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Experimental Research Article

Alteration of melatonin receptor expression associated with melatonin-mediated amelioration of oxidative stress in the spleen of hyperthyroid mice

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ABSTRACT

Introduction: Excessive synthesis of thyroid hormone in hyperthyroidism is related to the imbalance of oxidative status in living organisms. Melatonin mediates its effects either directly through scavenging free radicals or indirectly through the activation of melatonin receptors (MT1 and MT2). The present study investigated the involvement of melatonin receptors in the melatonin-mediated attenuation of hyperthyroidism-induced oxidative stress in the spleen of laboratory mice.

Methods: The hyperthyroidism was induced by L-thyroxine (0.6µg/g B. wt.) supplementation. The experimental mice were supplemented with melatonin (25 µg/100g B. wt.) subcutaneously. Oxidative stress, melatonin receptor expression in the spleen tissues, and circulatory levels of thyroid hormone were determined.

Results: L-thyroxine treatment caused a significant increase in serum T3 and T4 levels. Melatonin supplementation caused a significant decrease in serum T3 and T4 levels in L-thyroxine-treated mice. L-thyroxine treatment increased MDA levels and suppressed catalase and SOD enzyme activities. Melatonin treatment caused suppression of MDA levels and an increase in SOD and catalase activities. L-thyroxine treatment caused significant suppression in MT1 receptor expression and a significant increase in MT2 receptor expression. Melatonin supplementation significantly induced the MT2 receptor protein expression in the spleen tissues of experimental mice.

Conclusion: This study suggests that alterations in MT2 melatonin receptor expression may be associated with melatonin-mediated attenuation of oxidative stress in the spleen tissues of hyperthyroid mice.

Keywords:
Hyperthyroidism
Oxidative stress
Melatonin
MT1
MT2 receptors

Introduction

Hyperthyroidism is an endocrine disorder in which excess thyroid hormone {thyroxine (T4) and triiodothyronine (T3)} is synthesized and secreted by the thyroid gland into the blood (Leo et al., 2016). The iodine-containing Thyroxine (tetraiodothyronine, or T4) prohor-

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mone is released into the blood from the thyroid gland. Enzymatic removal of iodine results in the formation of T3 which is the main bioactive form of thyroid hormone (Niedowicz et al., 2021). Hyperthyroidism is a common condition with a global prevalence of 0·2–1·3% (Wiersinga et al., 2023). The excess of thyroid hormones results in suppression of thyrotropin (TSH) production in the pituitary (Costilla et al., 2019). Elevated thyroid hormone induces an overall increase in metabolism which affects almost all tissues of the body (Mullur et al., 2014). The number and activity of mitochondrial respiratory chain components were altered by circulating T3 and T4 hormones (Jezek and Hlavata, 2005) as these hormones are involved in the modulation of the mitochondrial respiratory process (Taleux et al., 2008). Upregulation of thyroid hormones can result in mitochondrial respiratory perturbation, and an increase in ROS generation (Guerrero et al., 1999). ROS are toxic due to their high reactivity potential and cause oxidative damage in cellular macromolecules such as proteins, lipids, and DNA (Brix et al., 1998). However, every cell contains a biochemical defense system known as the antioxidant system and when ROS generation exceeds the antioxidant capacity of cells, it causes oxidative damage (Petrulea et al., 2012). Antioxidants can scavenge free radicals thus neutralizing the harmful effects of free radicals (Rastogi and Haldar, 2018).

Thyroid hormones regulate the synthesis and degradation of antioxidant enzymes (Chainy and Sahoo, 2020). It is found that oxidative stress is related to dysfunction (Rastogi and Haldar, 2018), dysregulation, or suppression of immune function (Dhabhar, 2014). The spleen is a vital immune organ (Grisanti et al., 2011). It is reported that oxidative stress is related to the impairment of splenic immunity (Zhang et al., 2022a).

In the cells of the immune system, the thyroid hormone modulates the production of various cytokines and factors to modify the immune function and responses (Guria et al., 2015). The spleen is the largest immune organ and plays an important role in immunity throughout life. In the medical context, hyperthyroidism is associated with the enlargement of the spleen (Ilardo et al., 2021). L-thyroxine-induced experimental hyperthyroidism altered lymphocyte count in the spleen of rats. In these rats, splenic CD4 cell number was reduced whereas the proportion of blood CD4 cells was elevated (Robinson et al., 2014). Hyperthyroidism showed elevated splenic natural killer cells, and T lymphocytes in both long-term and short-term treatment with thyroxine in mice (Watanabe et al., 1995).

Melatonin, an indoleamine is a modulator of immune functions and a potent antioxidant. Melatonin is an effective direct free radical scavenger and an indirect antioxidant (El-Sokkary et al, 2002) and has general immunoenhancing effects in studied organisms (Ahmad et al., 2012). Melatonin mediates its function directly by scavenging free radicals or indirectly via activation of its MT1 and MT2 receptors. MT1 and MT2 are G-protein coupled receptors and are localized in various tissues and organs of the body including immune organs and cells. Exogenous melatonin attenuated the metabolic stress-induced suppression of the splenocyte proliferation index in mice (Sutradhar et al., 2022). Further, the report also suggested the involvement of MT1 and MT2 receptors in melatonin-mediated modulation of splenocyte proliferation in hyperthyroidic mice (Laskar and Singh, 2018). Reports are suggesting the antioxidative and immune-enhancing properties of indoleamine melatonin in studied experimental conditions, but a few reports are suggesting the association of MT1 and MT2 receptors expression in melatonin-mediated attenuation of oxidative stress in thyroid hyperactivity. Therefore, in the present study, we have investigated the alteration of MT1 and MT2 receptor expressions associated with melatonin-mediated amelioration of oxidative stress in the spleen tissues of experimentally induced hyperthyroid mice.

Material and Methods

All the experiments with animals and their maintenance were done according to the institutional practice and within the framework of CCSEA (Committee for Control and Supervision of Experiments on Animals) and the Act of Government of India (2007) for animal welfare.

Animal model

Swiss albino mice were considered for the present study. Healthy mice colonies were housed in ambient laboratory conditions with light (12L:12D), temperature (25±2ºC), and humidity (55±5%). Five mice per group were kept in polycarbonate cages (43 cm x 27 cm x 14 cm) while avoiding population stress. Mice were fed with mice feed and water *ad libitum***.**

Healthy male Swiss-albino mice were selected from the housed colony and were divided into four groups having 5 mice each-

Group I: Control (Con)
Group II: Thyroxine (T4)
Group III: Melatonin (Mel)
Group IV: Thyroxine + Melatonin (T4+Mel)

Group I of mice received ethanolic saline (0.01% ethanol). Group II of mice received a subcutaneous injection of L-Thyroxine (Sigma-Aldrich Chemicals, St. Louis, USA), 0.6 µg/g B. wt./day (Shinohara et al., 2000). Melatonin (Sigma-Aldrich Chemicals, St. Louis, USA) was subcutaneously injected in the third mice group with a dose of 25 µg/100 g B. wt./day in the evening hours (4:30-5:00 p.m.) (Singh et al., 2015). The fourth group of mice received L-thyroxine and Melatonin simultaneously. Melatonin was dissolved in a trace amount of absolute alcohol and then diluted in normal saline. L-thyroxine was dissolved in a few drops of 0.01N NaOH and then diluted in normal saline. All the supplementations were given for 30 consecutive days. After 24 h of the last administration, experimental mice were sacrificed and trunk blood was collected in the sterilized syringe. The blood was allowed to clot, and the serum was separated and stored at -20^{o}C until analysis. The spleen was dissected out on ice. Each spleen was divided and processed for reverse transcription PCR analysis, immunohistochemical study, western blot analysis, and oxidative stress analysis.

Parameters Studied

Hormonal Analysis

Serum levels of T3 and T4 hormones were analyzed by commercial ELISA Kits (Diagnostic Automatation Inc, CA, USA). For T3, sensitivity was 0.2ng/mL, detection range was 0-10 ng/mL, and specificity was 96.30%. For T4, specificity was 96.30%, sensitivity was 0.05µg/mL, and detection range was 0-30µg/dL.

Oxidative Stress

a) Lipid peroxidation Assay in the splenic tissue

A product of lipid peroxidation i.e., malondialdehyde (MDA) was measured in splenic tissue of experimental mice following the method of Ohkawa et al. (1979). It is measured by the reaction of MDA with thiobarbituric acid (TBA). Splenic homogenate (10%) was prepared in phosphate buffer and 0.1 ml of this was allowed to mix with 3.3 ml of TBA reagent which contains 8% SDS, 20% acetic acid (pH 3.5), 0.8% TBA, and 0.8% butylated hydroxyl-toluene. The above mixture was boiled and the supernatant was collected. The collected supernatant was then subjected to optical density measurement at 532 nm. Lipid peroxidation of experimental tissues was expressed as nmol TBARS formed per mg protein

b) Determination of superoxide dismutase (SOD) activity in spleen

Splenic tissues of experimental mice were analyzed for SOD activity (EC 1.15.1.1) by following the method of Das et al. (2000). Phosphate buffered saline (pH = 7.4) was used to prepare 10% homogenate of spleen tissue collected from experimental mice. 0.1 ml of the tissue homogenate was mixed with a reaction mixture (1.4 ml) containing 50 mM phosphate buffer (pH 7.4), 20mM L-methionine, 1% Triton-X-100, 10 mM hydroxylamine hydrochloride, 50 mM EDTA. The mixture was exposed to 20 W fluorescence after addition of 50 mM riboflavin. 1 ml Griess reagent was added to the above mixture and optical density was measured at 543 nm. The amount of SOD inhibiting 50% of nitrite formed under the assay conditions was determined as one unit of SOD enzyme activity. SOD activity was expressed as U/mg protein.

c) Determination of catalase (CAT) activity in spleen

Splenic tissue of experimental mice was analyzed for catalase activity (EC 1.15.1.1) by following the method of Sinha (1972) and modified by Hadwan, (2016). Splenic tissue homogenate (10%) of experimental mice were prepared in phosphate buffer (pH = 7.4). After centrifugation, the supernatant was added to a reaction mixture that contained H_2O_2 and potassium dichromate and centrifuged after boiled in a water bath. The supernatant was collected and subjected to optical density measurement at 570 nm. The decrease in H_2O_2 was calculated, and catalase enzyme activity was expressed as the amount of H_2O_2 degraded per min.

Immunohistochemistry of MT1 and MT2 receptors

MT1 and MT2 receptor protein localization by immunohistochemistry was done in the spleen of experimental mice following the modified methods of Singh et al. (2017). Slides were coated with gelatine and 5µm thick sections of spleen tissue were mounted. Antibod-

ies against MT1 and MT2 receptors protein [Mel1AR (MT1); sc13186 and Mel1BR (MT2); sc13177, goat polyclonal, Santacruz Biotech, USA, diluted 1:100] were used for the detection. HRP enzyme-catalyzed DAB+H_2O_2 reaction was employed for visualization of antigen-antibody interaction. Micrographs of stained sections were taken under the 40X objective of Leica Microscope DM2500.

Western blot analysis

Western blot analysis was performed to estimate the expression of MT1 and MT2 melatonin receptor proteins in the splenic tissue of experimental mice. 100 mg protein aliquots were resolved by 10% (w/v) SDS polyacrylamide gel electrophoresis followed by electrotransfer to nitrocellulose membrane (Santa Cruz Biotech, USA). The nitrocellulose membranes were blocked for 2 h in 5% skimmed milk solution in PBST [PBS containing 0.1% Tween-20]. The membranes were incubated overnight at 4ºC in primary antibody [Mel1AR (MT1); sc13186 and Mel1BR (MT2); sc13177, goat polyclonal, Santacruz Biotech, USA, diluted 1:200] followed by horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit IgG). The immune interactions were detected by using Super Signal West Pico Chemiluminescent Substrate (#34080, Thermo Scientific, Rockford, USA). Further, stripping buffer (10% sodium azide) was used to strip the nitrocellulose membranes, which were then incubated with β-actin antibodies at a dilution of 1:1000 (sc-130656, goat polyclonal, Santacruz Biotech, USA) as internal loading control. Immunodetection of β-actin was performed with horseradish peroxidase-conjugated goat anti-rabbit IgG (dilution 1:5000). Bands were quantified by measurement of optical density using Scion Image Analysis Software (Scion Corporation, MD, USA). Values were expressed as the ratio of the specific signal density to the β-actin signal and expressed as the % control value (Treeck et al., 2006).

Reverse Transcription PCR of MT1 and MT2 Receptors

RNA isolation, cDNA synthesis, and PCR

PureZol (Biorad, USA) was used to isolate the total RNA of the spleen, and synthesis of cDNA was done by using iScript cDNA synthesis kit (Biorad, USA). Specific forward and reverse primer of MT1 (Forward 5'-CCGGAACACTCCAGTACGAT-3'and reverse 5'-GGACCAGGACCCATATCCTT-3' give 150 bp PCR product), MT2 (Forward 5'-TTTATGGGTCCT-GACCAG-3'and reverse 5'-CCCTGTCGCTCCT-CAGTAAG-3' give 121bp PCR product), and GAPDH (Forward 5'-AACTTTGGCATTGTGGAAGG-3'and reverse 5'-ACACATTGGGGGTAGGAACA-3' give 132 bp PCR product) were used to amplify cDNA. The PCR products were resolved on a 2% agarose gel, and the optical density of the PCR product bands was analyzed by ImageJ software. The MT1R and MT2R expression was expressed in terms of optical density relative to GAPDH.

Statistical Analysis

Statistical analysis of the data was performed using SPSS 17.0 (SPSS Corp., USA) program with one-way ANOVA followed by Tukey's multiple range tests for multiple comparisons. The data was presented as Mean±SEM. The differences were considered significant when $P<0.05$.

Results

Effect of hyperthyroidism and melatonin on serum T3 and T4 levels

T4 (Figure 1a) and T3 (Figure 1b) hormone levels were significantly ($P<0.01$) increased in thyroxine-treated mice. Melatonin treatment caused a significant ($P<0.01$) decrease in circulatory T3 and T4 hormone levels in comparison to the control group. Melatonin supplementation along with thyroxine treatment caused a significant ($P<0.01$) decrease in circulatory T3 and T4 levels in experimental mice in comparison to thyroxine-treated mice.

Effect of hyperthyroidism and melatonin on lipid peroxidation

L-thyroxine treatment significantly ($P<0.01$) increased the lipid peroxidation level (nmol TBARS formed/mg protein) in the spleen of mice compared to the control mice. Melatonin treatment significantly ($P<0.01$) decreased lipid peroxidation levels in splenic tissue in comparison with the control mice. Melatonin along with L-thyroxine treatment significantly ($P<0.01$) decreased lipid peroxidation levels in spleen tissue in comparison with the L-thyroxine treated group of mice (Figure 2a).

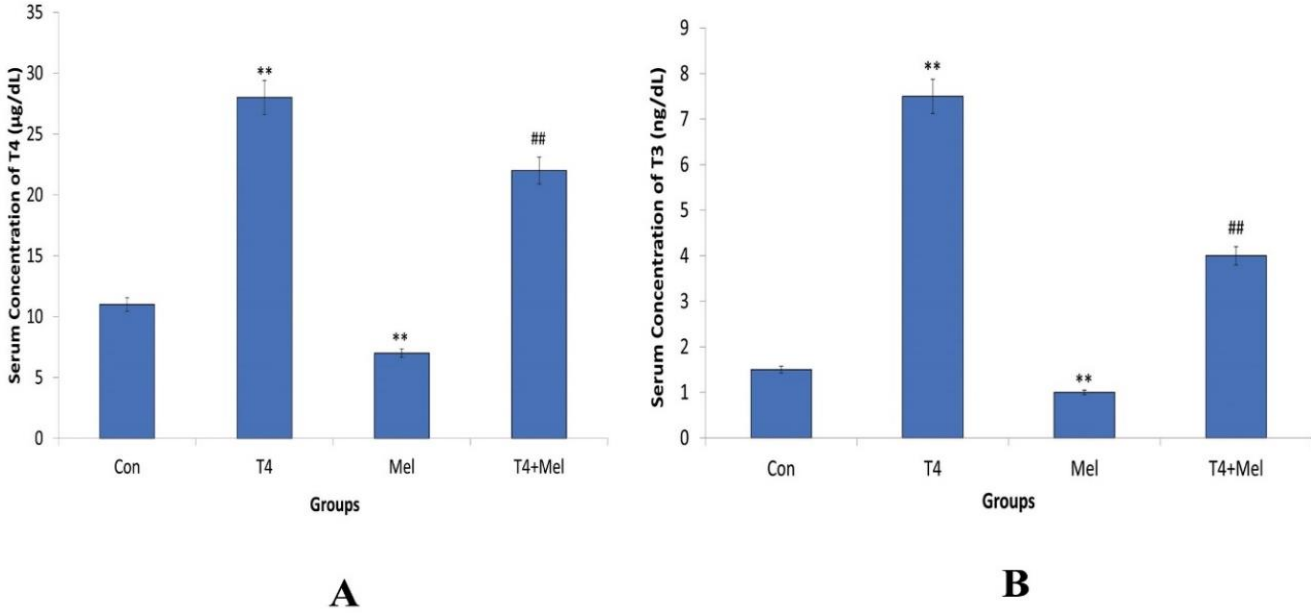

FIGURE 1. Effect of hyperthyroidism and melatonin on serum concentration of (A) T4 (µg/dL) and (B) T3 (ng/dL) in experimental mice. Histograms represent Mean ± SEM, with n=5 for each group. Con = control, T4 = L-Thyroxine treated, Mel = Melatonin treated, T4+Mel = L-Thyroxine treated + Melatonin. ** P<0.01for T4 vs Con and Mel vs Con; ## P<0.01 for T4+Mel vs T4.

Effect of hyperthyroidism and melatonin on SOD activity

Treatment with L-thyroxine caused a significant (P< 0.01) decrease in SOD enzyme activity (U/mg protein) in spleen tissue in comparison with control mice. Melatonin treatment significantly (P<0.01) increased SOD enzyme activity in spleen tissue in comparison with control mice. Melatonin treatment along with L-thyroxin significantly (P<0.01) increased SOD enzyme activity in spleen tissue in comparison with L-thyroxin-treated mice (Figure 2b).

Effect of hyperthyroidism and melatonin on catalase activity

Treatment with L-thyroxine caused a significant (P<0.01) decrease in catalase enzyme activity (kU/mg protein) in spleen tissue compared to control mice. Melatonin treatment significantly (P<0.01) increased the catalase enzyme activity in spleen tissue in comparison with control mice. Melatonin treatment along with L-thyroxine significantly (P<0.01) increased catalase enzyme activity in spleen tissue in comparison with the L-thyroxine-treated group of mice (Figure 2c).

Effect of hyperthyroidism and melatonin on immune reactivity of MT1 and MT2 receptors

Immunoreactivity of MT1 antisera (Figure 3) was noted throughout the spleen of the control group of mice. Weak immunoreactivity of MT1 antisera was noted in the spleen of the thyroxine-treated and melatonin-supplemented group of mice. Melatonin-supplemented thyroxine-treated mice also showed weak immunoreactivity to MT1 antisera in the spleen tissue. MT2 receptor antisera immunoreactivity (Figure 4) was observed in the spleen of the control group. Thyroxine treatment showed strong immunoreactivity to MT2 receptor antisera in the spleen tissue. Melatonin supplementation caused highly strong MT2 receptor antisera immunoreactivity in the spleen among all the experimental groups. Melatonin supplementation in thyroxine-treated mice resulted in stronger immunoreactivity to MT2 receptor antisera in the spleen.

Effect of hyperthyroidism and melatonin on MT1 and MT2 receptor protein expression

MT1 receptor protein expression (Figure 5) was significantly (P<0.01) decreased in thyroxine-treated mice compared to the control group. Melatonin supplementation in thyroxine-treated mice showed significant (P<0.01) suppression of MT1 receptor protein expression in comparison with the melatonin-treated group of mice. MT2 receptor protein expression (Figure 6) was

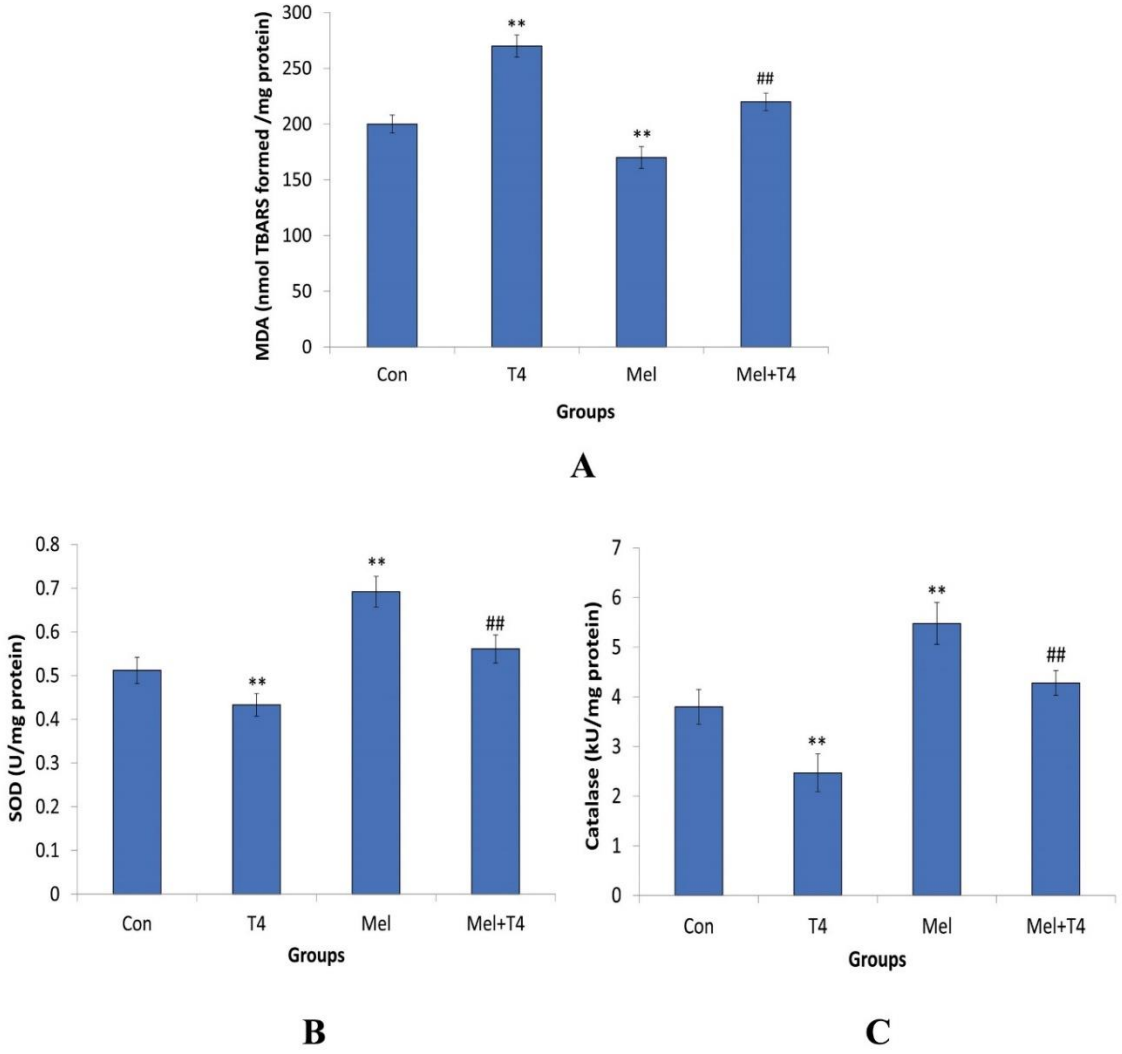

FIGURE 2. Effect of hyperthyroidism and melatonin on (A) lipid peroxidation (MDA level), (B) SOD activity, and (C) catalase activity in spleen tissue of laboratory mice. Histograms represent Mean ± SEM, with n=5 for each group. Con = control, T4 = L-Thyroxine treated, Mel = Melatonin treated, T4+Mel = L-Thyroxine treated + Melatonin. ** P<0.01for T4 vs Con and Mel vs Con; ## P<0.01 for T4+Mel vs T4.

significantly (P<0.01) increased in the thyroxine-treated group, melatonin-treated group, melatonin, and thyroxine group compared to the control group. Melatonin supplementation highly increased the MT2 receptor protein expression in the spleen tissue among all experimental groups of mice.

Effect of hyperthyroidism and melatonin on reverse transcriptase-PCR analysis of MT1 and MT2 receptors

MT1 receptor mRNA expression (Figure 7) was significantly (P<0.01) decreased in the spleen of thyroxine-treated mice, melatonin-treated mice, and melatonin and thyroxine-treated mice in comparison with control mice. MT2 receptor mRNA expression (Figure 8) was significantly (P<0.01) increased in the spleen of thyroxine-treated mice, melatonin-treated mice, and melatonin and thyroxine-treated mice compared to the control mice. Significantly high levels of MT2 receptor mRNA expression were noted in the spleen of melatonin-treated mice among other groups of mice.

Discussion

Hyperthyroidism is a pathological condition typically characterized by excessive thyroid hormone production in the body. Experimental hyperthyroidism is mainly generated by the supplementation of Levothyroxine (Zhang et al., 2022b). The high circulatory thyroid hormone induces over-metabolic activities which leads generation of reactive oxygen species in the organisms. Melatonin is a known antioxidant and has immunoenhancing effects. The present study investigated the alteration of melatonin receptor expression and its

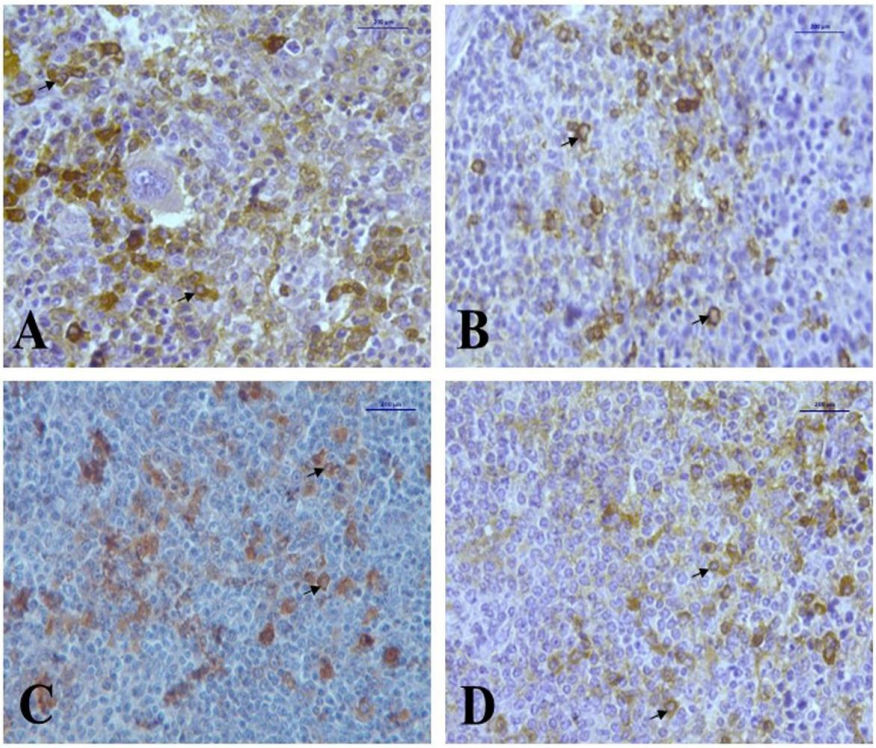

FIGURE 3. Effect of hyperthyroidism and melatonin on the immunoreactivity of MT1 receptor antisera in the spleen of laboratory mice. A = control, B = L-Thyroxine treated, C = Melatonin treated, D = L-Thyroxine treated + Melatonin. Micrographs were taken under the 40X objective. Arrowhead (➔) showed MT1 receptor antisera reactivity in splenocytes. The magnification bar showed 200µm.

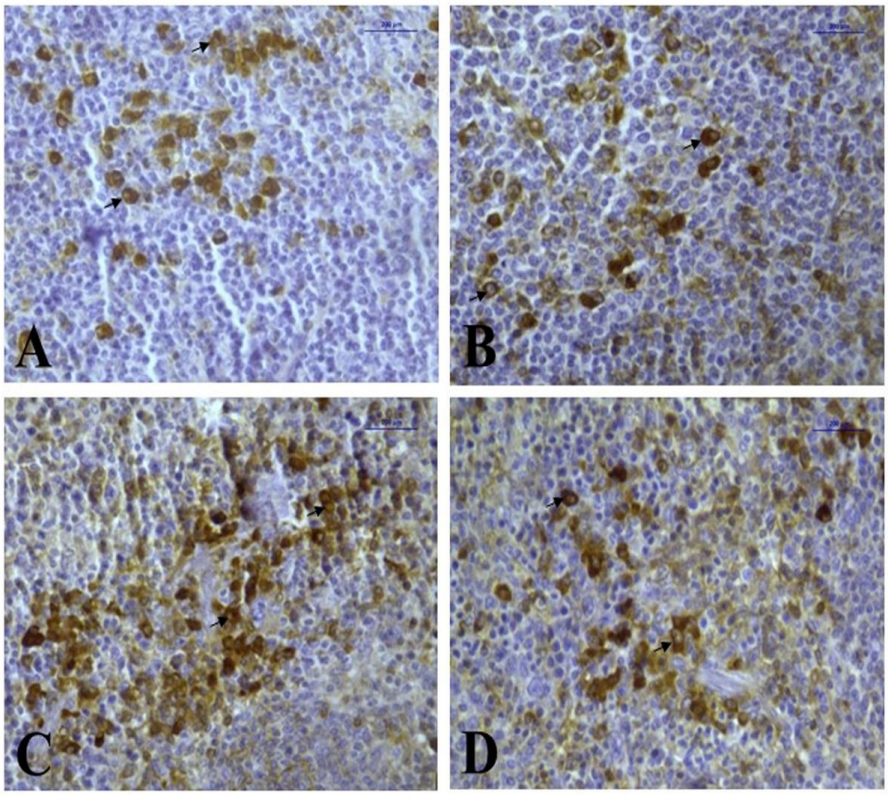

FIGURE 4. Effect of hyperthyroidism and melatonin on the immunoreactivity of MT2 receptor antisera in the spleen of laboratory mice. A = control, B = L-Thyroxine treated, C = Melatonin treated, D = L-Thyroxine treated + Melatonin. Micrographs were taken under the 40X objective. Arrowhead (➔) showed MT2 receptor antisera reactivity in splenocytes. The magnification bar showed 200µm.

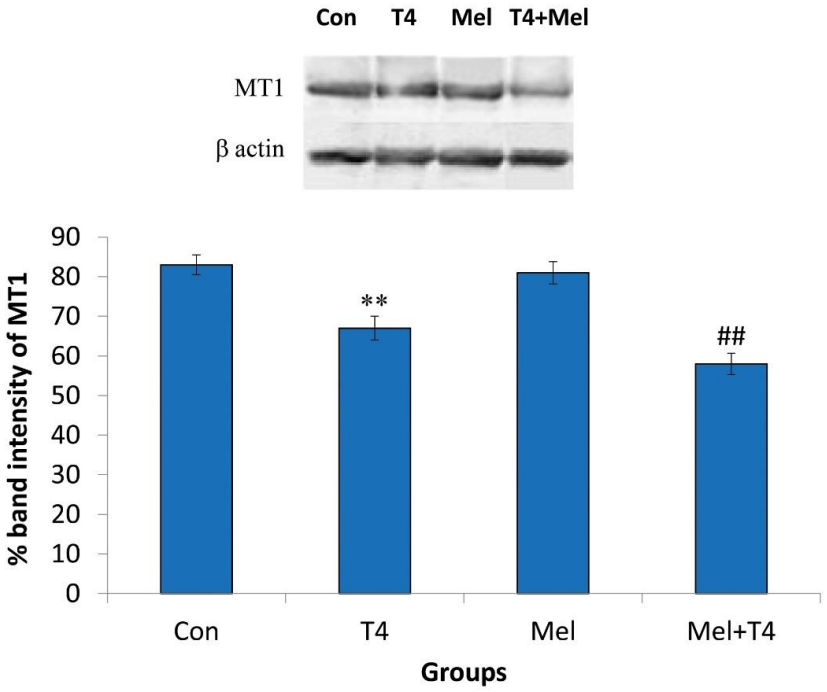

FIGURE 5. Effect of hyperthyroidism and melatonin on MT1 receptor protein expression in spleen of laboratory mice. Histograms represent Mean ± SEM, n=5 for each group. Con = control, T4 = L-Thyroxine treated, Mel = Melatonin treated, T4+Mel = L-Thyroxine treated + Melatonin. ** P<0.01 for T4 vs Con; $^{\#\#}$ P<0.05 for T4+Mel vs T4.

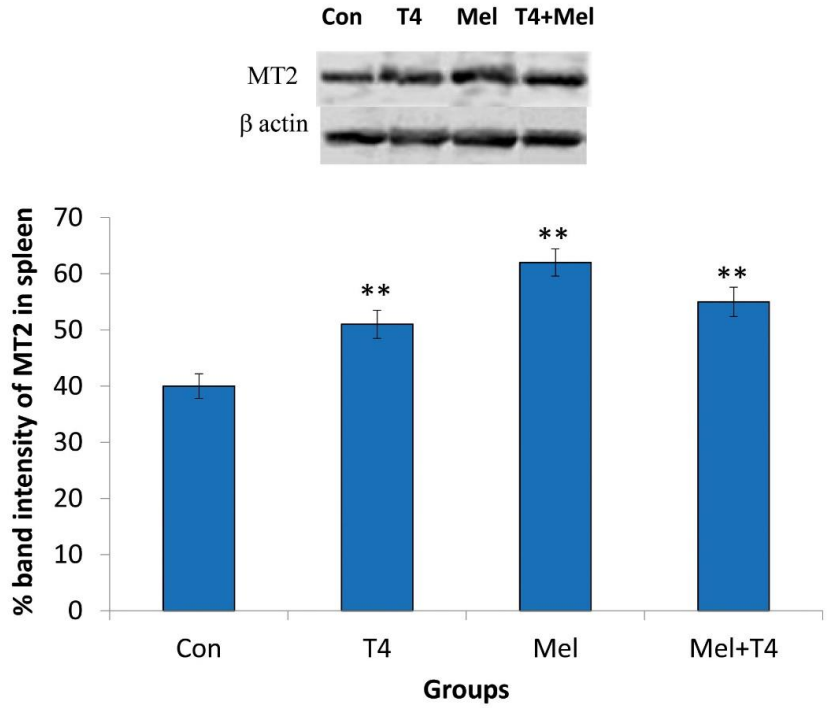

FIGURE 6. Effect of hyperthyroidism and melatonin on MT2 receptor protein expression in spleen of laboratory mice. Histograms represent Mean ± SEM, n=5 for each group. Con = control, T4 = L-Thyroxine treated, Mel = Melatonin treated, T4+Mel = L-Thyroxine treated + Melatonin. ** P<0.01 for T4 vs Con, Mel vs Con, and Mel+T4 vs Con.

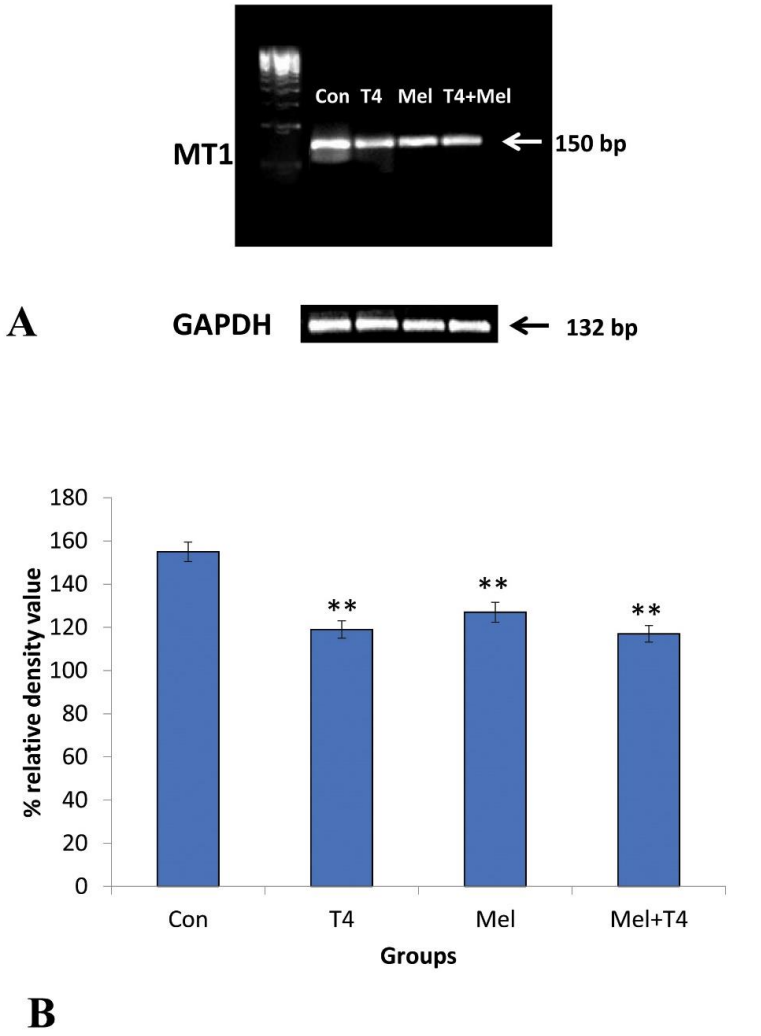

FIGURE 7. Effect of hyperthyroidism and melatonin on MT1 mRNA expression in spleen of laboratory mice. Histograms represent Mean ± SEM, with n=5 for each group. Con = control, T4 = L-Thyroxine treated, Mel = Melatonin treated, T4+Mel = L-Thyroxine treated + Melatonin. ** $P<0.01$ for T4 vs Con, Mel vs Con, and Mel+T4 vs Con.

association with melatonin-mediated attenuation of hyperthyroidism-induced oxidative stress in the spleen tissue of mice. L-thyroxine treatment caused an increase in circulatory levels of thyroid hormones T3 and T4, indicating experimental hyperthyroidism. Earlier reports suggested that L-thyroxine induces hyperthyroidism in laboratory mice (Laskar and Singh, 2018). Melatonin treatment lowers the T3 and T4 hormone levels in hyperthyroidic mice. Similar findings were reported in male rats by Ramadan et al. (2021). Further melatonin supplementation to the L-thyroxine-treated mice caused significant suppression of T3 and T4 hormone levels in the experimental mice. Ramadan et al., 2021 reported a lowering of circulatory T3 and T4 levels by melatonin supplementation in hyperthyroid male rats. Melatonin administration mainly during the evening, suppresses mitotic activity which has an inhibitory effect on the function of the thyroid gland leading to low levels of T3 and T4 hormones (Sewerynek et al., 1999).

Hyperthyroidism accelerates mitochondrial free radical production thus inducing a change in the antioxidant protective system of rat tissues (Asayama and Katu, 1990). In the present study, L-thyroxine treatment increased the lipid peroxidation level in the spleen of hyperthyroidic mice. Studies showed that hyperthyroidism tends to increase lipid peroxidation in the spleen of rats. Increased thyroid hormones in hyperthyroid rats caused an accelerated metabolic rate associated with a decrease in the antioxidant enzyme activity (Pereira et al., 1994). In the present study, decreased antioxidant enzyme defense in terms of suppressed SOD and catalase enzyme activities was observed in hyperthyroidic

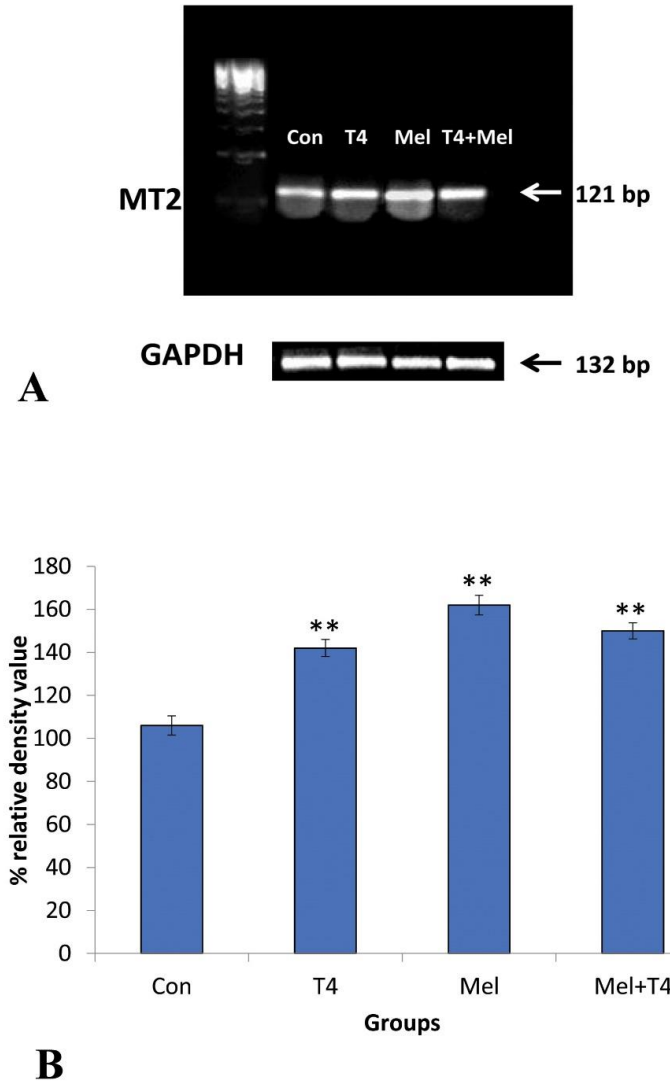

FIGURE 8. Effect of hyperthyroidism and melatonin on MT2 mRNA expression in spleen of laboratory mice. Histograms represent Mean ± SEM, with n=5 for each group. Con = control, T4 = L-Thyroxine treated, Mel = Melatonin treated, T4+Mel = L-Thyroxine treated + Melatonin. ** P<0.01 for T4 vs Con, Mel vs Con, and Mel+T4 vs Con.

mice. The studies suggested reduced catalase activity in the spleen of hyperthyroidic mice (Pereira et al., 1994), and a decreased SOD activity in the liver of rats following L-thyroxine treatment (Giavarotti et al., 1998). The study also suggested a reduced level of SOD activity in the kidney of hyperthyroid rats (Sawant et al., 2003). Further, the study of Pereira et al., (1994) also suggested the regulation of antioxidant enzyme activities in lymphoid organs by thyroid hormones in rats. The increased ROS production due to accelerated metabolism in hyperthyroidism may be responsible for reduced antioxidant enzyme (SOD and catalase) activities in the splenic tissues of studied mice. The hyperthyroid state reduces the antioxidant status of an organism by reducing the mRNA and protein activities of critical antioxidant enzymes (Chattopadhyay et al., 2007).

The present study showed that melatonin administration caused significant suppression of lipid peroxidation in the spleen of control mice. Reiter et al., (1997) reported depletion of the lipid peroxidation in the liver of control rats treated with melatonin. Our study showed a significant increase in catalase and SOD enzyme activity in the spleen of melatonin-treated mice. The study suggested that melatonin increases antioxidant defense by potentiating catalase and SOD enzyme activities in experimental rats (Rodriguez et al., 2004; Chainy and Sahoo, 2020). The present study showed that melatonin administration to hyperthyroid mice significantly de-

creased the lipid peroxidation levels in the spleen. Studies suggested that melatonin supplementation prevented lipid peroxidation in cardiac tissue of hyperthyroid rats (Mogulkoc et al., 2006). Our previous findings suggested that melatonin caused the restoration of catalase and SOD enzyme activity in the spleen of diabetic mice (Sutradhar et al., 2022). Further, studies also showed that melatonin restored SOD activity in the heart of T3-treated hyperthyroidic rats (Ghosh et al., 2007).

Melatonin mediates its action via the activation of G-protein coupled MT1 and MT2 receptors in the target tissues. Melatonin receptors are present in various tissues including the spleen of Swiss albino mice (Singh et al., 2015). In the present study, immunoreactivity of MT1 and MT2 receptors antisera were observed in the spleen of experimental mice. Weak reactivity of MT1 antisera and strong reactivity of MT2 antisera was observed in the spleen of thyroxine-treated, melatonin-treated, and melatonin-treated L-thyroxine-supplemented mice. Western blot analysis showed significant suppression in MT1 protein intensity in thyroxine-treated, melatonin-treated L-thyroxine-supplemented mice. Reverse-transcription PCR analysis showed significant suppression of MT1 receptor mRNA level in thyroxine-treated, melatonin-treated, and melatonin-treated L-thyroxine-supplemented mice. The MT2 protein and mRNA expression were significantly increased in thyroxine-treated, melatonin-treated, and melatonin-treated L-thyroxine-supplemented mice. A study by Catala et al, (1988) demonstrated that increased T3 levels may modulate melatonin synthesis by the pineal gland in a light-dark condition-dependent manner. The MT2 receptor protein and mRNA expression were upregulated in the melatonin-treated group. Our previous studies suggest that exogenous melatonin supplementation modulates MT1 and MT2 receptor expression in the spleen in an age-dependent manner (Singh et al., 2015). Melatonin supplementation in thyroxine-treated mice caused suppression of MT1 receptor protein and mRNA expression. However, MT2 receptor expression was considerably increased in the mice group treated with both melatonin and thyroxine compared to the experimentally induced hyperthyroid group. Our previous study suggested that melatonin treatment affects MT2 receptor protein expression but not MT1 receptor protein expression in the spleen (Laskar and Singh, 2018). MT2 receptor protein and mRNA expression significantly increased in the thyroxine-treated group compared to the control group. The increased circulatory T4 levels caused metabolic stress in hyperthyroid mice and the MT2 receptor expression might be associated with stressed conditions in the spleen tissues of studied mice. Our previous study also suggested an increase in MT2 receptor protein expression in the spleen tissues of heat-stressed mice (Acharjee and Singh, 2023). A recent report suggested increased circulatory melatonin levels in T4-treated PCOS rats (Ghosh et al. 2022). This might help melatonin to protect against experimental hyperthyroidism-induced changes in the spleen of experimental mice.

Conclusion

The findings of the present study showed experimental hyperthyroid-induced oxidative stress in the spleen tissue of experimental mice. Hyperthyroidism caused an increase in lipid peroxidation and suppression of the antioxidant defense system, down-regulation of melatonin MT1 receptor, and upregulation of MT2 receptors in the spleen tissue of mice. Melatonin ameliorated L-thyroxine-induced oxidative stress and upregulated the MT2 receptor protein and mRNA expression in spleen tissues. Therefore, the present study concludes that alteration in MT2 receptor expression may be associated with melatonin-mediated attenuation of hyperthyroid-induced oxidative stress in the spleen tissue of mice.

Acknowledgment

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethics approval

Ethics approval and consent to participate – All the experiments with animals and their maintenance were done in accordance with the institutional practice and within the framework of CCSEA (Committee for Control and Supervision of Experiments on Animals) and the Act of Government of India (2007) for animal welfare with IAEC approval no. TU/IAEC/2013/V/5-3.

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