Advances in Bioresearch
Adv. Biores., Vol 6 (1) January 2015:07-14
©2015 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV 7.20 [Poland]

Advances in Bioresearch

ORIGINAL ARTICLE

Effect of Exogenous Melatonin on Thyroxine (T_4), Thyrotropin (TSH) Hormone Levels and Expression patterns of Melatonin Receptor (MT1 and MT2) Proteins on Thyroid gland during Different age groups of Male and Female Swiss albino Mice

Prashanjit Laskar[1], Samik Acharjee[2], Shiv Shankar Singh[3*]
[1]Molecular Endocrinology Research Laboratory, Department of Zoology, Tripura University, Suryamaninagar, Tripura, India
[2]Molecular Endocrinology Research Laboratory, Department of Zoology, Tripura University, Suryamaninagar, Tripura, India
[3]Molecular Endocrinology Research Laboratory, Department of Zoology, Tripura University, Suryamaninagar, Tripura, India
Corresponding author's email: shivssbhu@yahoo.co.in

ABSTRACT
The pineal gland hormone melatonin regulates diverse endocrine activities such as thyroid hormone regulation during different physiological condition of the organism. Melatonin exerts most of physiological activities through its membrane bound MT1 and MT2 receptors. The present research was carried out to study the effects of exogenous melatonin on thyroxine (T_4), thyrotropin (TSH) hormone levels and expression patterns of melatonin receptor (MT1 and MT2) proteins on thyroid gland during different age groups (2 months, 4 months and 8 months) of male and female Swiss albino mice. In male mice group, melatonin treatment can cause the inhibition of T_4 hormone via unaltered TSH level during 2 months and 4 months age group, but in 8 months it increased the level of T_4 as well as TSH level. In female mice group, melatonin elevated the level of T_4 through unaltered the level of TSH during all age groups of mice. However melatonin also significantly decreased the MT1 receptor proteins expression of thyroid gland in both male and female mice. Whereas MT2 receptor proteins of thyroid gland were expressed differentially due to melatonin supplementation during different age groups of male and female mice. The findings of our present study suggested that melatonin age and sex differentially effects on T_4 hormone. The present study also suggested that melatonin might be prefers its MT2 receptors present on thyroid gland for regulation of T_4 hormone secretion which also depends on age and sex (male or female) of Swiss-albino mice.
Key Words: *Melatonin, Melatonin Receptors (MT1, MT2), Thyroid, T_4, TSH.*

Received 21/11/2014 Accepted 02/01/2015

How to cite this article:
Prashanjit L, Samik A, Shiv Shankar S. Effect of Exogenous Melatonin on Thyroxine (T_4), Thyrotropin (TSH) Hormone Levels and Expression patterns of Melatonin Receptor (MT1 and MT2) Proteins on Thyroid gland during Different age groups of Male and Female Swiss albino Mice. Adv. Biores., Vol 6 [1] January 2015: 07-14. DOI: 10.15515/abr.0976-4585.6.1.714

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine secreted from the pineal gland. It implicated various physiological activities such as circadian rhythm control of mammalian and other vertebrates [1]. Besides these, this hormone has antioxidant [2, 3, 4], anti-aging [5, 6], anti-proliferative and potentially, anti-carcinogenic activities. It also appears to play key roles in the regulation of the endocrine system. Among the many more regulatory process of melatonin on endocrine activities, it has also involved in secretory and growth processes of the thyroid gland [7].

Although, the pineal gland is considered the main site of melatonin synthesis, many extra-pineal tissues have been identified as melatonin synthesizers. Among these extra-pineal tissues, thyroid gland is one of them [8]. Many investigators have suggested the role of melatonin through synthesis from parafollicular

cells might be protecting the follicular cells of the thyroid gland against oxidative damage during both physiological and pathological processes [9, 10, 11, 4].

Thyroid gland is represents as one of the major endocrine regulators of cell and tissue metabolic activity and changes of the secretions of thyroid gland hormone can induce many pathological changes in the organism. Thyroid hormones are mainly involved in the regulation of numerous physiological actions including lipid and carbohydrate metabolism, oxygen consumption and also in other activities such as development, reproduction and growth [12, 13].

Many of melatonin's physiological actions are mediated through the interaction with specific membrane bound receptors, MT1 and MT2 [14, 15]. The MT1 and MT2 melatonin receptors are classified as unique subtypes based on their molecular structure [16] and chromosomal localization [17]. Melatonin receptors have been found in various tissues throughout the endocrine system.

There are several reports suggesting the modulatory (stimulatory/inhibitory) effect of melatonin in the regulation of circulating levels of thyroxine (T_4) hormone. But it was still unknown, what is the expression pattern of melatonin receptor (MT1 and MT2) proteins in thyroid gland during the effects of melatonin in modulation of thyroxine hormone. The present work was mainly investigated the effects of melatonin on thyroxine hormone with the observed level of TSH hormone and consequent expression patterns of melatonin receptor (MT1 and MT2) proteins during different age groups of male and female Swiss albino mice.

MATERIAL AND METHODS

Animal procurement and Maintenance

Healthy laboratory Swiss albino male and female mice were housed at ambient laboratories conditions. Mice were kept in groups of seven (n=7) in polycarbonate cages (43cm x 27cm x 14cm) to avoid the crowding effect and fed with mice feed and water *ad libitum*. Mice were procured and acclimatized for 1 month at ambient laboratory conditions before experimentation in normal day-light (12L : 12D) condition. All the experiments on the animals were conducted in accordance with institutional practice and within the framework of the revised Animal (Specific Procedure) Act of 2007 of Govt. of India on animal welfare. The study protocol was approved by institutional animal ethics committee with ethical clearance no. TU/IAEC/2013/V/5-3.

Experimental Design

Both male and female experimental Swiss-albino mice were classified into three different age groups (2 months, 4months and 8 months). Each age group was further divided into two groups- control and melatonin treated groups. Each groups containing 7 mice. Control group of mice were received ethanolic saline (0.01% ethanol), 0.1 ml/day. Melatonin treated mice group were received melatonin 25 µg/100 g BW/day for consecutive 30 days. All the administrations were made subcutaneously during evening hour (4:30-5:00 pm). Melatonin (Sigma-Aldrich Chemicals, St. Louis, USA) dilution was prepared by dissolving melatonin in few drops of ethanol then diluted to desire concentration with normal saline (0.9% NaCl). All of these mice groups were sacrificed after 24 hours of last administration. Mice were decapitated without anesthesia. Thyroid glands were dissected out and fixed in Bouin's solution. For western blot analysis thyroid glands were dissected out and immediately kept at -40^{o}C. The trunk blood was collected and separated serum was stored at -40^{o}C.

Hormonal Analysis

Serum T_4 and TSH hormone analysis was done by commercial ELISA Kits (Diagnostic Automatation Inc, CA, USA). For T_4, detection range 0-30µg/dl, specificity 96.30% and sensitivity was 0.05µg/ml. For TSH, detection range 0-40 µIU/ml, specificity 100% and sensitivity was 0.20µIU/ml.

Immunohistochemical Staining

Immunohistochemical study of thyroid gland was done following the procedure of Savaskan et al [18]. 5µm thick paraffin sections were mounted on 3% gelatine coated slide. Sections were deparaffinised and rehydrated with alcohol grades. The sections were placed in PBS for 30 minutes and endogenous peroxide activity was blocked by 0.3% H_2O_2 in methanol for 30 minutes at room temperature. Sections were washed thrice with PBS and placed in blocking solution (horse blocking serum, diluted 1:100 in PBS, PK-6200, Vector Laboratories, Burlingame, CA). Then sections were incubated with primary antibodies (Mel 1AR; sc-13186 and Mel 1BR; sc-13177, goat polyclonal, Santacruz Biotech, USA, diluted 1:100) overnight at 4^{o}C. Sections were washed thrice with PBS and were incubated with biotinylated secondary antibody (Vectastain ABC Universal Kit, PK-6200, Vector Laboratories, Burlingame, CA, dilution 1:200). Sections were washed thrice with PBS and incubated with preformed AB complex reagent for 30 minutes. The antigens were visualized using the 0.03% peroxidase substrate 3, 3-diaminobenzidine (DAB; Sigma-Aldrich Chemicals, St. Louis, USA) and counter stained with Ehrlich's haematoxylin. Sections were dehydrated and mounted with DPX. Microphotographs of the stained sections were taken under 40X

objective of Olympus microscope. To test the specificity of the used antibodies, the primary antibodies were not added in control sections. The control sections were incubated with same dilution of normal serum for overnight at 4^0C and following morning the immunohistochemical protocol was followed under the same condition.

Western Blot analysis

Thyroid samples were homogenized and lysed in RIPA buffer (1% (v/v) NP-40, 0.1% w/v) sodium dodecyl sulphate (SDS) in PBS containing aprotonin, sodium orthovanadate and phenyl methyl sulphonyl fluoride (PMSF) and quantified by Lowry method (1951). Aliquots containing 100 µg proteins were resolved by 10% (w/v) SDS polyacrylamide gel electrophoresis followed by electrotransfer to nitrocellulose membrane (Santa Cruz Biotech, USA). Immune detection was carried out by using anti-Mel 1AR, anti-Mel 1BR (Mel 1AR; sc-13186 and Mel 1BR; sc-13177, goat polyclonal, Santacruz Biotech, USA, diluted 1:200) and β-actin antibody (sc-130656, rabbit polyclonal, Santacruz Biotech, USA, diluted 1:500) diluted in PBS contained 5% skimmed milk and 0.01% Tween-20 followed by incubation with horseradish peroxidase conjugated secondary antibodies (goat anti-rabbit IgG for β-actin antisera; diluted 1:1000 and rabbit anti-goat IgG for Mel 1AR and Mel 1BR antisera; diluted 1:1000). The immune interactions were detected by using Super Signal West Pico Chemiluminescent Substrate (# 34080, Thermo Scientific, Rockford, USA). Bands were quantified by measurement of optical density using Scion Image Analysis Software (Scion Corporation, MD, USA). Values were expressed as ratio of the density of the specific signal to β-actin signal and expressed as the % control value [19]. Each sample corresponds to tissue from a single animal and at least four gels corresponding to each subunit and experimental conditions were analyzed.

Statistical Analysis

Statistical analysis of the data was performed by one way ANOVA followed by Student's Newman-Keul's multiple range tests. The differences were considered significant when $p< 0.05$.

RESULTS

Estimation of T_4 and TSH

Hormonal analysis (Table 1 and Table 2) showed that decrease of serum T_4 hormone in 2 months and 4 months male mice due to administration of melatonin. Whereas, in 8 months male mice, level of T_4 hormone was increased after administration of melatonin. It was also noted that melatonin treatment does not changes the serum TSH level in 2 months and 4 months experimental male mice group. But melatonin treatment was increases the serum TSH level in 8 months experimental male mice group. It was also noted that serum T_4 hormone level was increased after administration of melatonin in all studied age groups of female mice. Whereas, melatonin does not changes the TSH hormone level in all experimental age groups of female mice.

Immunohistochemical observation

Immunohistochemical staining (Fig. 1 and Fig. 2) showed immune reactivity of MT1 and MT2 melatonin receptors in the thyroid gland of male and female mice. Both MT1 and MT2 immune reactivity was noted on follicular cells as well as on C-cells or parafollicular cells of the thyroid gland in case of both male and female mice. In negative immunohistochemical control sections no reaction were detected.

Western blot analysis

MT1 melatonin receptor proteins expression

Significant decrease of MT1 melatonin receptor proteins expression was noted in 2 months, 4 months and 8 months melatonin treated both male and female mice group in comparison to their respective control groups. (Fig. 3; Fig. 5).

MT2 melatonin receptor proteins expression

Significant decrease of MT2 melatonin receptor proteins expression was noted in 2 months and 8 months melatonin treated male mice group in comparison to their respective control groups. But in 4 months, significant increase of MT2 receptor proteins expression was noted in melatonin treated male mice group in comparison to its control group. (Fig. 4).

Whereas, in female, MT2 melatonin receptor proteins expression was significantly increased in 2 months and 8 months melatonin treated mice group in comparison to their respective control group. But in 4 months, MT2 receptor protein expression was significantly decreased in melatonin treated female mice group in comparison to its control group. (Fig. 6).

Table 1. Serum T_4 and TSH hormone concentrations of control and experimental (Melatonin treated) male mice of 2 months, 4 months and 8 months age groups. Results shown are the mean±S.E.M. for each group. Control vs experimental (Melatonin treated) differences were considered when p<0.05.
** = P<0.01.

Age groups (Male mice)	Thyroxin, T_4 (ng/ml)		Thyrotropin, TSH (µIU/ml)	
	Control	Melatonin Treated	Control	Melatonin Treated
2 months	18± 2.0	10± 2.3**	0.9± 0.032	0.95± 0.021
4 months	16± 3.0	12± 2.5**	1.1± 0.022	1.15± 0.031
8 months	12± 1.9	18± 2.3**	0.85± 0.015	1.1± 0.023**

Table 2. Serum T_4 and TSH hormone concentrations of control and experimental (Melatonin treated) female mice of 2 months, 4 months and 8 months age groups. Results shown are the mean±S.E.M. for each group. Control vs experimental (Melatonin treated) differences were considered when p<0.05.
** = P<0.01.

Age groups (Female mice)	Thyroxin, T_4 (ng/ml)		Thyrotropin, TSH (µIU/ml)	
	Control	Melatonin Treated	Control	Melatonin Treated
2 months	11± 2.0	18.5± 2.5**	1.2± 0.012	1.25± 0.013
4 months	10± 2.1	19.5± 3.0**	1.25± 0.021	1.25± 0.015
8 months	9± 2.0	18± 2.5**	1.1± 0.016	1.15± 0.021

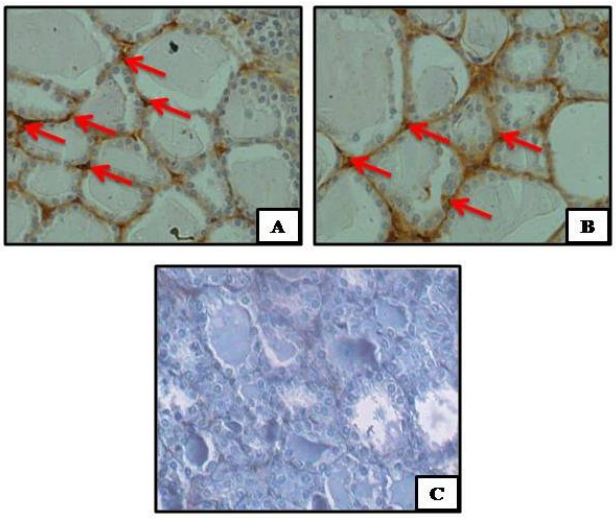

Figure 1. Immunostaining of MT1 melatonin receptors (A), MT2 melatonin receptors (B) and negative control (C) section in thyroid gland of male mice. Microphotographs were taken by Olympus Microscope under 40X objective.

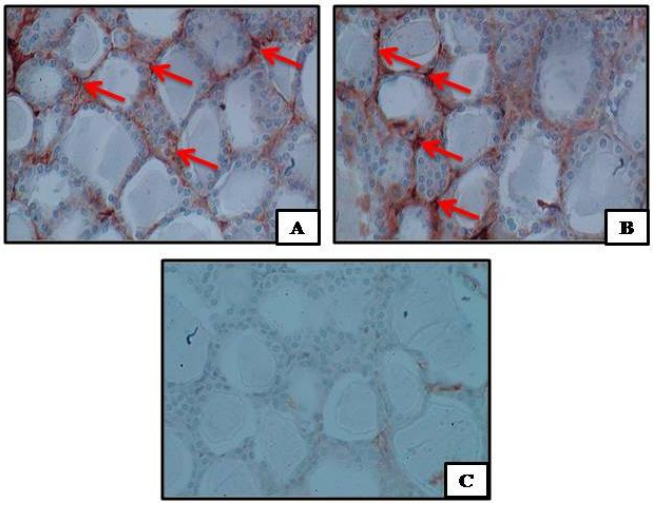

Figure 2. Immunostaining of MT1 melatonin receptors (A), MT2 melatonin receptors (B) and negative control (C) section in thyroid gland of female mice. Microphotographs were taken by Olympus Microscope under 40X objective.

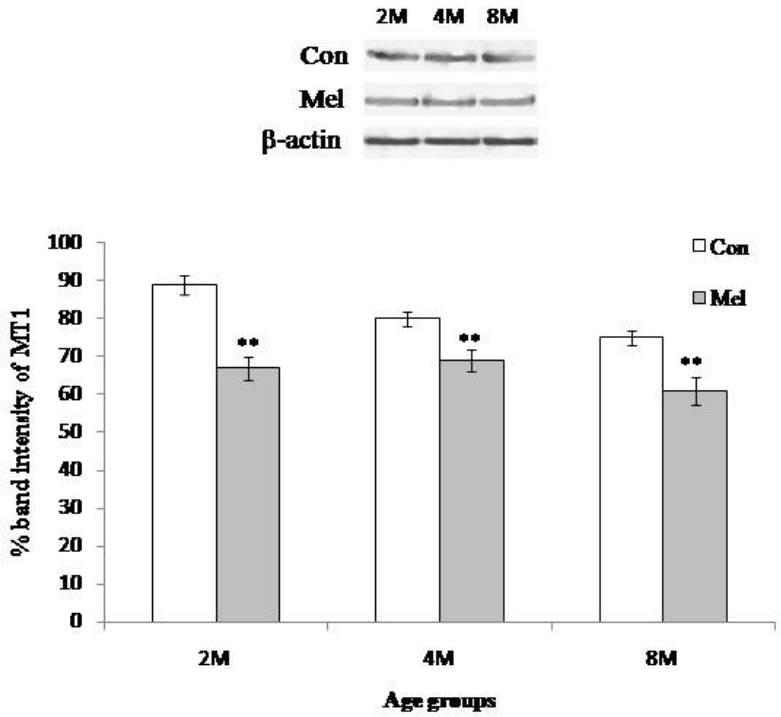

Figure 3. Western blot analysis of MT1 receptors in thyroid gland of 2 months (2M), 4 months (4M) and 8 months (8M) age group of control (Con) and melatonin treated (Mel) male mice. β-actin was used as loading control. Percent band intensity of MT1 receptors was showing following Scion Image analysis. Histogram represents Mean ± SEM. Control vs experimental (Melatonin treated) differences were considered when $p<0.05$.
** = $P<0.01$.

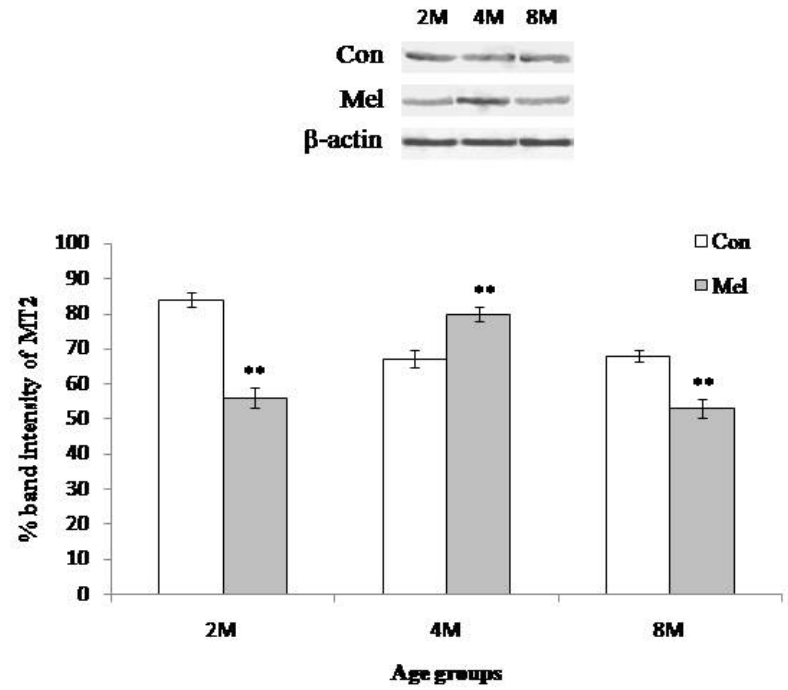

Figure 4. Western blot analysis of MT2 receptors in thyroid gland of 2 months (2M), 4 months (4M) and 8 months (8M) age group of control (Con) and melatonin treated (Mel) male mice. β-actin was used as loading control. Percent band intensity of MT2 receptors was showing following Scion Image analysis. Histogram represents Mean ± SEM. Control vs experimental (Melatonin treated) differences were considered when $p<0.05$.
** = $P<0.01$.

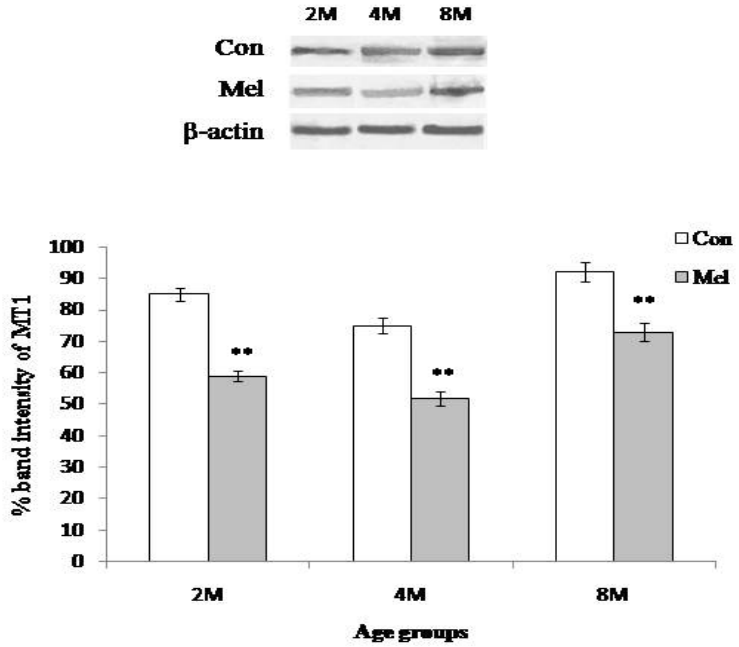

Figure 5. Western blot analysis of MT1 receptors in thyroid gland of 2 months (2M), 4 months (4M) and 8 months (8M) age group of control (Con) and melatonin treated (Mel) female mice. β-actin was used as loading control. Percent band intensity of MT1 receptors was showing following Scion Image analysis. Histogram represents Mean ± SEM. Control vs experimental (Melatonin treated) differences were considered when p<0.05.
** = P<0.01.

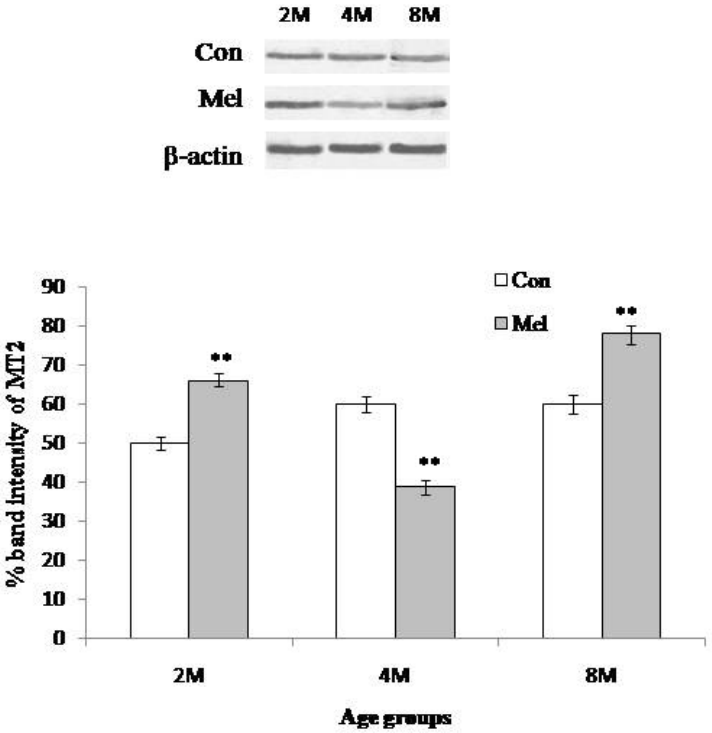

Figure 6. Western blot analysis of MT2 receptors in thyroid gland of 2 months (2M), 4 months (4M) and 8 months (8M) age group of control (Con) and melatonin treated (Mel) female mice. β–actin was used as loading control. Percent band intensity of MT2 receptors was showing following Scion Image analysis. Histogram represents Mean ± SEM. Control vs experimental (Melatonin treated) differences were considered when p<0.05.
** = P<0.01.

DISCUSSION

The functional link between melatonin and thyroid was well established by many investigators. In this study, exogenous melatonin was inhibiting the level of thyroxine (T_4) in 2 months and 4 months male mice group. Melatonin supplementation also reduced thyroxine hormone level of male adult Sprague–Dawley rats [20]. Melatonin induced inhibition of T_4 might be of significance in lowering the metabolic rate as well as in conserving energy reserves during these ages of male mice. It was also noted that melatonin was unable to change the level of TSH hormone during 2 months and 4 months male mice. In 2 months and 4 months male mice, melatonin inhibited the T_4 level via unaltered the TSH hormone level. But in 8 months male mice group, melatonin plays as a stimulatory effect on T_4 as well as on TSH hormone. During this progressive age (8 months) of male mice group, melatonin stimulating effect was both on thyroid gland and pituitary via stimulation of TSH levels. The observed increase in T_4 levels following melatonin treatment due to increase in TSH secretion or sensitivity of thyroid follicles to TSH was also reported [21] or it might be due to a direct thyrotropic action of melatonin. On the other hand, in female mice group, melatonin plays as a stimulatory effect on T_4 hormone without changing in the TSH hormone levels during all age groups. Prolonged evening administration of melatonin produced a significant increase of thyroxin hormone with unaltered levels of TSH was also reported in women [22]. In case of female mice, melatonin age independently modified the levels of thyroxine. These findings suggested the positive correlation existing between levels of melatonin and thyroxine during different age groups of female mice.

The presence of melatonin receptors on thyroid gland also strengthens the relationship between melatonin and thyroid gland. In present study immunohistochemical staining showed presence of MT1 and MT2 melatonin receptors in follicular and parafollicular cells of thyroid gland of both male and female mice. Melatonin receptors immunopositivity in follicular and parafollicular cells in rat thyroid gland was also reported [11].

In this study, western blot analysis showed the reduced expression patterns of MT1 receptor proteins after prolonged exposure of exogenous melatonin during all studied experimental age groups of male and female mice. Melatonin acts upon thyroid gland might be through down regulation of its MT1 receptor proteins during different age groups of male and female mice. Whereas, melatonin treatment causes down regulation of MT2 receptor proteins in 2 months, 8 months male mice groups and in 4 months female mice group. Prolonged treatment of melatonin also causes up regulation of MT2 receptor proteins in 4 months male and 2 months, 8 months female mice groups. Melatonin mediated effects on its MT2 receptor proteins expression might be sex (male or female) dependently regulates during different ages of mice.

Melatonin plays an important role in the regulation of circulating level of thyroxine hormone. The effects of melatonin on thyroxine hormone secretion may be either direct or indirect and the corresponding mechanisms are still to be established. The stimulatory and inhibitory effects of melatonin on thyroxine hormones depend on age and sex (male/female) of the mice. The age and sex, these two factors might be influencing on the effect of melatonin through TSH mediated regulation of circulatory thyroxine hormone. The current study also suggested that melatonin action on thyroxine hormone might be mediated through its receptors present on thyroid gland. Melatonin might be prefers its MT2 receptors on thyroid gland for regulation of thyroxin hormone secretion. Although MT2 receptors mediated effects of melatonin on thyroid gland depend on age and sex (male or female) of the mice. However, further investigation will also be needed through using different melatonin receptors (MT1 and MT2) inhibitors which elucidated the individual contribution of each melatonin receptors in relation to thyroxin hormones status during different ages of male and female mice.

CONFLICT OF INTERESTS

The authors have no conflict of interests to disclose.

ACKNOWLEDGMENTS

Financial support from the University Grants Commission (UGC), New Delhi, India is gratefully acknowledged.

REFERENCES

1. Reiter, R.J. (1991). Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr. Rev., 12 (2):151–180.
2. Martinez-Cruz, F., Pozo, D., Osuna, C., Espinar, A., Marchante, C., & Guerrero, J.M. (2002). Oxidative stress induced by phenylketonuria in the rat: Prevention by melatonin, vitamin E, and vitamin C. J. Neurosci. Res., 69 (4): 550-558.

3. Reiter, R.J., Paredes, S.D., Manchester, L.C., & Tan, D.X. (2009). Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. Crit. Rev. Biochem. Mol. Biol., 44 (4): 175-200.
4. Mogulkoc, R., Baltaci, A.K., Oztekin, E., Aydin, L., & Sivrikaya, A. (2006). Melatonin prevents oxidant damage in various tissues of rats with hyperthyroidism. Life. Sci., 79 (3): 311-315.
5. Reiter, R.J., Tan, D.X., Mayo, J.C., Sainz, R.M., & Lopez-Burillo, S. (2002). Melatonin, longevity and health in the aged: an assessment. Free. Radic. Res., 36 (12): 1323-1329.
6. Tajes, M., Gutierrez-Cuesta, J., Ortuno-Sahagun, D., Camins, A., & Pallas, M. (2009). Anti-aging properties of melatonin in an in vitro murine senescence model: involvement of the sirtuin 1 pathway. J. Pineal. Res., 47 (3): 228-237.
7. Lewinski, A., & Karbownik, M. (2002). Melatonin and the thyroid gland. Neuro. Endocrinol. Lett., Suppl 1: 73-78.
8. Kvetnoy, I.M. (1999). Extra pineal melatonin: location and role within diffuse neuroendocrine system. Histochem. J., 31 (1): 1-12.
9. Karbownik, M., & Lewinski, A. (2003). The role of oxidative stress in physiological and pathological processes in the thyroid gland; possible involvement in pineal-thyroid interactions. Neuroendocrinol. Lett., 24 (5): 293-303.
10. Rao, M.V., & Chhunchha, B. (2010). Protective role of melatonin against the mercury induced oxidative stress in the rat thyroid. Food. Chem. Toxicol., 48 (1): 7-10.
11. Garcia-Marin, R., Miguel, de, M., Fernandez-Santos, J.M., Carrillo-Vico, A., Utrilla, J.C., Morillo-Bernal, J., Diaz-Parrado, E., Rodriguez-Prieto, I., Guerrero, J.M., & Martin-Lacave, I. (2012). Melatonin-synthesizing enzymes and melatonin receptor in rat thyroid cells. Histol. Histopathol., 27 (11): 1429-1438.
12. Flood, D.E., & Langlois, V.S. (2014). Crosstalk between the thyroid hormone and androgen axes during reproductive development in *Silurana tropicalis*. Gen. Comp. Endocrinol. 203 (1): 232-240.
13. Forhead, A.J., & Fowden, A.L. (2014). Thyroid hormones in fetal growth and prepartum maturation. J. Endocrinol. 221 (3): R87-R103.
14. Dubocovich, M.L., Cardinali, D.P., Delagrange, P.R.M., Krause, D.N., Strosberg, D., & Sugden, D. (2000). Melatonin Receptors (Eds. Girdlestone, D.) The IUPHAR Compendium of Receptor Characterization and Classification (2nd Ed), London, IUPHAR Media, p. 270-277.
15. Dubocovich, M.L., Rivera-Bermudez, M.A,, Gerdin, M.J., & Masana, M.I. (2003). Molecular pharmacology, regulation and function of mammalian melatonin receptors. Front. Biosci., 8: d1093–1108.
16. Reppert, S. M., Weaver, D. R., & Godson, C. (1996). Melatonin receptors step into the light: cloning and classification of subtypes. Trends. Pharmacol. Sci., 17 (3): 100–102.
17. Slaugenhaupt, S. A., Roca, A. L., Liebert, C. B., Altherr, M. R., Gusella, J. F., & Reppert, S. M. (1995). Mapping of the gene for the Mel1a-melatonin receptor to human chromosome 4 (MTNR1A) and mouse chromosome 8 (Mtnr1a). Genomics. 27 (2): 355–357.
18. Savaskan, E., Wirz-Justice, A., Olivieri, G., Pache, M., Krauchi, K., Brydon, L., Jokers, R., Muller-Spahn, F., & Meyer, P. (2002). Distribution of melatonin MT1 receptor immunoreactivity in human retina. J. Histochem. Cytochem., 50 (4): 519-526.
19. Treeck, O., Halder, C., & Ortmann, O. (2006). Antiestrogens modulate MT1 melatonin receptor expression in breast and ovarian cancer cell lines. Oncol. Rep., 15 (1): 231-235.
20. Baltaci, A.K., Mogulkoc, R., Kul, A., Bediz, C.S., & Ugur, A. (2004). Opposite effects of zinc and melatonin on thyroid hormones in rats. Toxicology., 195 (1): 69–75.
21. Grau, E.G., Brown, C.L., & Stetson, M.H. (1985). Photoperiodic regulation of thyroid responsiveness to TSH in *Fundulus heteroclitus*. J. Exp. Zool. 234 (2): 199–205.
22. BELLIPANNI, G., DI MARZO, F., BLASI, F., & DI MARZO, A. (2005). Effects of melatonin in perimenopausal and menopausal women: our personal experience. Ann. N. Y. Acad. Sci., 1057: 393-402.