Aims & Objectives
2. Aim

To investigate whether melatonin acts on its own or in conjugation with allogeneic thymus graft on the onset of puberty in male juvenile rats.

2.1 Objectives

1. To administer exogenous melatonin to intact juvenile male rat pups either alone, or in presence of allogeneic thymus graft and to observe the age on descent of testes for the confirmation of the onset of puberty.

2. To note the changes in body weight, male reproductive organs and thymus weight, on the day of descent of testes and also pancreas weight in melatonin treated rats.

3. To study the histometric changes in
   a. Testes,
   b. Epididymides,
   c. Thymus on the day of descent of testes.

4. To study and correlate the changes of serum hormonal profile of
   a. Follicle Stimulating Hormone,
   b. Leutenizing Hormone,
   c. Prolactin,
   d. Testosterone and
e. Growth hormone with gross and histological changes.

2.2 Hypothesis

Exogenous melatonin alters the onset of puberty in male juvenile rats either alone or in association with allogeneic thymus graft.
3. REVIEW OF LITERATURE

The pineal gland is the subject of considerable investigation as it proves to be a productive experimental model for studying circadian rhythms\(^\text{16}\).

The hormone melatonin (MT), an indoleamine secreted by the pineal gland is of considerable interest for its circadian regulation of a variety of physiological and neuroendocrine processes. The first demonstration of biological activity of melatonin was reported in 1917 when McCord and Allan discovered that extracts of bovine pineal gland caused blanching of Rana pipien tadpole skin\(^\text{17}\). This bioassay was used to isolate and purify melatonin from pineal extracts and also led to the elucidation of its chemical structure as 5-methoxy-N-acetyltryptamine\(^\text{18}\). Melatonin has been identified in the pineal glands of all mammalian and avian species. In the former it also exists in other tissues in a low concentration including peripheral nerve and urine\(^\text{19}\).

3.1 Biosynthesis and metabolism of melatonin – Melatonin Rhythm

The circulating precursor for pineal melatonin is tryptophan which is converted to 5-hydroxytryptophan by hydroxylase, whose activity is high in the pineal. The 5-hydroxytryptophan is decarboxylated by l-aromatic aminoacid decarboxylase to form the amine, 5-hydroxytryptamine (serotonin). The conversion of 5-hydroxytryptamine to melatonin is initiated by its acetylation, in the presence of the enzyme serotonin N-acetyltransferase to yield N-acetyl serotonin\(^\text{18}\). N-acetyl serotonin is transformed to melatonin through the action of hydroxy-indole-o-methyl transferase (HIOMT)\(^\text{20}\).

HIOMT is highly localized in the pineal glands of mammals and birds. Small amounts of the enzyme are also present in the retina of the rat. In other classes (reptiles, amphibia and fish) HIOMT is also found in the eye, brain as well as in the pineal\(^\text{19,21}\).
The half life of circulating melatonin is approximately 10 min\(^22,23,24\) and it gets rapidly cleared by the liver with the help of a microsomal enzyme that oxidizes most of it to 6-hydroxymelatonin. This is then conjugated with sulfonic or glucuronic acid and excreted in the urine\(^19,25\). Alternatively, melatonin is converted to 5-methoxytryptamine by deacetylation, which is deaminated to 5-methoxyindoleacetic acid and 5-methoxytryptophol\(^26,27,28\) which takes place in retina in addition to liver. Melatonin is metabolized by indoleamine 2,3-dioxygenase to L-kynurenine by cleavage of the indole ring in the choroids plexus of brain\(^29\). It also gets richly concentrated in organs like ovary, pituitary and sympathetic ganglia\(^19\).

The activity of HIOMT is inhibited by continuous illumination and is stimulated by continuous darkness in rats\(^19\). Effect of light on melatonin synthesis occurs diurnally under normal lighting conditions; pineal HIOMT activity is lowest at the end of light period and with the onset of darkness activity rises sharply\(^30\). It was shown that information about environmental light reaches the rat pineal in the following pathway:

Retina $\rightarrow$ inferior accessory optic tract $\rightarrow$ medial forebrain bundle $\rightarrow$ medial terminal nucleus of the accessory optic system $\rightarrow$ preganglionic sympathetic tract in the spinal cord $\rightarrow$ superior cervical ganglia $\rightarrow$ post ganglionic sympathetic fibres $\rightarrow$ parenchymal cells of the pineal\(^16\).

As the age advances pineal melatonin synthesis and elevation of serum melatonin is known to be reduced in several species. In Wistar rats pineal HIOMT activity falls (17-55\%) after 18 months of age, but nocturnal arylalkylamine NAT activity in the pineal is not significantly altered. This gives an indication that in old age the capacity of the pineal to synthesize melatonin is reduced\(^31\). Gonadal
hormones influence the weight of the pineal gland and its ability to synthesize melatonin\textsuperscript{19}.

Melatonin is also synthesized in the vertebrate retina and harderian gland, and its synthesis in these organs is obeyed by the daily rhythm\textsuperscript{32,33,34,35}. Although retina has high capacity to synthesize melatonin, plasma melatonin rhythm is not significantly affected by it\textsuperscript{36,37,38,39,40}. This may be due to the rapid catabolism of melatonin in the retina and to 5-methoxytryptamine by the enzyme arylacylamidase\textsuperscript{26,27}. Thus indicating that melatonin synthesized in the retina serves mainly the local purposes.

Melatonin is a lipophilic compound and freely diffuses through biological membranes of cells from where it is produced and it readily crosses haematoencephalic barrier. Melatonin concentration in circulating blood reflects the changes of pineal melatonin concentration\textsuperscript{22,41}. Melatonin synthesis at extra pineal sites is independent of pineal production of melatonin\textsuperscript{42}.

During the day synthesis of melatonin is low and at night it is high which shows marked daily rhythm in its synthesis\textsuperscript{41,43}. In mammals, the entrainment of the endogenous clock and the overt circadian rhythms is involved by the melatonin\textsuperscript{44,45}. In non-mammalian vertebrates, the melatonin rhythm is necessary for the clock function; in several species, pinealectomy brings about the attenuation of free running daily rhythms, and rhythmic melatonin infusions restore the rhythmicity\textsuperscript{46,47,48,49,50,51}. Daily rhythm of melatonin in the circulation serves as the normal signal of the daily light/dark cycle. The biochemical basis of this rhythm is serotonin-N-acetyltransferase [arylalkylamine N-acetyltransferase, AA-NAT] which is considered as melatonin rhythm generating enzyme. The nocturnal rise in enzyme activity ranges from 7 to 150 fold on a species to
species basis, in rat, AA-NAT m-RNA levels exhibit a 150 fold rhythm, which reflects cyclic AMP dependent regulation of expression of the AA-NAT gene$^{52}$.

Also, serotonin content (precursor of melatonin), was reported to exhibit a marked 24-hour cycle in the rat pineal with peak levels of serotonin at about midday with a rapid fall soon after night fall$^{53}$. The daily rhythm in pineal serotonin is endogenous (circadian) but is synchronized by environmental lighting. The circadian rhythms of serotonin content in the rat pineal appear as early as 6 days after birth$^{16}$.

In all species, regardless of whether they are day active or night the melatonin increase occurs during night. This makes melatonin rhythm an endocrine marker of night. Duration of melatonin increase is controlled by photoperiod; on long photoperiods as in summer duration of the melatonin increase is short and on short photoperiods the melatonin increase pulse is long$^{54,55,56,57,58}$.

The circadian timing system generates the rhythm in melatonin production by the mammalian pineal gland. The components of that system which mediate the function are visual projections through the retinohypothalamic tract to the circadian pacemaker, the suprachiasmatic nucleus (SCN) of the hypothalamus; The SCN which generates a circadian signal transmits the same by SCN projections to the paraventricular nucleus; Paraventricular nucleus projections to the upper thoracic intermediolateral cell column of spinal cord; Preganglionic sympathetic fibres to the superior cervical ganglion; Postganglionic sympathetic fibers from the superior cervical ganglion to the pineal$^{59}$. 
In the newborn rat and birds the environmental lighting can reach the pineal gland by an extra retinal pathway but not in the adult rat^{16,60}.

By cutting sympathetic innervation to the pineal gland the circadian rhythms in pineal serotonin and NAT can be abolished. This gives an indication that there may be differences in the release of noradrenaline from sympathetic nerves during the day and night. Also it was found that turnover of noradrenaline in the sympathetic nerves innervating the pineal exhibits 24-hour rhythm. Utilization of noradrenaline is more at night than during the day. This rhythm in turnover persisted in blinded rats and was abolished in continuous light. All these suggest that the circadian rhythm in the pineal cell is under the influence of diurnal release of the neurotransmitter noradrenaline. The circadian rhythms of pineal gland are generated by changes in N-acetyltransferase activity which is controlled by the beta-adrenergic receptor. The beta-adrenergic receptor is stimulated by the sympathetic nerves through the release of noradrenaline^{16}.

Biosynthesis of melatonin is primarily regulated by a sympathetic innervation through the release of norepinephrine; in addition, sympathetic fibers may co-localize with other neuroactive factors like Neuropeptide Y (NPY), which is found in the nerve fibers of the pineal gland^{61}. Norepinephrine (NE) stimulates the rat pineal with a dose-related increase of melatonin release, independently from the phase of the day^{62}. Regulation of pineal melatonin synthesis by NE is brought about by its action at beta-adrenergic receptors on pinealocytes^{63}.

In the rat, increase in intracellular c-AMP levels results in the massive changes in NAT activity. An important aspect of the temporal control of melatonin production is the programmed down-regulation of responses to noradrenergic stimulation once the initial surge of c-AMP is produced. Other enzyme functions, including tryptophan hydroxylase and HIOMT activities are influenced by the
noradrenergic activation of the pineal gland. Exposure to very short duration (1 ms) of single high intensity light pulse at night time leads to a significant reduction in the NAT-activity and melatonin content in rats. So the rat pineal is capable of responding to very short light flashes of high intensity.

Suppression of melatonin by light depends on the wavelength of light and the circadian phase; a drop in circulating melatonin due to light exposure at night is related to the intensity (brightness), wavelength (colour) of light. Melatonin rhythm is disturbed by the visible and ultraviolet light as well as extremely low frequency electric and magnetic fields. Decreased melatonin content along with decreased serotonin NAT activity and catecholamine levels are also observed by stimulation of paraventricular nucleus during night time. The duration of the melatonin signal is the critical parameter of the melatonin rhythm, rather than circadian timing.

The administration of a single injection of melatonin to rat decreases the pineal norepinephrine turnover suggesting that endogenously released melatonin may be a regulatory signal for sympathetic synapses. Studies also show that melatonin may entrain directly a circadian pace maker controlling the NAT rhythm and affect its own rhythmic production. The exogenous melatonin exerts an acute regulatory action on the pineal melatonin synthesis by reducing the amplitude of the rhythm of endogenous melatonin and the effect is not circadian time dependent. Melatonin pulse may exert duplicate effects, a) it entrains the circadian rhythms and also the rhythm of sensitivity to melatonin and b) it induces the photoperiodic response when high melatonin concentration matches with the sensitive period.

Melatonin may have a role in maternal-foetal entrainment. In the early postnatal period melatonin induced entrainment may be important, when the
closed eyelids of the offspring do not allow the regular entrainment by the light-dark cycle. Maternal milk and placental blood both exhibit melatonin rhythm\textsuperscript{75,76}. Photoperiod modulates pineal melatonin rhythm in neonatal rats. A nyctohemeral difference in NAT activity is observed in 5 day old rats but until the second postnatal week rat pups do not show a significant difference in the day/night melatonin content and also the nocturnal light induced decrease. At the age of 13-17 days a daytime dark exposure elevates the pineal melatonin, which disappears by 21-day age, while nocturnal suppression by light persists, thus, indicating that the component of the circadian regulatory system matures at the end of 3\textsuperscript{rd} postnatal week. Plasma melatonin is detectable from 5 days postnatally reaches adult level at 21 days age, after having a transient rise with peak at 10 days of age\textsuperscript{77,78,79}. In old animals the reduction in serum melatonin is related to a reduced capacity of the pineal to synthesize melatonin, rather than an altered responsiveness of the gland to neural stimulation\textsuperscript{31}. Melatonin has been shown to phase shift the rhythms of sleep-wake cycle, body temperature cycle and plasma melatonin onset\textsuperscript{80,81,82,83}.

3.2 Melatonin and Reproduction

Reproduction occurs in various seasons of a year depending on the species. The main strategy is obviously to deliver offspring in the most favourable time. The changes in frequency of the gonadotropin-releasing hormone (GnRH) pulses, which regulate the release of gonadotropin hormones from pituitary and subsequently the function of reproductive organs is the primary factor for the seasonal rhythm in fertility\textsuperscript{84}.

The adaptation to the seasonal change of ambient conditions depends on the presence of intact pineal gland in most of the mammalian species. The seasonal changes either do not occur at all or lose their synchronization with the geophysical annual cycle in pinealectomized animals\textsuperscript{85,86,87}. In pinealectomized...
adult Syrian hamsters, daily melatonin administration mimicking the pattern of melatonin secretion on short days (long melatonin pulse) induces involution of the gonads, cessation of the estrous cycle, and decrease in reproductive hormone concentration\textsuperscript{88,89,90,91,92}. Similarly, long melatonin infusions in juvenile Siberian hamsters results in a marked inhibition of gonadal growth\textsuperscript{93,94}. However, in sheep, on the contrary, long melatonin infusions stimulate the reproductive activity and the mating behaviour\textsuperscript{95,96,97}.

Although the inhibitory effect of melatonin on the reproductive axis in mammals is widely known\textsuperscript{5}, its reproductive role in nonseasonal breeders, such as the laboratory rat, continues to be elusive. Exogenous melatonin seems to be ineffective in adult male rats\textsuperscript{6}.

Subcutaneous injections of melatonin (400 micrograms/100g wt/day) to adult rats for 14 days decreases testicular, accessory sex organs weight, suppresses spermatogenesis and serum levels of gonadotropins and testosterone\textsuperscript{7}. Daily injection of melatonin for 15 or 20 days to rats of age 20 up to 25,30,35 or 40 days result in abnormal progression of spermatogenesis and decrease the ability of leydig cells to produce testosterone and also a marked decrease in LH serum levels\textsuperscript{98}.

Pineal melatonin production in rat is partially controlled by gonadal hormones, which act through noradrenergic input. The response of pineal cells to adrenergic stimulation is also regulated by the gonadal steroids. Gonadal hormones may have a direct effect on pineal melatonin release and the effect may be time related which is evidenced by the increased release of melatonin by the glands removed only during dark span but not during the light span after treatment with testosterone and estradiol\textsuperscript{9,99}. Presence of melatonin binding sites in the rat testes have been demonstrated and the NAT activity are predominantly localized
in interstitial cells with a peak on day 40 indicating that rat testes are capable of synthesizing melatonin\textsuperscript{100}. In males, to maintain the amplitude of the nocturnal melatonin peak circulating testosterone seems to be necessary. This effect may be through noradrenergic input, as the changes in circulating steroid hormone levels are capable of bringing about acute changes of tyrosine hydroxylase activity in pineal sympathetic nerve endings\textsuperscript{9}. Gonadal response to melatonin infusions is independent of GnRH neuron number\textsuperscript{101}.

In rats, the gonadal and genital development and function of offspring is influenced by the maternal pineal, thus it is important for postnatal reproductive and somatic development. But this hypothesis is yet to be confirmed in humans\textsuperscript{102,103}. Treatment of melatonin between 6 and 3 days before birth are effective in stimulating postnatal reproductive development of the hamster offspring, indicating that there is a well delineated, sensitive period during prenatal development when melatonin can provide the foetus with a prenatal photoperiodic history\textsuperscript{104}.

Duration of melatonin pulse, may regulate seasonal reproduction in mice as evidenced by decreased combined testicular weight and seminal vesicle weight in animals receiving melatonin for 10 hours during either the day or night but not in those receiving for 5 hours or as two 5 hour pulses (separated by 3 hour)\textsuperscript{105}. The time of injection with respect to photoperiod, appears to be crucial since an obvious diurnal pattern in the sensitivity of the reproductive axis to melatonin has been noticed. Rivest \textit{et al.}, have demonstrated that melatonin acts most efficiently when administered either shortly before the onset of darkness or late in the light period\textsuperscript{106}. In all, what appear crucial are the age of the rats and the time of administration of melatonin\textsuperscript{107,108}. 
Increased testicular functions after exposure to long photoperiods and decreased functions after short photoperiod exposure is reported\textsuperscript{109}. Testicular weights of male rats did not show any effect after daily afternoon injections of 25 micrograms melatonin for 12 weeks exposed to long photoperiod (14L:10D) and to short photoperiod (2L:22D)\textsuperscript{110}. Serum testosterone was significantly increased in pinealectomised rats\textsuperscript{111}.

In females pineal NAT activity and melatonin levels are reduced during the night of proestrous. Estradiol but not progesterone, may be the ovarian hormone responsible for the inhibition of pineal melatonin synthesis observed in the normal cycling female rat during proestrous night\textsuperscript{101,112}. But in \textit{in vitro} study by Cardinali \textit{et al}., reported that rat pineal melatonin content was increased with estradiol, while decrease with testosterone and lastly no effect with progesterone\textsuperscript{113}.

Pineal gland plays a regulatory role in prolactin secretion. In both rats and humans melatonin is reported to increase serum prolactin concentration\textsuperscript{114,115}. After unilateral overiectomy melatonin suppressed PRL and LH levels and also prevented transient rise in FSH in Holtzman rats indicating that melatonin may act at the level of the hypothalamus or higher brain centers to suppress the FSH surge\textsuperscript{116}.

Melatonin acts rapidly at low concentrations to block GnRH induced release of LH in vivo and in vitro\textsuperscript{117,118} and also melatonin blocks the GnRH induced release of FSH\textsuperscript{119}. A striking feature of the effects of melatonin on LH and FSH release is that they are lost during the course of development. Although melatonin inhibits GnRH induced LH release by \textasciitilde{}50-60\% in 4 to 8 days old rats, the melatonin effect gradually decreases starting at day 10 and disappears almost completely after day 15 of age. These developmental changes correlate well with the time course of decrease of the melatonin receptor density\textsuperscript{120}.
In vitro studies reveal that 100 ng/ml FSH decreases pineal melatonin; 10 ng/ml LH increases pineal melatonin while small concentration (1 ng/ml) PRL increases melatonin content and release but 100 ng/ml significantly decreases content and release\(^{121}\).

Melatonin levels increases with age parallel to pineal growth until 6 weeks and significantly decreased after that until 8 weeks of age in female Sprague dawley rats. The pineal gland through melatonin signal controls the timing of the proestrous LH surge in the rat. In peripubertal female rats nocturnal melatonin synthesis can be modulated by estrogen, but not by progesterone and the decline in the melatonin synthetic activity during the pubertal period is attributed to the increasing levels of endogenous estrogen, which is secreted from the maturing ovary. The inhibitory effect of estrogen on the pineal melatonin synthesis may be through its action on multiple sites\(^{122,123,124}\).

Effects of exogenous melatonin on adult female rats vary from disrupting the estrous cycle to exerting a blockade of ovulation\(^{125,126,127}\). The time of injection, with respect to photoperiod, appears to be crucial since an obvious diurnal pattern in the sensitivity of the reproductive axis to melatonin has been noticed. Badawi and Wilkinson, despite adhering to different accepted time schedules, have denied actions of melatonin on the reproductive system in many strains of female laboratory rats, including Sprague-Dawley and Wistar\(^{128}\).

A direct metabolic effect of melatonin on oocyte and its inhibitory action on ovulation in \textit{in vitro} have been observed\(^{129}\). Melatonin treatment to food restricted rats resulted in significant diminishing of the morphometric indices of ovary but melatonin has failed to produce same effects in rats fed ad libitum, suggesting that food restriction sensitizes the pituitary-ovarian axis to
antagonadotropic melatonin action. In female rats, Pinealectomy or suprachiasmatic nuclei lesions both decreased plasma LH and FSH, which is restored to normal levels effectively by daily administration of melatonin. Melatonin can modulate gonadotropin secretion by acting on a dopamine mechanism independent of hypothalamic suprachiasmatic areas. An intact hypothalamus is essential for the expression of gonadotropic but not lactotropic responses to melatonin and/or photoperiod. The cells responsive to both gonadal steroids and melatonin may be involved in the seasonal variation in GnRH release.

Although melatonin controls seasonal reproductive cyclicity in some mammalian species, its role in women is controversial. Under certain experimental conditions melatonin in addition to its well known antagonadal effects, can exert a progonadal influence. Exogenous melatonin may influence ovarian hyperplasia and hypertrophy; also it may inhibit ovarian growth processes in rats, indicating that melatonin may exert different effects under different conditions. Night administration of melatonin to aging mice and transplantation of a young pineal gland into the rudiment of older mice and rats positively effect size and function of testes and maintenance of juvenile hippocampal and testicular LHRH receptors and beta-adrenergic receptors in the testes of old rats and mice. This demonstrates that for the regulation of sexual, reproductive physiology a pineal directed circadian function and cyclicity is fundamental. Aging of neural and gonadal sexual function can be delayed by proper intervention with melatonin. Melatonin treatment is capable of advancing the reproductive recrudescence in seasonally anoestrous ewes. Irrespective of the time of the year continuous melatonin administration to cyclic hinds (red deer) stimulates prolonged ovarian cyclicity.
Humans do not exhibit seasonal patterns of puberty and do not respond to the seasonal melatonin information but secrete melatonin in a pattern which reflects the environmental light-dark cycle. Marked variations in the magnitude of the nocturnal melatonin peak are observed throughout the life span of the humans. The highest levels occur in childhood and then fall during puberty, further during adulthood. But pineal output barely changes during childhood and adolescence and the increase in body mass brings about the decrease in circulating levels of melatonin during growth and sexual maturation. Effects of exogenous melatonin are variable, probably reflecting differences in dose and timing. Circulating testosterone down regulates pineal melatonin, this negative correlation observed appears to be independent of concomitant gonadotropins. Both gonadotropins and gonadal steroids modulates the melatonin secretion. Melatonin secretion is increased in untreated male with GnRH deficiency. Testosterone administration decreased melatonin secretion to normal levels.

3.3 Melatonin receptors

Specific high-affinity melatonin receptors mediate the effects of melatonin. These receptors have been identified and characterized in a number of tissues by in vitro autoradiography and conventional binding assays using (125I) iodomelatonin (I-MEL) as ligand. Species to species basis the pattern of distribution varies, however, some features of tissue distribution are constant. In mammals, a very discrete distribution of the melatonin receptors has been shown, whereas in non-mammalian vertebrates, the melatonin receptors are much more abundant.

The high affinity melatonin receptors are present in pars tuberalis (PT) of the pituitary and in suprachiasmatic nuclei of hypothalamus. The specific I-MEL binding is often found also in medial preoptic area, anterior hypothalamus, dorsomedial and ventromedial hypothalamic nuclei, parsdistalis, paraventricular...
and anteroventral thalamic nuclei, hippocampus, cerebral and cerebellar cortex, area postrema and retina\textsuperscript{150,151,152,153,154,155,156,157,158,159,160,161,162,163,164}.

Indirect data suggest the localization of the melatonin receptors in some tissues. Because melatonin inhibits GnRH-induced LH and FSH release from the cultured pituitary cells, it seems likely that receptors are present on the gonadotrophs\textsuperscript{165,166,167}. Moreover, melatonin also inhibits the LH release in enriched preparation of gonadotrophs\textsuperscript{166}. But the direct evidence regarding the binding of I-MEL on gonadotrophs is still lacking\textsuperscript{74}.

Melatonin receptor is membrane associated, belongs to a distinct group within the large super family of G protein-coupled receptors and coupled to GTP binding protein. Density of the melatonin receptors not only varies with species and location, but in several tissues which is influenced by the lighting regime, also with time of the day, and developmental or endocrine status\textsuperscript{168}.

Melatonin interacts with neuroendocrine system via high affinity binding sites, after constant light or pinealectomy the density of the receptors increased in rats but can be reversed without significant alteration in the plasma melatonin concentration by single melatonin injection. This effect is not a result of a receptor occupation, but a direct regulation of melatonin receptors by melatonin itself\textsuperscript{169,170}. The diurnal variations in \textsuperscript{125}I melatonin binding sites in the rat brain are not generated by the pineal but are affected by removal of the gland\textsuperscript{171}. Mennenga \textit{et al.}, report the binding of melatonin in the nucleus of pineal parenchymal cells and in the outer nuclear layer of the retina of rats; binding is greater at night than during the day, also in the rats kept on a light/dark cycle of 12:12h; melatonin binding site density is more in the evening as compared to the morning\textsuperscript{172,173}. Melatonin binding sites in membrane preparations of the mouse thymus suggests that melatonin have direct regulatory action an immune system mediated through
the melatonin binding sites. The subcellular distribution of binding sites in the
mouse thymus is in the order of nuclear > mitochondrial > microsomal > cytosolic
fraction and also there is an age related decrease in 2-(125 I) iodomelatonin
binding in the mouse thymus correlating with the involution of the thymus174.

The melatonin receptor mRNA is expressed in thymus, spleen and also in
all the lymphocyte subpopulations of the rat thymus175. Also binding of 2-(125 I)
iodomelatonin in rat spleen crude membranes which exhibits day-night variations
with highest binding observed during late light period and lowest at late night
supports the hypothesis of a regulatory role of melatonin on the immune system in
which melatonin down regulates its own binding site176.

Melatonin target sites may occur at several levels of the hypothalamic-
pituitary-gonadal hierarchy, including a direct melatonin action on the gonads177.
Melatonin binding sites in membrane preparation of immature rat testes has been
demonstrated by using 2-(125 I) iodomelatonin as a radioligand. The presence of
binding sites in immature rat testes, suggests a possible direct role of melatonin on
testicular steroidogenesis178. The importance of a gonadal steroid modulatory role
in the photic-dependent melatonin binding activity suggests that other types of
neuronal mechanisms might be involved in the regulation of neuroendocrine and
sociosexual behaviors in non-mammalian vertebrates179. In addition to all these
presence of melatonin binding sites have been demonstrated in brain membrane
preparations of chicken, testes, ovary, and retina of chicken, in quail gonads and
testes, ovary and thymus in ducks180,181,182,183,184.

3.4 Melatonin and Immune system

Thymus, the central organ of the immune system is one of the main targets
of melatonin. The endogenous opioid peptides produced by the endocrine system
as well as by the immune cells mediate the immunoenhancing effects of
melatonin. Also lymphokines such as gamma-interferon and interleukin-2 as well as thymic hormones can modulate the synthesis of melatonin in the pineal gland. Immune dysfunctions that characterize some diseases depend not only on the immune system per se, but also at least in part, on altered secretion of immunomodulating neurohormones, including melatonin and opioid peptides. Therefore, exogenous administration of neurohormones could potentially improve the immune status in humans.\textsuperscript{185,186}

Experimental evidence has revealed that melatonin is of major importance in the development of thymus.\textsuperscript{12} Decreased thymus weight in rats was observed after pinealectomy which was restored to normal levels after long-term administration of melatonin.\textsuperscript{15} The thymus gland and the cells that it regulates produce a number of soluble factors that are capable of indirectly modulating the immune system via reproductive neuroendocrine circuits, certain interferons have been found capable of suppressing estrogen and progesterone release. Thymosin beta-4, secreted by thymus has been found to stimulate the release of LHRH from hypothalamus and in turn pituitary LH.\textsuperscript{11}

Melatonin binding sites are characterized in partially purified rat thymus membranes. In rats, maximum melatonin binding is observed in new born; thereafter, binding decreases progressively during the first weeks of life and the lowest values reach in adult animals. The decrease in melatonin binding is due to changes in the binding capacity rather than to changes in the affinity of the receptor. So there is a physiological role for melatonin in regulating thymus activity early during postnatal life.\textsuperscript{13,14}
3.5 Melatonin and Pancreas, Insulin like growth factor

Melatonin receptors have been detected in pancreas. Also, melatonin is having an stimulatory effect on the functioning of the pancreas\textsuperscript{187,188}. Melatonin is reported as having a therapeutic effect on pancreas by reducing oxidative stress and preservation of pancreatic beta-cell integrity\textsuperscript{189}. It acts as an organoprotector in the pancreas through the activation of specific melatonin receptors and by scavenging reactive oxygen species\textsuperscript{190}. Insulin like growth factors (IGF), which share the common ancestry with insulin have been implicated in normal growth. It is said to have physiological role in the development of pancreas\textsuperscript{191}. Pinealectomy resulted in decreased growth hormone and IGF-1 concentrations, melatonin administration increased the same. Influence of exogenous melatonin is dependent on the endogenous melatonin concentrations\textsuperscript{192}. IGF – 1 is considered as a critical link between reproductive and other neuroendocrine functions by acting on the hypothalamus\textsuperscript{193}. 