the pineal goes to work. As melatonin production rises, one begins to feel less alert; body temperature starts to fall as well. Sleep seems more inviting. Then melatonin level drop quickly with dawning of a new day. Thus melatonin levels go hand in hand with light dark cycle. This hormone is the sleep trigger regulating the natural sleep cycle. It regulates the circadian rhythm that is the maintenance of biological clock of the brain at locus of suprachiasmatic nuclei (SCN) (34). Melatonin plays a role of a marker of phases in the clock (16, 35).

One of the first experiments conducted to elucidate the function of the pineal, extracts of pineal glands from cattle were added to water containing tadpoles. Interestingly, the tadpoles responded by becoming very light in color or almost transparent due to alterations in melanin pigment distribution. Although such cutaneous effects of melatonin are seen in a variety of "lower species", the hormone does not have such effects in mammals or birds. It reverses the darkening effect on skin. It is light sensitive molecule and prone to self-oxidation. In hundreds of investigations melatonin has been documented as a direct free radical scavenger and an indirect antioxidant as well as an important immunomodulatory agent. Besides being a highly effective direct free radical scavenger and indirect antioxidant, (36) melatonin has several features that make it of clinical interest (37). Melatonin is readily absorbed when it is administered via any route, it crosses all morphophysiological barriers, e.g. cellular membranes, blood-brain barrier and placenta, with ease, it seems to enter all parts of every cell where it prevents oxidative damage, it preserves mitochondrial function, and it has low toxicity. While blood melatonin levels are normally low, tissue levels of the indoleamine can be considerably higher and at some sites, e.g., in bone marrow cells and bile, melatonin concentrations exceed those in the blood by several orders of magnitude. Actions are pleiotropic, immunostimulatory (38) and cytoprotective mediated by membrane and nuclear receptors, other binding sites or chemical interactions (39). Antioxidative protection, safeguarding of mitochondrial electron flux, (40) and in particular, neuroprotection, have been demonstrated in many experimental systems (41). Melatonin has antitumor activities as well. The elevated endogenous melatonin can exert oncostatic effect via immunomodulation or alteration of reproductive hormones or antioxidant activity. Elevated glucocorticoid hormone levels potently suppress adult hippocampal cell proliferation and in contrast melatonin increase cell proliferation, this implies that glucocorticoid receptors expression is influenced by melatonin administration. Melatonin preserves mitochondrial homeostasis, (42) reduces free radical generation by enhancing mitochondrial glutathione levels, and safeguards proton potential and ATP synthesis by stimulating complex 1 and complex 4 activities (43). Melatonin is well known for its functional interactions with the neuroendocrine axis and with the circadian biorhythms (16). Very recently it has also been found to have neuroprotective and neuroregenrative effects in models of neuronal cell deaths in which excitotoxins are involved (44). Melatonin plays important role in many different physiological processes including, regulation of circadian rhythms, sleep promotion (45) and reproduction (46). It has an anti-inflammatory effect against ischemia/ reperfusion injury and stroke models (47) Melatonin is also effective in protecting the nuclear DNA and its associated histone proteins (48).Human lymphocytes also produce melatonin when attacked by a mitogen that is Phytohaemagglutinin (PHA). One of the

main targets of melatonin is thymus-central organ of immune system, (49) it plays an important role in immuno-neuro-endocrine network (38) and Immunocancer therapy (50).Neuroprotective and neuroregenrative effect of Melatonin have been tested and are being further studied for establishing it, as a drug molecule to be used in neurodegenerative diseases. When administered exogenously it suppress estrogen synthesis, possibly through reducing luteinizing hormone and FSH secretion. It also interferes with binding of estrogen to its receptors (22). Antagonism has been established between melatonin and the reproductive hormones. Melatonin exerts inhibitory effect at various levels of hypothalamic pituitary gonadal axis in brain. The effect of melatonin on reproductive systems can be summarized by saying that it is anti-gonadotropic (25). In other words, melatonin inhibits the secretion of the gonadotropic hormones, luteinizing hormone and follicle-stimulating hormone from the anterior pituitary. Much of this inhibitory effect seems due to inhibition of gonadotropin-releasing hormone from the hypothalamus, which is necessary for secretion of the anterior pituitary hormones. One practical application of melatonin's role in controlling seasonal reproduction is found in its use to artificially manipulate cycles in seasonal breeders

Powerful Antioxidant

It was 'Ianas' who first suggested that melatonin may have a role in scavenging free radicals(51).Melatonin has been shown to be highly effective in reducing oxidative damage in the central nervous system(52) this efficacy derives from its ability to directly scavenge a number of free radicals(16) and to function as an indirect antioxidant(53). In particular melatonin detoxifies the free radicals via electron donation (54). Its function as a free radical scavenger is likely facilitated by the ease with which it crosses the morphological barriers like the blood brain barriers to enter the nerve cells and sub cellular compartments (55).It scavenges hydroxyl, carbonate and various organic radicals, peroxynitrite and other reactive nitrogen species (56). Melatonyl radicals formed by scavenging combine with and, thereby, detoxify superoxide anions in processes terminating the radical reaction chains. Melatonin also enhances the antioxidant potential of the cell by stimulating the synthesis of antioxidant enzymes like superoxide dismutase, glutathione peroxidase and glutathione reductase, and by augmenting glutathione levels (52).Definitive evidence that melatonin function as a direct scavenger of hydroxyl radical (OH) was provided by Tan et al., in 1993. OH- is widely accepted as the most damaging molecule endogenously produced in aerobic organisms. The OH radical mutilates any molecule in the vicinity of where it is produced (57). In *in vitro* and *in vivo* experiments, melatonin has been found to protect cells, tissues and organs against oxidative damages induced by a variety of free radicals generating agents and processes, including the cyanide poisoning, glutathione depletion, ischemia reperfusion, kainic acid induced excitotoxicity and MPTP (1 methyl-4phenyl 1,2,3,6-tetrahydropyridine) (58). Melatonin as an antioxidant protects the nuclear DNA, membrane lipid and cytosolic proteins from oxidative damage but is also reported to alter the activities of enzymes that improve the total antioxidative defense capacity of the organism (59). Melatonin not only stimulates 'antioxidant cascade' but also scavenges resulting metabolite effectively. They claimed that melatonin was more efficient peroxyl radical scavenger as compared to vitamin E.

Melatonin's Action on antioxidative enzymes and gene expression

Melatonin stimulates several important antioxidative enzymes, their activity or their gene expression (60, 61) including Super Oxide Dismutase (SOD) Glutathione peroxidase (GPx)(62) and Glutathione reductase.(GRx).Experimental evidences show that melatonin promotes activity of GRx, thereby helping to maintain high levels of reduced glutathione. At a molecular level melatonin has been reported to increase tissue levels of messenger RNA for both manganese SOD as well as copper SOD. It was also documented in the same study that messenger RNA levels for GSH-Px were also augmented after melatonin administration. The amphiphilic property of melatonin and importance as a vital antioxidant depends on several characteristics such as: its lipophilic and hydrophilic nature, its ability to cross all barriers with ease and its availability to all tissues and cells(52). Levels of melatonin are two to three times of magnitude higher than maximal blood melatonin concentration in cerebrospinal fluid (63). Melatonin efficacy was compared with classical antioxidants in terms of pharmacologically protective agent against free radical damage and the in vivo studies reported that melatonin was found to be more effective at a very lower dose than other antioxidants (64).

The life extender role

Increased melatonin has significantly increased life span in the test animals. The systemic administration of melatonin stimulated neurogenesis, improved neuronal survival rate and facilitated behavioral recovery after transient focal cerebral ischemia in mice (65). It was shown that melatonin increases cell proliferation in the dentate gyrus region of maternally separated rats (66). Melatonin reduces the oxidative damage in the central nervous system effectively and improves the life of neurons due to its ease with which it crosses the blood brain barrier (35). Melatonin has antidepressant properties and the hormone can be recommended to improve the quality of life of cancer patients on chemotherapy (67). It has been proved that melatonin improves the quality of sleep and is helpful in maintaining the biorhythms at a pace (45).

Melatonin and the neurodegenerative diseases

Melatonin is a free radical scavenger, an antioxidant and immunomodulatory agent. Antioxidant properties of melatonin are connected with its neuroprotective activity in several degenerative disorders (68). The etiology of the neurodegenerative diseases, which are characterized by the progressive and irreversible destruction of specific neuronal populations, is complex and multifactorial (69, 70). One of causes of neurodegenerative damage in the nervous system is oxidative injury, which results

from an imbalance between free radical formation (71) and antioxidative mechanisms (72). The efficacy of melatonin in the inhibition of the oxidative stress was estimated (73) in various neurodegenerative disorders whose pathogenesis is associated with cytotoxic activity of free oxygen radicals, such as Alzheimer's or Parkinson's disease (74).The experimental findings related to the neuroprotective role of melatonin in particular, focuses on research directed at models of Huntington's disease, Alzheimer's disease (75) and Parkinsonism (43). The decline in melatonin production in aged individuals has been suggested as one of the primary contributing factors for the development of age-associated (76) neurodegenerative diseases, e.g., Alzheimer's disease (77). Melatonin has been shown to be effective in arresting neurodegenerative phenomenon seen in experimental models of Alzheimer's disease, Parkinsonism and ischemic stroke. Melatonin preserves mitochondrial homeostasis, reduces free radical generation, e.g., by enhancing mitochondrial glutathione levels, and safeguards proton potential (78) and ATP synthesis by stimulating complex IV and I activities (52). Therapeutic trials with melatonin have been effective in slowing the progression of Alzheimer's disease but not of Parkinson's disease. Melatonin's efficacy in combating free radical damage in the brain suggests that it may be a valuable therapeutic agent in the treatment of cerebral edema after traumatic brain injury. Different patterns of melatonin concentrations is observed at different stages of neurodegenerative diseases related to the age and the secretion patterns are being studied for the onset of neurodegenerative diseases (19, 79, 80)

Recent trends in Melatonin research

The recent research has been focused largely on the neuroprotective and neuroregenrative properties of Melatonin (81). Melatonin is a powerful antioxidant, lack serious toxicity and its natural occurrence has evoked hopes that it might be used as a promising drug in the near future for neurological disorders (82, 83). Melatonin directly scavenge free radicals, stabilizes and protects the nervous system. The recent researches have shown that melatonin could be wonder drug in near future for various ailments.(84)Melatonin versatility and combination of actions is making it a highly effective pharmacological agent for neuroprotection, neuroregenration and to treat and cure the neurodegenerative disorders, brain tumors, and cancers (85) as it is one of the safest product around with no observed late side effects or consequences. In recent research work melatonin is shown to prevent the delayed death of hippocampal neurons induced by enhanced excitatory neurotransmission and nitridergic pathways (86) Scientist have proved melatonin's role in cell proliferation in the dentate gyrus region of maternally separated rats (66). Researches are studying the mechanism of neuronal plasticity and stressor toxicity during aging and role of melatonin during these processes. Melatonin's role in ischemia reperfusion injury has been reported (65) that pretreatment with melatonin exerts anti-inflammatory effects against ischemia/reperfusion injury in a rat middle brain cerebral artery occlusion stroke model. The recent studies investigated whether systemic administration of melatonin stimulates endogenous neurogenesis, improves neuronal survival and facilitates behavioral recovery after transient focal cerebral ischemia in mice. The results demonstrated that, in all the evaluated regions of the brain including cortex, striatum, hippocampus and sub ventricular zone the number of proliferating cells of neural lineage was found significantly higher for melatonin treated animals than for the control groups. The number of surviving neurons was also significantly higher in melatonin treated animals (65). Researches are going on to investigate the role of melatonin in early developmental events of adult neurogenesis, apoptosis and neurophilic migration in adult hippocampus region. Melatonin pharmacological role as an agent against neuronal loss in experimental models of Huntington's disease, Alzheimer's disease and Parkinson's disease needs to be generated before the drug can find place in neurology clinics (87).The experimental findings are related to the neuroprotective role of melatonin and its high effectiveness in scavenging a number of free radicals such as the peroxyl radical, peroxy nitrite anion, nitric oxide, singlet oxygen etc. and reducing the oxidative damage in cells. The role of physiological levels of melatonin in forestalling oxidative damage in the brain is currently being tested.

In our laboratory attempt has been made to investigate the neuroprotective effects of melatonin. The results were compared to the herbal formulations known for their potent role in neuroprotection and melatonin was found to be more effective. Studies are being carried to assess the action of melatonin on the antioxidant enzyme profiles.

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