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Melatonin Prevents Dexamethasone-Induced Involution of the Thymus and Spleen in Mice

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Abstract: *Synthetic glucocorticoids like dexamethasone (DEX) are potent anti-inflammatory agents, but their prolonged use induces severe immunosuppression and characterized by profound lymphoid organ involution. Melatonin (MEL), an endogenous indolamine synthesized by the pineal gland, possesses well-documented immunomodulatory, antioxidant, and anti-apoptotic properties. Based on the above, it is hypothesised that Melatonin cotreatment may antagonize the immunosuppressive effects log-term Dexamethasone treatment and hence the present study was designed to investigates the potential of melatonin to antagonize Dexamethasone-induced involution of the thymus and spleen in a murine model. Thirty young adult male Parkes strain mice were divided into three groups: Control (vehicle), DEX-treated (400 µg/kg/day, i.p.), and DEX+MEL-treated (DEX 400 µg/kg/day, i.p. + MEL 750 µg/kg/day, s.c.). Treatments were administered daily between 16:30 and 17:30 for 30 consecutive days. Following the experimental period, lymphoid organs were dissected out, weighed, and histological changes were assessed via Haematoxylin and Eosin (H&E) staining. Log-term Dexamethasone administration induced a significant reduction in both thymic and splenic absolute weights. Co-administration of melatonin significantly restored the organ weights which were comparable to Control (vehicle) group. Histological analysis of DEX-treated mice revealed severe cortical thymocyte depletion and white pulp hypoplasia in the spleen. Co-administration of melatonin significantly preserved the microanatomical architecture of both the thymus and spleen. Histomorphology of lymphoid organs in DEX+MEL-treated group were comparable to Control (vehicle) group. In the present study, Melatonin effectively antagonized Dexamethasone-induced effects on lymphoid organs in young adult male albino mice (Parkes Strain), suggesting its therapeutic potential as a cytoprotective adjuvant during chronic corticosteroid therapy..*

Keywords: Melatonin, Glucocorticoids, Dexamethasone, Corticosteroid, Neuroimmunomodulation, Thymus, Spleen, Thymocytes, Involution, White pulp, Red pulp, Follicles, Mice

I. INTRODUCTION

Glucocorticoids are highly efficacious steroid hormones widely utilized in clinical medicine for their potent anti-inflammatory and immunosuppressive properties (Agnes et al., 2011). Synthetic analogs, such as dexamethasone (DEX), are routinely prescribed to manage autoimmune diseases, severe allergies, asthma, and inflammatory disorders, as well as to prevent allograft rejection (Coutinho & Chapman, 2011). However, the chronic administration of high-dose glucocorticoids is invariably accompanied by severe adverse physiological effects. One of the most profound consequences is the rapid and extensive involution of primary and secondary lymphoid organs, particularly the thymus and the spleen (Compton & Cidlowski, 1986). This iatrogenic immunosuppression significantly increases the patient's vulnerability to opportunistic infections and compromises overall immune surveillance.

The structural regression of lymphoid tissues following dexamethasone exposure is not merely a cessation of growth, but an active, hormonally driven process of cellular dismantling. In the thymus, glucocorticoids trigger massive apoptotic cell death, specifically targeting immature CD4+CD8+ double-positive (DP) thymocytes residing in the thymic cortex (Ashwell et al., 2000). This depletion disrupts the cortico-medullary architecture and halts the maturation

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of T-lymphocytes. Similarly, in the spleen, dexamethasone induces profound hypoplasia of the white pulp, driving apoptosis in mature peripheral lymphocytes and disrupting the structural integrity of lymphoid follicles (Gruver-Yates & Cidlowski, 2013). At the molecular level, dexamethasone binds to the cytosolic intracellular glucocorticoid receptor (GR), translocates to the nucleus, and modulates gene transcription. This process upregulates pro-apoptotic proteins (such as Bax and Bim), downregulates anti-apoptotic factors (such as Bcl-2), and simultaneously generates high levels of intracellular reactive oxygen species (ROS), leading to severe oxidative stress within the lymphoid microenvironment.

Mitigating the cytotoxic side effects of synthetic glucocorticoids without neutralizing their primary therapeutic benefits remains a significant challenge in pharmacology. Melatonin (N-acetyl-5-methoxytryptamine), an indolamine synthesized primarily by the pineal gland during the dark phase of the circadian cycle, has emerged as a promising cytoprotective candidate. Originally recognized solely for its role in synchronizing circadian and circannual rhythms, melatonin is now established as a highly pleiotropic molecule with profound immunomodulatory, antioxidant, and anti-apoptotic properties (Carrillo-Vico et al., 2013). The presence of specific high-affinity melatonin receptors (MT1 and MT2) on the membranes of various immunocompetent cells, including thymocytes and splenocytes, underscores the existence of a direct neuroendocrine-immune signaling axis (Guerrero & Reiter, 2002).

Accumulating evidence suggests that melatonin functions as an endogenous functional antagonist to glucocorticoids, particularly under conditions of stress or pharmacological overload. Melatonin's high lipophilicity allows it to readily cross biological membranes and enter subcellular compartments, where it acts as a potent, broad-spectrum free radical scavenger, neutralizing the ROS generated by glucocorticoid metabolism (Reiter et al., 2017). Furthermore, exogenous melatonin has been shown to counteract glucocorticoid-induced apoptosis by preventing the collapse of the mitochondrial membrane potential and shifting the intracellular balance back toward cell survival (anti-apoptotic Bcl-2 expression) (Sainz et al., 2003). While previous studies have documented melatonin’s ability to buffer against environmental stress-induced immunosuppression, the specific morphological and histological antagonism of exogenous melatonin against high-dose, synthetic dexamethasone toxicity in a controlled murine model requires more precise structural elucidation.

Given the severe impact of glucocorticoid therapy on immune integrity, investigating pharmacological adjuvants that preserve lymphoid architecture is of high clinical relevance. Therefore, the present study was designed to investigate whether concurrent exogenous melatonin administration can prevent or significantly antagonize Dexamethasone-induced effects on the thymus and spleen in young adult male mice (Parkes strain). By administering treatments during the evening hours—coinciding with the natural circadian surge in both endogenous pineal melatonin synthesis and hypothalamic-pituitary-adrenal (HPA) secretory activity, this study aims to maximize receptor-mediated efficacy over a continuous 30-day period. We hypothesize that melatonin will preserve absolute lymphoid organ weights and maintain the microanatomical integrity of the thymic cortex and splenic white pulp against dexamethasone-induced atrophy and degradation.

II. MATERIALS AND METHODS

Animals and Housing

Young adult male albino mice (Parkes Strain, 8 weeks of age, weighing 30 ± 3 g) were procured from the Central Drug Research Institute (CDRI) in Lucknow, India. The animals were housed in PVC cages (290 × 320 × 390 mm) under standardized laboratory conditions, including a 12:12 hour light/dark cycle (lights on at 07:00), a controlled temperature of 21 ± 2 ºC, and a relative humidity of 55 ± 5%. Mice were provided ad libitum access to purified water and standard pelleted rodent chow. All animals were acclimatized to the laboratory environment for one week prior to the commencement of the experiment. The care and handling of the animals were according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

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Experimental Design and Treatment

A total of 30 mice were randomly assigned to three equal groups (n = 10 per group). To coincide with the circadian increase in endogenous pineal secretory activity, all treatments were administered during the evening hours (16:30–17:30) for 30 consecutive days.

Group	Designation	Treatment Protocol
Group I	Control (CONT)	Received 0.1 ml vehicle for DEX (0.9% normal saline, i.p.) and vehicle for MEL (alcoholic saline, 0.01% ethanol, s.c.) for 30 consecutive days.
Group II	DEX	Treated with dexamethasone (400 µg/kg BW/day, i.p.) for 30 consecutive days.
Group III	DEX+MEL	Treated with dexamethasone (400 µg/kg BW/day, i.p.) concurrently with melatonin (750 µg/kg BW/day, s.c.) for 30 consecutive days.

Tissue Collection and Histological Processing

Upon completion of the 30-day treatment period, mice were decapitated and lymphoid organs (thymus and spleen) were rapidly dissected, cleared of adjacent adipose and connective tissues, blotted dry, and weighed. Tissues were immediately immersed in Bouin's fluid for overnight fixation. Following fixation, the organs were washed thoroughly to remove excess picric acid and processed conventionally for paraffin embedding. Tissue blocks were sectioned at a thickness of 5–7 µm, mounted on glass slides, deparaffinized, and stained with Hematoxylin and Eosin (H&E). The stained sections were examined and photographed under a light microscope to assess microanatomical alterations.

Statistical Analysis

The results are expressed as Mean ± S.E ($n \geq 5$). Statistically significant differences between the treatment groups were analyzed using one-way analysis of variance (ANOVA) followed by post hoc comparison using the Tukeys test. The differences of the means were considered significant when $p \leq 0.05$. For all statistical analysis, the Statistical Package of Social Science version 10.0 (SPSS Inc.) was used.

III. RESULTS

Effect on Lymphoid Organ Weights

Administration of dexamethasone (Group II) for 30 days resulted in a marked and statistically significant decrease in the absolute weights of both the thymus and spleen compared to the vehicle-treated control group (Group I). A 68% reduction of thymus weight together with a significant ($P \leq 0.01$) drop in thymus/body weight ratio (relative weight) from that of the control group was noticed. Conversely, the concurrent administration of melatonin (Group III) significantly attenuated this weight loss. In the melatonin cotreated group (DEX+MEL), the thymus weight was comparable to that of CONT, reduced only by 14% (Fig. 1a).

Spleen weight was reduced by 43%, which was highly significant compared to CONT value ($P \leq 0.01$); the relative spleen weight was also significantly dropped (Fig. 1b). The spleen weight in cotreated (DEX+MEL) group was significantly more ($P \leq 0.05$) compared to DEX group and was comparable to that of CONT (Fig. 1b).

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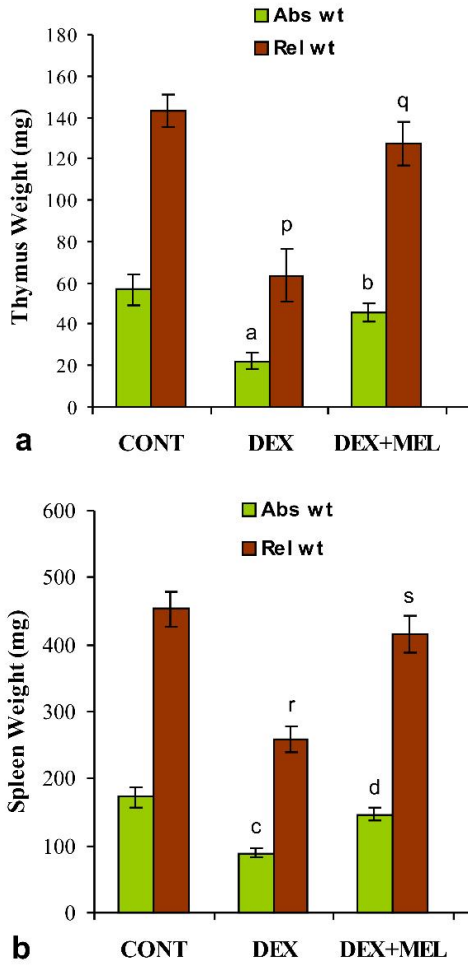

Figure 1 *Graph showing absolute (Abs) and relative (Rel) weights of the thymus (a) and spleen (b) under dexamethasone (DEX) and melatonin (MEL) treatments. CONT: Control. Data represented as Mean ± SEM (n ≥ 5). (a & p) p < 0.01 DEX vs CONT, (b & q) p < 0.05 DEX vs DEX+MEL.*

Histopathological Observations of the Thymus

Under chronic DEX treatment thymic histology was distinctly altered as compared to CONT (Table 1, Fig. 2a-f). The involution was prominent with significant reduction of cortical width (Fig. 2d) compared to CONT (Fig. 2a). The cortical degeneration was distinct. The density of thymocytes in the cortex as well as in medulla was less (Fig. 2d-f) and the corticomedullary junction became indistinct as compared to CONT (Fig. 2a-c). A significant reduction (P≤0.05) of size of thymocytes in cortex as well medulla was observed (Table 1). Number of darkly stained pyknotic nuclei was more in the cortical region compared to CONT. Enlarged blood vessels, colloid accumulation and fatty infiltration were also observed (Fig. 2e). Thymic cortex histology of MEL cotreated group resembled that of the control (Fig. 3a-c). Corticomedullary junction was distinct as the cellular density of the cortex was more as compared to DEX group. The size of the thymocytes in cortex as well as medulla (Table 1, Fig. 3b, c) was significantly more (P≤0.05) as compared to DEX.

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Parameters	Experimental Groups		
	CONT	DEX	DEX + MEL
Thymocyte Size			
Cortex (µm)	3.98 ± 0.19	3.41 ± 0.15 a	3.78 ± 0.11 b
Medulla (µm)	3.99 ± 0.16	3.13 ± 0.27 a	3.76 ± 0.15 b
Splenocyte Size			
White Pulp (µm)	4.86 ± 0.13	4.13 ± 0.06 a	4.65 ± 0.29 b
Red Pulp (µm)	4.78 ± 0.11	4.21 ± 0.09 a	4.67 ± 0.17 b

Table 1 *Morphometry of Thymus and Spleen under Dexamethasone (DEX) and Melatonin (MEL) treatments. Data are expressed as Mean ± S.E (n ≥ 5). a p < 0.01 DEX vs CONT, b p < 0.05 DEX vs DEX+MEL.*

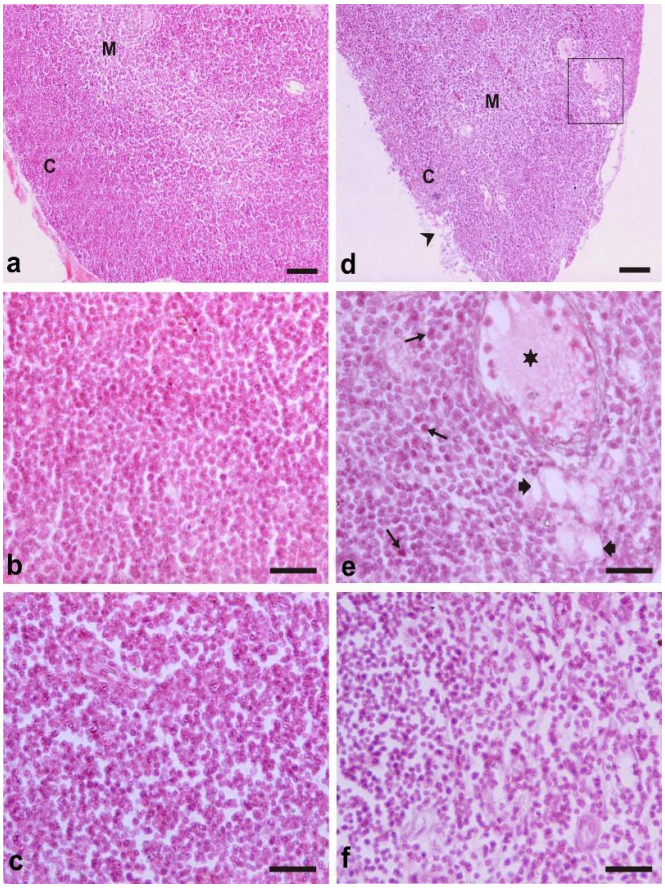

Figure 2 Cross sections showing thymic histology of control (CONT, a-c) and dexamethasone (DEX, d-f) groups. Note cortical degeneration (➤), thymocyte pyknosis (➤), fatty infiltration (◆), colloid accumulation (*), and reduction of thymocytes in DEX group (d, e). Also note the reduction of size and density of medullary thymocytes (f). C, Cortex; M, Medulla. Hematoxylin and Eosin. Bar 50 µm (a, d); 20 µm (b, c, e & f).

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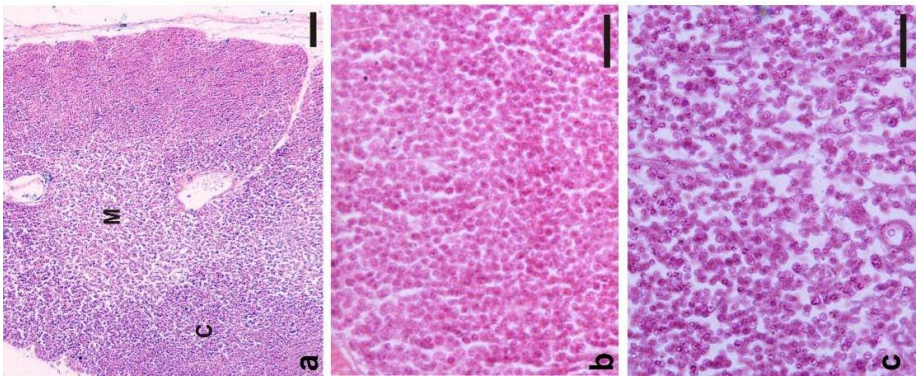

Fig. 3 Cross sections showing thymic histology under melatonin (MEL, a-c) treatments. Note distinction of cortex (C) and medulla (M) in melatonin treated group (a, c). Density of thymocytes in cortex (b) and medulla (c) was increased in the MEL treatment group compared to DEX. Hematoxylin and Eosin. Bar 50 µm (a); 20 µm (b, c).

Histopathological Observations of the Spleen

Distinct zonation of red pulp (RP, red blood cell storage and degradation) and white pulp (WP, lymphoid) area with a clear marginal zone as observed in control spleen (Fig. 4a, b) was distorted and became indistinct on DEX treatment (Fig. 4e, f). The WP area was reduced with less number of follicles. The normal follicular organization was changed due to reduction of cellular density of WP that resulted in indistinct marginal zone (Fig. 4f). The splenocyte size of both WP and RP was also significantly ($P \leq 0.05$) reduced (Table 1 Fig. 4g, h). In DEX+MEL group, the zonation between WP and RP became distinct (Fig. 5a, b). The overall area of WP was more with more numbers of follicles as compared to DEX group.

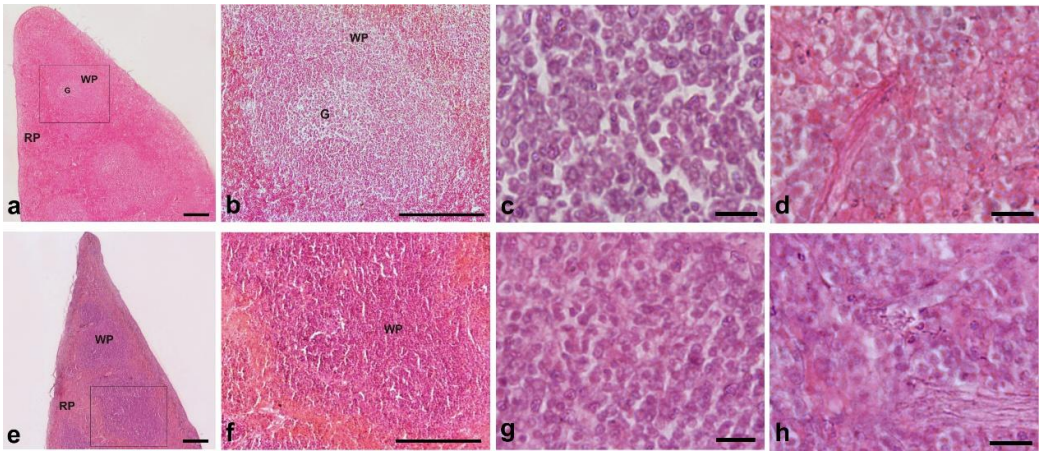

Figure 4 *Cross sections showing spleen histology of control (CONT, a-d) and dexamethasone (DEX, e-h) groups. Note well organized follicles with distinct red pulp (RP) and white pulp (WP) area and germinal centers (G) in CONT (a, b) which was disrupted in DEX (e, f) group. Also note reduction of splenocyte number and size both in RP and WP (g, h) from that of the CONT (c, d). Hematoxylin and Eosin. Bar 100 µm (a, b, e & f); 20 µm (c, d, g & h).*

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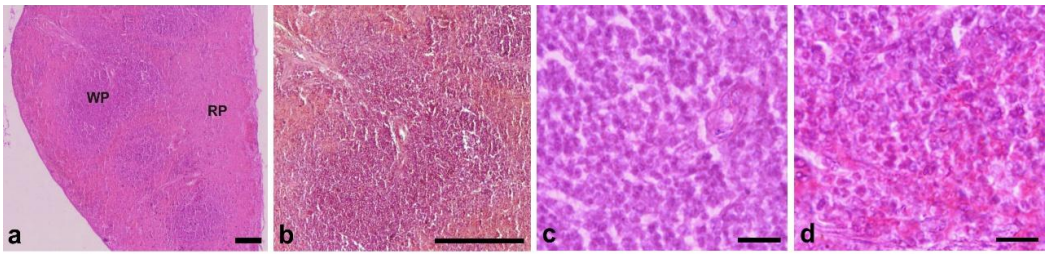

Figure 5 *Cross sections showing spleen histology under melatonin (MEL, a-d) treatments. Note distinction of white pulp (WP) and red pulp (RP) in cotreated (a, b) group. Also note increase of splenocyte density and size both in WP and RP of cotreated (c, d). Hematoxylin and Eosin. Bar 100 μm (a, b); 20 μm (c, d).*

IV. DISCUSSION

The pineal hormones melatonin is shown to modulate the immune components exerting their immunoenhancing properties. In addition to antagonization of the adverse effects of glucocorticoids analogue dexamethasone, the immune favorable actions of melatonin also have been demonstrated in mice not administered with synthetic glucocorticoids. The antagonization of the immunosuppressive effects of dexamethasone by melatonin has been elucidated in the present study through weight analysis, histopathology of lymphoid organs thymus and spleen. Dexamethasone-induced significant decrease in thymus and spleen weight indicated their hypofunctional state as weight of any organ signifies its functional activity (Rooman et al., 1999; Biolatti et al., 2005). These results are in accordance to the studies where extraneous or stress mediated glucocorticoid elevation reported to induce thymus (Cohen, 1992; Maestroni et al., 1993; Zucker et al., 1994) and spleen involution (Ben Nathan et al., 1995; Haldar et al., 2004). DEX-induced lymphoid organ involution was reflected in their histopathological alterations. Severe atrophy of the thymic cortex with depletion of cortical thymocytes was observed as demonstrated on corticosteroid treatment (Compton et al., 1992; Cohen, 1992; Sun et al., 1992; Zucker et al., 1994; Tarcic et al., 1998). Few studies have shown spleen involution (Ben-Nathan et al., 1995) and a significant reduction in splenocyte proliferation after treatment with therapeutic doses of corticosteroids (Haldar et al., 2004). The reduction of splenocyte was evident in the present study as the marginal zone between red pulp and white pulp area of spleen became indistinct with the reduced cellular density in later. DEX-induced lymphocyte apoptosis might have resulted in the reduced density of WP as reported earlier (Burton and Kehrli, 1996; Thompson, 1999).

Immunosuppressive effect of DEX was antagonized by melatonin. The weights of both thymus and spleen in the cotreatment (DEX+MEL) group were comparable to their control counterparts. Antagonizations of suppressive effects of DEX by melatonin hormone also have been reflected in the thymus and spleen histology. Though there is persistence of some adverse effects, most of the DEX-related histopathological alterations of thymus and spleen were prevented on cotreatment of melatonin. The cellular density of the thymic cortex was increased along with a significant increase of thymocyte size. The WP area of spleen was increased with increase in follicles numbers and their size. That the melatonin counteracts the DEX-induced suppression of lymphoid organs has been demonstrated through the histological study of thymus and spleen in different vertebrates (Maestroni et al., 1993; Haldar et al., 2004). The weight analysis and histology of lymphoid organs of the group treated with MEL alone also indicated immune favorable role. Hyperplasia of lymphoid organs and inhibition of the programmed cell death under MEL administration has been reported (Csaba and Barath, 1975; Sainz et al., 1995; Provinciali et al., 1996).

Inspite of significant number of studies demonstrating MEL action in counteracting the negative effects of GC on immune system, the mechanisms by which melatonin exert its effects are not well understood. High affinity melatonin receptors have been localized on thymocytes and splenocytes (Lopez-Gonzales et al., 1992; Martin-Cacao et al., 1993; Rafii-El-Idrissi et al., 1995). It has also been reported that MEL down-regulates the GC receptors in lymphoid organs thereby prevents the GC-induced apoptosis (Persengiev et al., 1991, 1992; Sainz et al., 1999, 2003). Thus, suppression

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of DEX effect might be due to the direct action of MEL on thymus and spleen which further led to the down-regulation of GC receptors.

V. CONCLUSION

In conclusion, the present study demonstrates that exogenous melatonin acts as a potent functional antagonist against dexamethasone-induced involution of the thymus and spleen in mice. Ultimately, the preservation of the cortico-medullary junction in the thymus and the white pulp in the spleen suggests that melatonin protects both the maturation environment for T-cells and the peripheral zones required for antigen response. Melatonin shows significant promise as a therapeutic adjuvant to mitigate the immunosuppressive side effects of chronic glucocorticoid therapy, preserving systemic immune integrity.

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