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Effects Of Long-Term Glucocorticoid Treatment On Immune Components In Mice

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Abstract:

Corticosteroids play crucial roles in regulating the development, growth, metabolism, and immune function of the body. Long-term glucocorticoid treatment in mice has been shown to have significant effects on immune components in mice. Dexamethasone, a potent synthetic corticosteroid, exerts broad immunosuppressive and anti-inflammatory effects in mice. It is widely used as a drug for the treatment of a range of metabolic diseases and inflammatory disorders in humans as well as in farm animals. Major concern has emerged about the adverse effects of long-term glucocorticoid (dexamethasone) treatment. In the present study the effects of long-term dexamethasone treatment on immune components mainly histomorphology of lymphoid organs and hematological parameters in adult male mice was assessed. Involution of thymus and spleen was prominent with a significant reduction in organ weight and organ/body weight ratio. Thymus histology showed severe atrophy and fatty infiltration. The size and density of thymocytes in cortex as well as in medulla was reduced. In spleen, disorganized white pulp with reduced splenocyte density and size was observed. Significant decrease in the number of total leukocyte and lymphocyte was observed. An opposite trend was noted in neutrophil count which increased significantly after dexamethasone treatment. A dramatic alteration in the neutrophils to lymphocytes ratio was also observed. The results suggest that long-term glucocorticoid (dexamethasone) treatment, having significant effects on different immune components, may be a risk factor for the development of immune related disorders.

Key words: Glucocorticoids (GCs), Dexamethasone, Lymphoid organ, Thymocytes, Splenocytes, Leukocytes, Immunosuppressive, Mice.

Introduction

Glucocorticoids (GCs) are steroid hormones with widespread effects. GCs play crucial roles in regulating the development, growth, metabolism, and immune function of the body (Grbovic and Radenkovic, 2005). Long-term glucocorticoid treatment in mice has been shown to have significant effects on immune components in mice. Dexamethasone (DEX) is a synthetic glucocorticoid and is widely used as a drug for the treatment of a range of metabolic diseases and inflammatory disorders in

humans as well as in farm animals. It is a proven immune suppressive and anti-inflammatory agent (Cohn, 1997; Turnbull and Rivier, 1999,). Hence it is widely used in the treatment of metabolic diseases and inflammatory disorders in humans as well as in farm animals (Munk et al., 1984; Wilckens and DeRijk, 1997; Mandell, 2000). The most commonly used glucocorticoid (GC) drugs include prednisone, betamethasone, beclomethasone propionate, prednisolone, hydrocortisone, and dexamethasone (Grbovic and Radenkovic, 2005; Coutinho and Chapman, 2011). Dexamethasone affects all the major system of the body and hence the major concern has emerged about the possible adverse effects of the long-term dexamethasone treatment. Immune system is one of the most sensitive systems to the dexamethasone. Numerous studies have investigated the effect of dexamethasone treatment on thymus and spleen, the major lymphoid organs, and alterations in other immune parameters. Thymic involution due to dexamethasone exposure is well reported (Cohen, 1992; Compton et al., 1992; Zucker et al., 1994). Dexamethasone-induced spleen involution and effect on splenocyte proliferation has been studied (Ben Nathan et al., 1995; Haldar et al., 2004). Studies have also reported that dexamethasone exposure suppresses cellular and humoral immune function (Ader and Cohen, 1993; Dhabhar and McEwen, 1997; Anderson et al., 1999; Moire et al., 2002). Thus, dexamethasone mediated change in the function of circulating immune cells has been reported as opposed to change in their number and distribution (Dhabhar et al., 1996; Dhabhar and McEwen, 1997). However, the functional responses of leukocytes are less sensitive to the effect of dexamethasone treatment than other hematological parameters like TLC and DLC (Anderson et al., 1999).

Reports on dexamethasone effect on immune system have been derived mainly from in vitro studies. Much of the work has been fragmentary, limited either to the lymphoid organs (thymus/spleen) or to cellular and humoral immune functions. Studies related to the long-term in vivo effect of dexamethasone on different lymphoid organs and blood parameters as a whole are very few. In vivo studies are mainly focused to the effect of stress-induced elevated glucocorticoid level on immune system. Since synthetic glucocorticoids differ from the natural glucocorticoids in their potency and affinity for glucocorticoid receptor subtypes (Dhabhar and McEwen, 1999), the in vivo effect of dexamethasone on immune system may be different from the effect of stress-induced elevated glucocorticoid level on immune system. In addition, heterogeneity in receptor expression among various components such as thymus and spleen has been reported (McEwen, 1998; Dhabhar, 2002). This suggests that the response to dexamethasone accordingly might be different. In view of these facts the present study was carried for the elucidation of the overall impacts of chronic dexamethasone treatment on the histomorphology of both the main immune organs (thymus and spleen) as well as on the haematological parameters in adult male mice. Growth suppressing effects of dexamethasone have also been reported in many species including mice (Silbermann et al., 1976), rats (Ortoft, 1998) and man (Allen et al., 1996; Ortoft, 1998).

Materials and Methods

Animals

Male adult mice (Parkes Strain) used in the present study were obtained from Central Animal House, CDRI, Lucknow. Animals were housed in PVC cages (290x320x390 mm) and were maintained on a 12-hour light/dark cycle at a constant temperature (23 ± 2^0C) and humidity (50% to 55%). All the animals were having free access to water and mice feed ad libitum. They were acclimatized to the laboratory condition for one weak prior to use. 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. The experimental protocols were approved by the Institutional Animal Ethical Committee of the University of Allahabad, India.

Experimental Design

Twenty adult male mice (32 g BW) were divided into two groups of ten each. Group I mice were given intraperitoneal injection of dexamethasone 21-phosphate (400 µg/kg BW/day, i.p,) for thirty consecutive days in sterile pyrogen free saline. Group II mice received an equal volume of sterile pyrogen free saline for the same period and are treated as control group (CONT). All the injections were given at evening hours between 4:15pm to 4:45pm. Mice of both the groups were weighed on every second day till the end of the experiment. On the termination of the experiment, animals of both the groups were sacrificed by decapitation. Thymus and spleen were immediately dissected out, freed from the adjacent tissues, blotted and weighed. The tissues were then fixed in Bouin's fluid for further histological study.

Haematological Analysis

Cardiac blood samples were collected in microcentrifuge tubes containing anticoagulant ($EDTANa_2$). The white blood cell count was obtained by haemocytometer using Turks solution (glacial acetic acid, 1ml; gentian violet, 1% aqueous, 1ml. distilled water to 100ml.) to hemolise the red blood cells. The lymphocyte and neutrophil number/percentage was obtained by microscopic examination of peripheral blood smears stained by general Leishmann stain.

Bone marrow leukocyte count

Bone marrow strips from femur bones were flushed into a sterilized test tube with the help of syringe using phosphate buffer. After homogenization, a small drop of the suspension was put on the clean slide; a thin film was prepared and dried. The slide was stained with Leishmann stain and observed under microscope for DLC.

Histology

After overnight fixation the tissues were washed thoroughly and processed conventionally for paraffin embedding. Sections of 5-7µm thickness were cut and stretched on glass slides. After deparaffinization sections were stained with hematoxylin and counter stained with eosin for basic histopathological analysis under light microscope

Statistical Analysis

The results are expressed as Mean±S.E (n=10). Statistically significant difference between the treatment groups (CONT vs. DEX) were analyzed using student's *t*-test. The differences of the means were considered significant when $p \leq 0.05$. For all statistical analysis, a computer statistics package SPSS V 10.0 for windows was used.

Results

Effect on Lymphoid Organ Weight

Long-term dexamethasone treatment for thirty consecutive days resulted in a remarkable weight loss of thymus and spleen (Table 1). A reduction of 72% in the thymus weight of DEX group was observed. Statistically significant difference was detected between the thymus/body weight ratios of DEX and CONT group. Spleen weight also decreased significantly (45%) due to dexamethasone treatment. There was significant difference between spleen/body weight ratios of DEX group and CONT group (Table 1).

Parameters	Experimental Groups		
	CONT	DEX	
Thymus Weight (mg)			
Absolute (mg)	69.75 ± 7.56	20.25 ± 2.14	***
Relative (mg/100 g BW)	190.21±21	58.39±10.8	**
Thymocyte Size			
Cortex (µm)	3.98 ± 0.19	3.41 ± o.15	*
Medulla (µm)	3.99 ± 0.16	3.03 ± 0.27	**
Spleen Weight (mg)			
Absolute (mg)	192.2 ± 14.6	99.2 ± 6.5	**
Relative (mg/100 g BW)	602±39.09	372±22.01	**
Splenocyte Size			
Follicles (µm)	4.91 ± 0.12	4.18 ± 0.09	*

Table 1 Effect of dexamethasone treatment on lymphoid organ (thymus & spleen) weight and size of thymocytes and splenocytes. Data are expressed as mean±S.E Asterisks indicate significant differences between the groups (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, student's *t* test).

Effect on Lymphoid Organ Histology

Chronic dexamethasone treatment has a profound effect on the histology of thymus and spleen (Table 1, Figs. 1 & 2). Severe atrophy of thymus and fatty infiltration was observed in DEX group as compared to control group (CONT). The overall corticomedullary ratio was changed due to reduced cortical area. The size (Table 1) and density of thymocytes in the cortex and medulla was reduced when compared to control mice (Fig 1). The corticomedullary junction became indistinct in DEX group due to reduced density in cortex. More condensed and darkly stained nuclei in the cortical region indicative of apoptotic cell death were observed in DEX group compared to CONT group (Fig. 1). Control mice showed normal spleen histology with distinct area of red pulp (red blood cell storage and degradation) and white pulp (lymphoid area). In the white pulp areas follicles were well organized with distinct germinal centers and a clear marginal zone (Fig 2). In the dexamethasone treated animals' zonation between white pulp and red pulp became indistinct. The overall area of white pulp was reduced as compared to CONT group. The normal follicular organization was changed with reduced cell size (Table 1) and density compared to control (Fig 2).

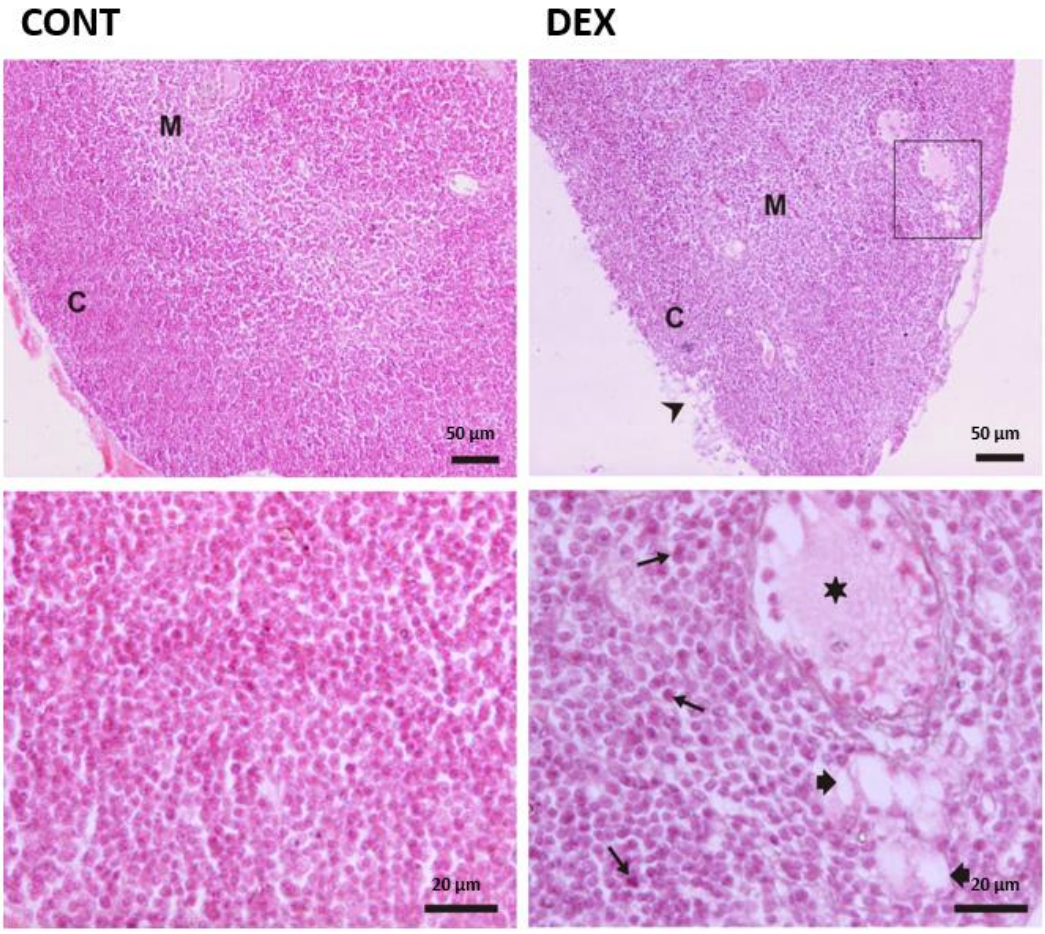


Fig. 1 Histology of thymus gland from CONT group and DEX group mice. Thymus of adult mice showing divisions in the cortical (C) and medullary (M) areas with clear corticomedullary junction. Thymus of DEX group mice showing reduced cortical area, fatty infiltration and indistinct corticomedullary junction. The density of thymocytes in DEX group mice decreased in both the thymic compartments ie. Cortex and medulla compared with the corresponding thymic regions in CONT group mice. More condensed and darkly stained nuclei indicative of apoptotic cell death are observed in the cortical region of DEX treated animals. Sections are stained with hematoxylin and eosin, original magnification ×100, ×400.

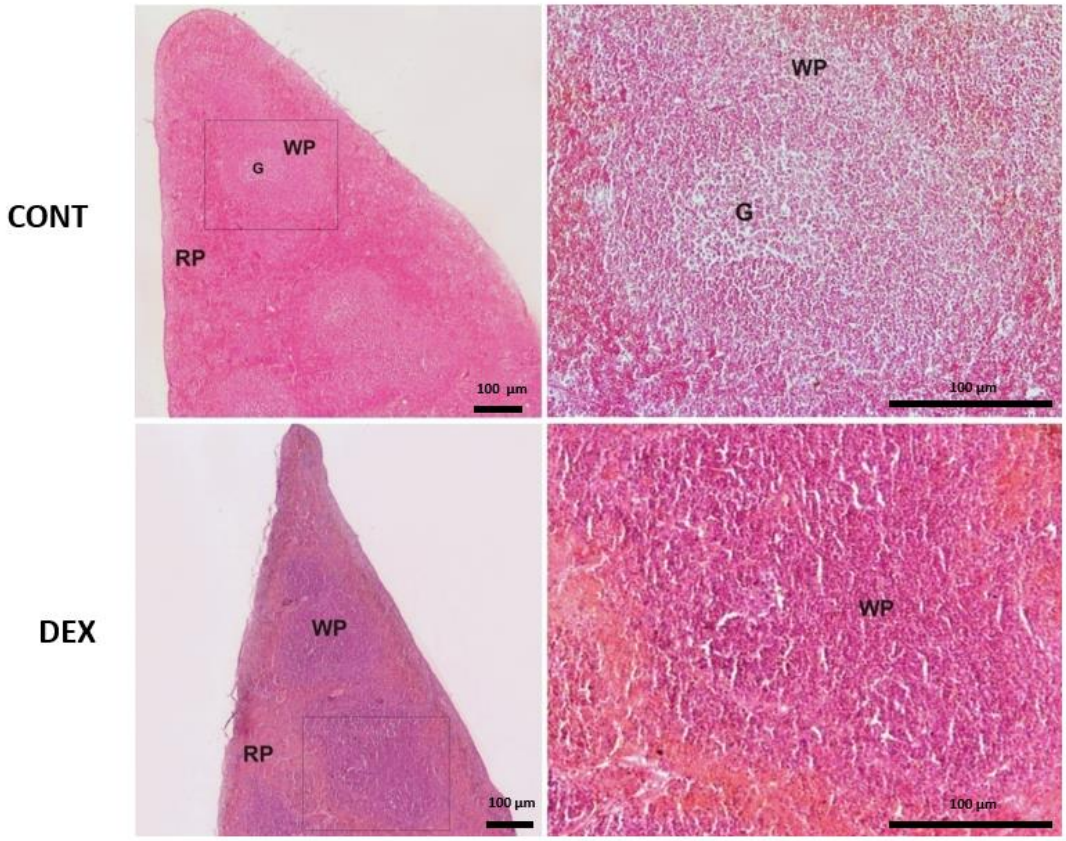


Fig. 2 Histology of spleen from CONT group and DEX group mice. Spleen of adult mice showing red pulp (RP) and white pulp with well-developed follicles (F) and germinal centers (G). Spleen of DEX group mice showing less differentiated red and white pulp, reduced white pulp area and disorganized follicular arrangement. Cellular size and density in white pulp (follicles) area seems to be decreased compared to CONT group. Sections are stained with hematoxylin and eosin, original magnification ×50 & ×500.

Effect on Haematological Parameters

Total Leukocyte, Lymphocyte and Neutrophil Count

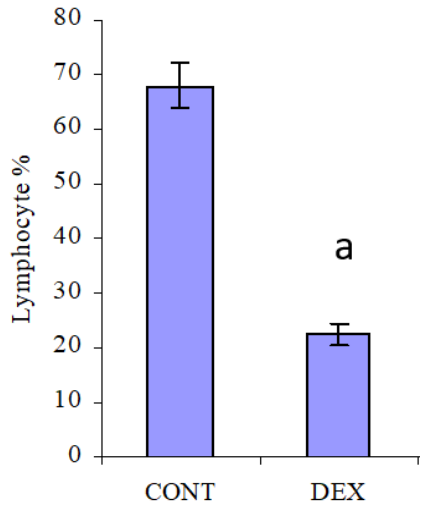
The total leukocyte and lymphocyte count were reduced by 44.70% and 64.37% respectively in DEX group compared to CONT group (Table 2). However, the neutrophil number increased significantly in DEX group compared to CONT group (+41.50%).

Leukocyte number ($1000/mm^3$)	Experimental Groups	
	CONT	DEX
TLC	18.56 ± 0.86	9.51 ± 0.23 **
Lymphocyte	12.02 ± 0.68	2.43± 0.19 ***
Neutrophil	4.42 ± 0.17	6.65 ± 0.15 *

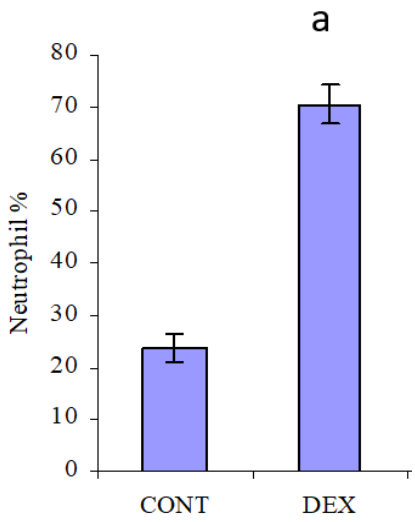
Table 2 Hematological parameters under Dexamethasone (DEX) treatments. Data are expressed as mean±S.E Asterisks indicate significant differences between the group (* P≤0.05, ** P≤0.01, *** P≤0.001, student's *t* test).

Differential Leukocyte Count

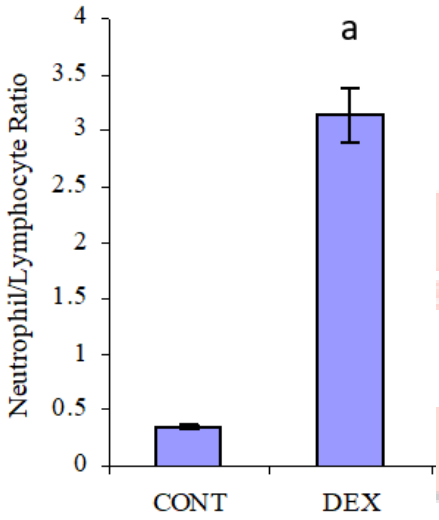
The proportion of neutrophil increased from 20.66% in CONT group to 50.33% in DEX group while the proportion of lymphocytes decreased from 78.5% in SAL group to 47.33% in DEX group (Fig. 3A&B). An increased neutrophil/lymphocyte ratio is observed in DEX group compared to CONT group (Fig. 3C). Long-term dexamethasone treatment has no effect on the percent count of eosinophils, basophils and monocytes in the present study (Data not shown).



(A)



(B)



(C)

Fig. 3 *Effect of dexamethasone treatment on percent count of blood leukocytes, Lymphocytes (A), Neutrophils (B), and Neutrophils/ Lymphocytes ratio (C). Data are expressed as mean±S.E (N=10). Asterisks indicate significant differences between the groups (∗ P≤0.05, ∗∗ P≤0.01, student's t test).*

Bone Marrow Leukocyte Count

A significantly decreased percentage (51%) of bone marrow lymphocyte was observed in DEX group as compared to CONT group (Fig. 4).

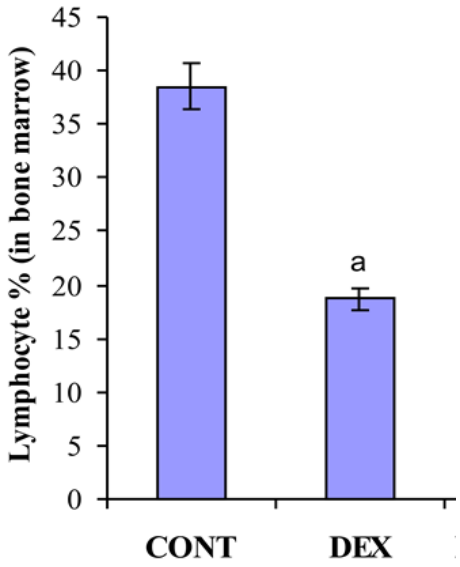


Figure 4 *Graph presenting percent count of lymphocytes in bone marrow under dexamethasone (DEX) treatments. Data expressed as Mean ± S.E ($n \geq 10$). a, $p < 0.01$ DEX vs CONT.*

Discussion

Glucocorticoids (GCs) are steroid hormones with widespread effects. GCs play crucial roles in regulating the development, growth, metabolism, and immune function of the body (Grbovic and Radenkovic, 2005). Long-term glucocorticoid treatment in mice has been shown to have significant effects on immune components in mice. Dexamethasone is a potent synthetic analogue of hydrocortisone which pharmacologically mimics the effects of adrenal glucocorticoids. Though widely being used in medicine for the treatment of a range of metabolic diseases and inflammatory disorders (Munk et al., 1984; Wilckens and DeRijk, 1997; Mandell, 2000), it has also been associated with generalized adverse effects of immunosuppression and consequent exacerbation of infectious diseases (Callow & Parker 1969; Ilott et al.1997). Dexamethasone exerts a profound catabolic effect on experimental animals and in man. The growth suppressive effects have been reported by many authors in several species (Silbermann et al., 1976; Allen et al., 1996; Ortoft, 1998). In the present study significantly less body weight gain was observed in DEX group compared to CONT group. Body weight loss due to dexamethasone treatment has been reported by many authors (Yang et al., 1994; Lo et al., 1996), but body weight gain was observed in DEX treated mice in the present study (Data not shown). This may be due to the higher doses of dexamethasone used in those studies. But the growth rate was less in DEX group and it was more affected in the last two weeks of the experiment. This growth suppressing effect of dexamethasone could be explained by considering the reported inhibitory effect of dexamethasone on growth hormone secretion (Guistin and Wehrenberg, 1992). Corticosteroids are also known to mobilize the carbohydrate, fat and protein, and hence can affect the weight gain (Horber et al., 1990; Dinneen et al., 1993; Djurhuus et al., 2002).

The thymus and spleen showed a significant decrease in their weight following the dexamethasone treatment for thirty consecutive days. This resulted in a significant reduction of thymus/body weight and spleen/body weight ratios. Our results are in agreement with the earlier reports that corticosteroid treatment or stress induced glucocorticoids caused thymus (Cohen, 1992; Maestroni et al., 1993; Zucker et al., 1994) and spleen involution (Ben Nathan et al., 1995; Haldar et al., 2004). The lymphoid organ

size and weight were presumed by many researchers to reflect changes in organ functions (Rooman et al., 1999; Biolatti et al., 2005).

Histologically a profound change was observed both in the thymus and spleen due to dexamethasone treatment. Severe atrophy of the thymic cortex was noted with reduced cellular density. Coticomedullary junction became indistinct because of depletion of cortical thymocytes in DEX group. These results are in line with the earlier studies denoting that stress or treatment with corticosteroids may result in thymic involution (Compton et al., 1992; Cohen, 1992; Sun et al., 1992; Maestroni et al., 1993; 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). In the present study zonation between red pulp and white pulp became indistinct in the spleen of DEX group. The cellular density in the white pulp area became reduced. Since white pulp area is populated with T- lymphocytes, the reduced density may be caused by the apoptosis of lymphocytes due to dexamethasone as reported earlier (Burton and Kehrli, 1996; Thompson, 1999). The drastic histological alterations also suggestive of the change in organ functions.

The haematological parameters, investigated in the present study as the absolute number and relative proportion of blood leukocytes also provide an important representation of immune status of animals. A significant decrease in the number of circulating leukocytes (-42.70%) and lymphocytes (-65.37%) was observed following a long term (30 days) treatment of dexamethasone. An opposite trend was noted in neutrophil count which increased significantly (+40.60%, $p \leq 0.05$). This neutrophilia in DEX group paralleled with the lymphopenia lead to a dramatic alteration in the ratio of neutrophils to lymphocytes compared to CONT group. The pronounced effect of dexamethasone treatment on leukocyte number and percentage in the current experiment is characteristic of the response reported earlier (Lan et al., 1998). This finding is also consistent with many reports indicating the effect of glucocorticoids on leukocyte number (Dhabhar et al., 1996; Dhabhar and McEwen, 1997). The observed change in leukocyte number and percentage could be the result of dexamethasone-induced change in leukocyte turnover (production/destruction) or of dexamethasone-induced change in leukocyte distribution. Glucocorticoids are known to induce lymphopenia by causing cells to redistribute to secondary lymphoid organs (Dhabhar and McEwen, 1997; Mashaly et al., 1998) and this could be one of the reasons for the decreased leukocyte and lymphocyte noted in the present study. The pronounced neutrophilia observed in DEX group is in accordance with earlier reports that corticosteroids cause delay in apoptosis of neutrophils and prolongs their survival in circulation (Dale et al., 1974; Cox, 1995). Corticosteroids have also been reported to decrease the egress of neutrophils from blood to inflammatory sites (Bishop et al., 1968) and hence may result in increased neutrophil number/percentage in blood, observed in the present study.

In conclusion the findings of the present study indicates that in vivo treatment of mice with dexamethasone affects growth rate, lymphoid organs histopathology and hematological parameters especially neutrophil/lymphocyte ratio. Hence long-term use of dexamethasone may be a risk factor for the development of immune related disorders because of its effects on different immune components.

References

1. Ader, R., and N. Cohen, 1993: Psychoneuroimmunology: Conditioning and stress. Ann. Rev. Psychol. **44**, 53-58.

2. Agnes E. Coutinho, Karen E. Chapman, 2011: The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Molecular and Cellular Endocrinology, 335, 1, 2-13.

3. Allen, D.B., 1996: Growth suppression by glucocorticoid therapy. Endocrinol. Metab. Clin North Am. **25**, 699-717

4. Anderson, B.H., D.L. Watson, and I.G. Colditz, 1999: The effect of dexamethasone on some immunological parameters in cattles. Vet. Res. Commun. **23**, 399-413

5. Ben-Nathen, D., G.J.M. Maestroni, S. Lusting, and A. Conti, 1995: Protective effect of melatonin in mice infected with encephalitis virus. Arch. Virol. **140**, 223-230.

6. Biolatti, B., E. Ballo, F.T. Cannizzo, G. Zancanaro, M. Tarantola, M. Dacasto, M. Cantiello, M. Carletti, P.G. Bioletti, and G. Barborino, 2005: Effect of low-dose dexamethasone on thymus morphology and immunological parameters in veal calves. J. Vet. Med. **52**, 202-208.

7. Bishop, G.R., A.W. Athens, D.R. Boggs, H.R. Warner, G.E. Cartwright, and M.M. Wintrobe, 1968: Leukocyte studies 13, a non steady state kinetic evolution of the mechanism of corticosterone-induced granulocytosis. J. Clin. Invest. **47**, 249-260.

8. Burton, J.L., and M.E. Kehrli, 1996: Effect of dexamethasone on bovine circulating T-lymphocyte populations. J. Leuk. Biol. **59**, 90-99.

9. Callow, L.L., and R.J. Parker, 1969: Cortisone-induced relapsed in Babesia argentina infection in cattle. Am.J.Vet. Res. **45,** 103-104.

10. Cohn, L.A., 1997: Glucocorticoids as immunosuppressive agents. Semin. Vet. Med. Surg. **12**, 150-156.

11. Cohen, J.J., 1992: Glucocorticoid-induced apoptosis in the thymus. Semin. Immunol. **4**, 363-369.

12. Compton, M.M., and J.A. Cidlowski, 1992: Thymocyte apoptosis: a model of programmed cell death. Trends Endocrinol. Metab. **3**. 17-23.

13. Coutinho, Agnes E., and Karen E. Chapman, 2011: The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Molecular and cellular endocrinology 335.1, 2-13.

14. Cox, G., 1995: Glucocorticoid treatment inhibits apoptosis in human neutrophils. J. Immunol. **154**, 4719-4725.

15. Dale, D.C., A.S. Fauci, and S.M. Wolff, 1974: Alternative day prednisone:leukocyte kinetics and susceptibility to infections. N. Eng. J. Med. **291**, 1154-1158.

16. Dhabhar, F.S., 2002: Stress induced augmentation of immune function-the role of stress hormones, leukocyte trafficking, and cytokines. Brain Behav. Immun. **16**, 785-798.

17. Dhabhar, F.S., A.H. Miller, B.S. McEwen, and R.L. Spencer, 1996: Stress-induced change in blood leukocyte distribution-role of adrenal steroid hormones. J. Imuunol. **57**, 1638-1644.

18. Dhabhar, F.S., and B.S. McEwen, 1997: Acute stress enhances while chronic stress suppresses immune function in vivo: A potential role for leukocyte trafficking. Brain Behav. Immun. **11**, 286-306.

19. Dhabhar, F.S., and B.S. McEwen, 1999: Enhancing versus suppressive effect of stress hormones on skin immune functions. PNAS, USA, **96**, 1059-1064.

20. Dinneen, S., A. Alzaid, J. Miles, and R. Rizza, 1993: Metabolic effect of nocturnal rise in cortisol on carbohydrate metabolism in normal humans. J. Clin. Invest. **92**, 2283-2290.

21. Djurhuus, C.B., C.H. Gravholt, S. Nielsen, A. Mengel, J.S. Christiansen, O.E. Schmitz, and N. Moller, 2002: Effect of cortisol on lypolysis and regional intestinal glycerol levels in humans. Am. J. Physiol. Endocrinol. Metab. **283**, E172-E177.

22. Giustina, A., and W.B. Wehrenberg, 1992: The role of glucocortocoids in the regulation of growth hormone secretion. Trends Endocrinol. Metab. **3**, 306-311.

23. Grbović L; Radenković M, 2005: Therapeutic use of glucocorticoids and immunosuppressive agents. Srp Arh Celok Lek, 133 Suppl 1, 67-73.

24. Haldar, C., S. Rai, and R. Singh, 2004: Melatonin blocks dexamethasone-induced immunosuppression in seasonally breeding rodent Indian palm squirrel, *Funambulus pennanti*. Steroids, **69**, 367-377.

25. Horber, F.F., and M.W. Haymond, 1990: Human growth hormone prevents the protein catabolic side effects of prednisone in humans. J. Cln. Invest. **86**, 265-272.

26. Ilott, M.C., J.S. Salt, R.M. Gaskell, and R.P. Kiching, 1997: Dexamethasone inhibits viertus production and the secretory Ig A response in esophageal-pharyngeal fluid in cattles persistently infected with foot and mouth disease virus. Immunol. Cell Biol. **72**, 398-405.

27. Jolien Vandewalle, Astrid Luypaert, Karolien De Bosscher, Claude Libert, 2018: Therapeutic Mechanisms of Glucocorticoids. Trends in Endocrinilogy & Metabolism, 29, 1, 42-54.

28. Jamela Jouda., 2016: The effect of long-term oral dexamethasone on blood cells counts and brain regions of young mice. Advances in Natural and Applied Sciences. 10(12), 63-71.

29.

30. Lan, H.C., P.G. Reddy, M. A. Chambers, G. Walker, K.K. Srivastava, and J.A. Forguson, 1998: Effect of stress on interleukin-2 receptor expression by bovine mononuclear leukocytes. Vet. Immunol. Immunopathol. **49,** 241-249.

31. Lo, H.C., P.S. Hinton, H. Yang, I.G. Unterman, and D.M. Ney, 1996: Insulin-like growth factor I, but not growth hormone attenuates dexamethasone-induced catabolism in parenterally fed rats. J. Parent. Ent. Nutri. **20**, 171-177.

32. McEwen, B.S., 1998: Glucocorticoid receptors are differentially expressed in the cells and tissues of the immune system. Cell Immunol. **186**, 45-54.

33. Mandell, D.C., 2000: Ophthalmic emergencies. Clinical Techniques in Small Animal Practice. **15**, 94-100.

34. Maestroni, G.J.M., 1993: The immunoendocrine role of melatonin. J. Pineal Res. **14**, 1-10.

35. Moir, N., O. Roy, and L. Gandey, 2002: Effect of dexamethasone on distribution and functions of peripheral mononuclear blood cells in pneumonic calves Vet. Immunol. Immunopathol**. 87**, 459-466.

36. Moshaly, M. J., G. Trout, I.I.I. Hendrick, l. Al-Sokhi, and A. Gehad, 1998: The role of neuroendocrine interactions in the initiation of humoral immunity in chickens. Domest. Anim. Endocrin. **15**,409-422.

37. Munk, A., P.M. Guyre, and N.J. Holbrook, 1984: Physiological functions of Glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. **5**, 25-44

38. Ohkarur, Y., N. Arairo, H. Ohno, S. Sato, Y. Sakakibara, H. Suzuki, S. Aritoshi, S. Akimoto, T. Ban, J. Tanihata, K. Thchiyashiki and K. Imaizumi, 2009. Acute and Subacute Effects of Dexamethasone on the Number of White Blood Cells in Rats. Joumal of Health Science, 56, 215-220.

39. Ortoft, G., H. Gronbaek, and H. Oxlund, 1998: Growth hormone administration can improve growth in glucocorticoid injected rats without affecting the lymphocytopenic effect of the glucocorticoid. Growth Horm. IGF Res. **8**, 251-264.

40. Rooman, R., G. Koster, R.Bloeman, R. Gresnigt, and S.C. Bull-offer, 1999: The effect of dexamethasone on body and organ growth of normal and IGF-II transgenic mice. J. Endocrinol. **163**, 543-552.

41. Silbermann, M., U. Kleienhaus, E. Livne, and T. Keder, 1976: Retardation of bone growth in triamcinolone-treated mice. J. Anat. **121**, 515-535.

42. Sun, X., M.D. Dinsdale, R.T. Snowden, G.M. Cohen, and D.N. Skilleter, 1992: Characterization of apoptosis in thymocytes isolated from dexamethasone treated rats. Biochem. Pharmacol. **44**, 2131-2137.

43. Tarcic, N., H. Ovadia, D.W. Weiss, and J. Weildenfeld, 1998: Restraint stress-induced thymic involution and cell apoptosis are dependent on endogenous glucocorticoids. J. Neuroimmunol. **82**, 40-46.

44. Thompson, F.B., 1999: Mechanism of T-cell apoptosis induced by glucocorticoids. Trends Endocrinol. Metab. **10**, 353-358.

45. Turnbull, A.V., and C.L. Rivier, 1999: Regulation of the hypothalamic-pituitary axis by cytokines: actions and mechanism of actions. Physiol Rev.**79**, 1-71.

46. Wayne J. Aston, Danika E. Hope, Alistair M. Cook, Louis Boon, Ian Dick, Anna K. Nowak, Richard A. Lake and W. Joost Lesterhuis. 2019: Dexamethasone differentially depletes tumour and peripheral blood lymphocytes and can impact the efficacy of chemotherapy/checkpoint blockade combination treatment. Onco-immunology. 8, 11, e1641390_1-8.

47. Wilckens, T., and R. DeRijk, 1997: Glucocorticoids and immune system: unknown dimensions and new frontiers. Immunol. Today, **18**, 418-424.

48. Yang, H., M. Grahn, U.S. Schalch, and D.M. Ney, 1994: Anabolic effect of IGF-I co-infused with total parentaral nutrition in dexamethasone treated rats. Am. J. Physiol. Endocrinol. Metab. **266**, E690-E698.

49. Zucker, R.M., K.H. Elstein, D.J. Thomas, and J.M. Rogers, 1994: Tribulin and dexamethasone induce apoptosis in rat thymocytes by mutually antagonistic mechanism. Toxicol. Appl. Pharmacol. **127**,163-170.