IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 12, Issue 1, December 2021
Impact Factor: 4.819

Effect of Chronic Dexamethasone Treatment on Histomorphology of Main Lymphoid Organs in Mice

Brijesh Kumar Mishra
Department of Zoology, H.N.B. Govt. P.G. College, Naini, Prayagraj, India
mishrabrijesh.au@gmail.com

Abstract: *Glucocorticoids (GCs) are one of the most important regulatory hormones in the human body. Glucocorticoid hormones have been widely used in clinical practice as potent anti-inflammatory and immunosuppressive agents. Dexamethasone is a synthetic glucocorticoid. It is frequently used as a medication to treat a variety of inflammatory conditions and metabolic problems in both humans and farm animals. The side effects of long-term dexamethasone treatment have become a major source of concern. In the present study the effect of chronic dexamethasone treatment on histomorphology of main lymphoid organs 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. The results of the present study highlight the effects of DEX treatment on histopathology of lymphoid organs thymus and spleen. Hence long-term use of dexamethasone may be a risk factor for the development of immune related disorders.*

Keywords*:* Glucocorticoids (GCs), Dexamethasone, Lymphoid organ, Thymocytes, Splenocytes, Atrophy, Mice

I. INTRODUCTION

Glucocorticoids (GCs) were discovered in the 1940s and are one of the most important regulatory hormones in the human body (Vandewalle et al., 2018). Glucocorticoid hormones have been widely used in clinical practice as potent anti-inflammatory and immunosuppressive agents. Dexamethasone (DEX), the synthetic analogue of glucocorticoid, is a proven immune suppressive and anti-inflammatory agent (Cohn, 1997; Turnbull and Rivier, 1999). Dexamethasone, a corticosteroid, exhibits similarities to a naturally produced hormone synthesised by the adrenal glands. It is commonly used as a replacement for this substance in situations where the body's synthesis is inadequate. 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). 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 have 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).

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 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 the main immune organs, thymus and spleen in adult male mice.

II. 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^{0}C) 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.

Experimental Design

Sixteen adult male mice (35 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 (SAL) for the same period and are treated as control group. All the injections were given at evening hours between 4:45pm to 5:00pm. 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 thymus and spleen were then fixed in Bouin's fluid for further histological study.

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 a clean 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=8). Statistically significant difference between the treatment groups (SAL 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.

III. RESULTS

Effect on Lymphoid Organ Weight

Chronic dexamethasone treatment for thirty consecutive days resulted in a remarkable weight loss of thymus and spleen (Table 1). A reduction of 70% in the thymus weight of DEX group was observed (Fig. 1A). Statistically significant difference was detected between the thymus/body weight ratios of DEX and SAL group (Fig. 1B). Spleen weight also decreased significantly (43%) due to dexamethasone treatment (Fig. 2A). There was significant difference between spleen/body weight ratios of DEX group and SAL group (Fig. 2B).

IJARSCT

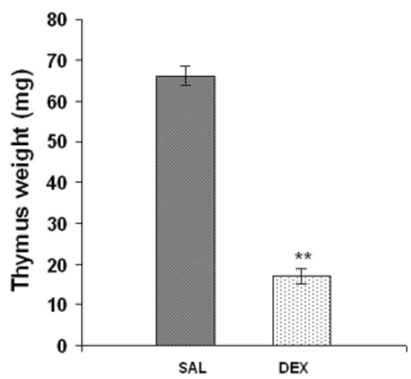
International Journal of Advanced Research in Science, Communication and Technology

International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal

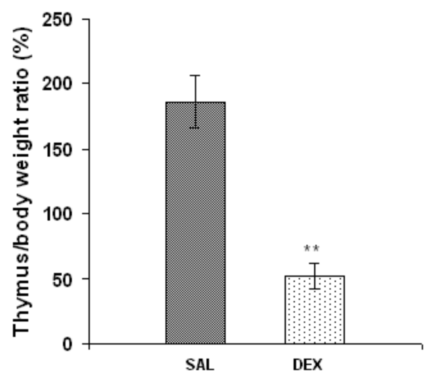
ISSN: 2581-9429 Volume 12, Issue 1, December 2021 Impact Factor: 4.819

	Experimental Groups		
Parameters	SAL	DEX	
Thymus Weight			
Absolute (mg)	66.11±2.36	17.01±1.79	***
Relative (mg/100 g BW)	186.22±20.2	52.33±10.4	**
Thymocyte Size			
Cortex (µm)	3.95±0.07	3.48±0.05	*
Medulla (µm)	3.99±0.06	3.13±0.07	**
Spleen Weight			
Absolute (mg)	218.34 ±16.4	117.64±17.5	**
Relative (mg/100 g BW)	615.04±43.4	361±21.5	**
Splenocyte Size			
Follicles (µm)	4.86±0.13	4.13±0.06	*

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≤0.05, ** P≤0.01, *** P≤0.001, student's *t* test).



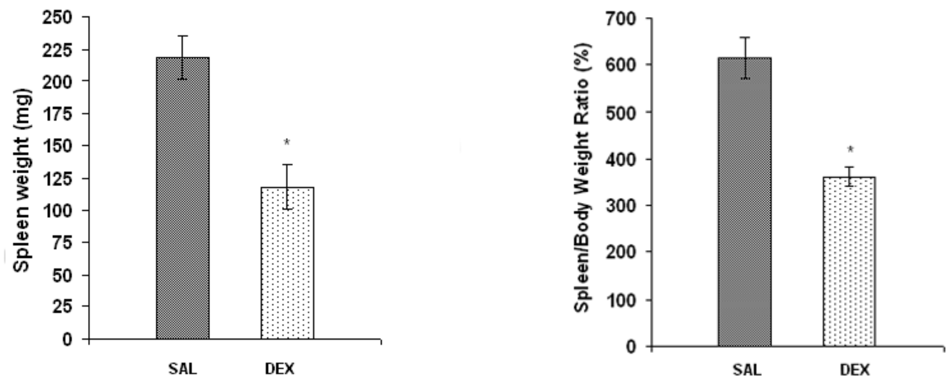
(A)



(B)

Fig. 1 Thymus weight (A) and Thymus/body weight ratio (B) in control (SAL) and dexamethasone (DEX) treated mice. Data are expressed as mean±S.E (N=10). Asterisks indicate significant differences between the groups (student's *t* test).

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 12, Issue 1, December 2021 Impact Factor: 4.819



(A) (B)

Fig. 2 spleen weight (A) and Spleen/body weight ratio (B) in control (SAL) and dexamethasone (DEX) treated mice. Data are expressed as mean±S.E (N=6). Asterisk indicates significant differences between the groups (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. 3&4). Severe atrophy of thymus and fatty infiltration was observed in DEX group as compared to SAL control (Figs. 3a, b). The overall corticomedullary ratio was changed due to reduced cortical area (Fig 3b). The size (Table 1) and density of thymocytes in the cortex and medulla was reduced when compared to control mice (Fig 3c-f). 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 SAL group (Figs. 3c, d). 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 4a). In the dexamethasone treated animals, zonation between white pulp and red pulp became indistinct (Fig 4b). The overall area of white pulp was reduced as compared to SAL group. The normal follicular organization was changed with reduced cell size (Table 1) and density compared to control (Fig 4c, d).

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 12, Issue 1, December 2021
Impact Factor: 4.819

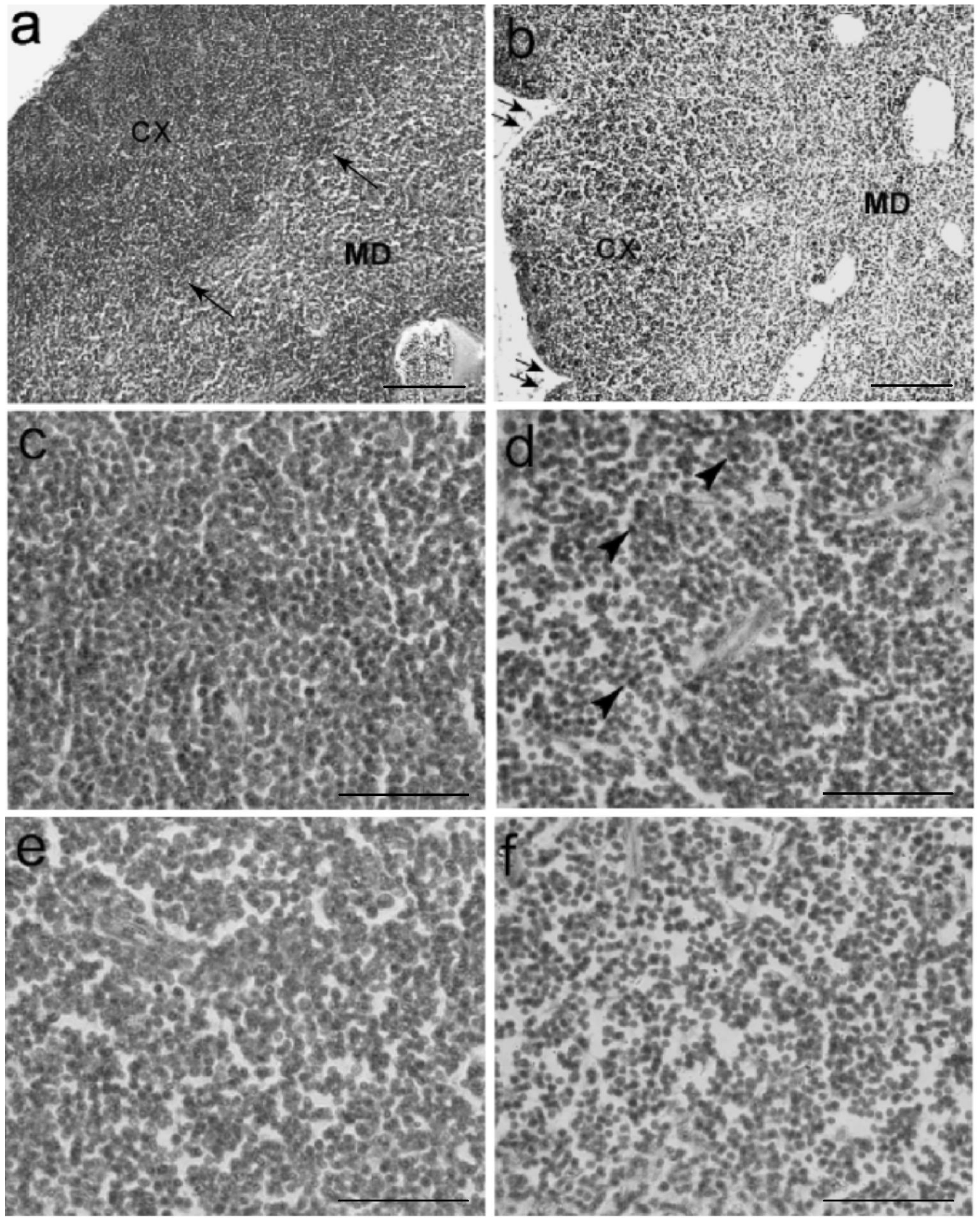


Fig. 3 Histology of thymus gland from SAL group (a, c, e) and DEX group (b, d, f) mice. a. Thymus of adult mice showing divisions in the cortical (CX) and medullary (MD) areas with clear corticomedullary junction (arrow). b. Thymus of DEX group mice showing reduced cortical area, fatty infiltration (arrow) and indistinct corticomedullary junction. The density of thymocytes in DEX group mice decreased in both the thymic compartments ie. Cortex (d) and medulla (f) compared with the corresponding thymic regions in SAL group mice (c and e respectively). More condensed and darkly stained nuclei (arrow heads), indicative of apoptotic cell death are observed in the cortical region of DEX treated animals (d). Sections are stained with hematoxylin and eosin, original magnification ×100 (a, b), ×400 (c-f). Bar 50µm (a, b), 20µm (c-f).

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 12, Issue 1, December 2021
Impact Factor: 4.819

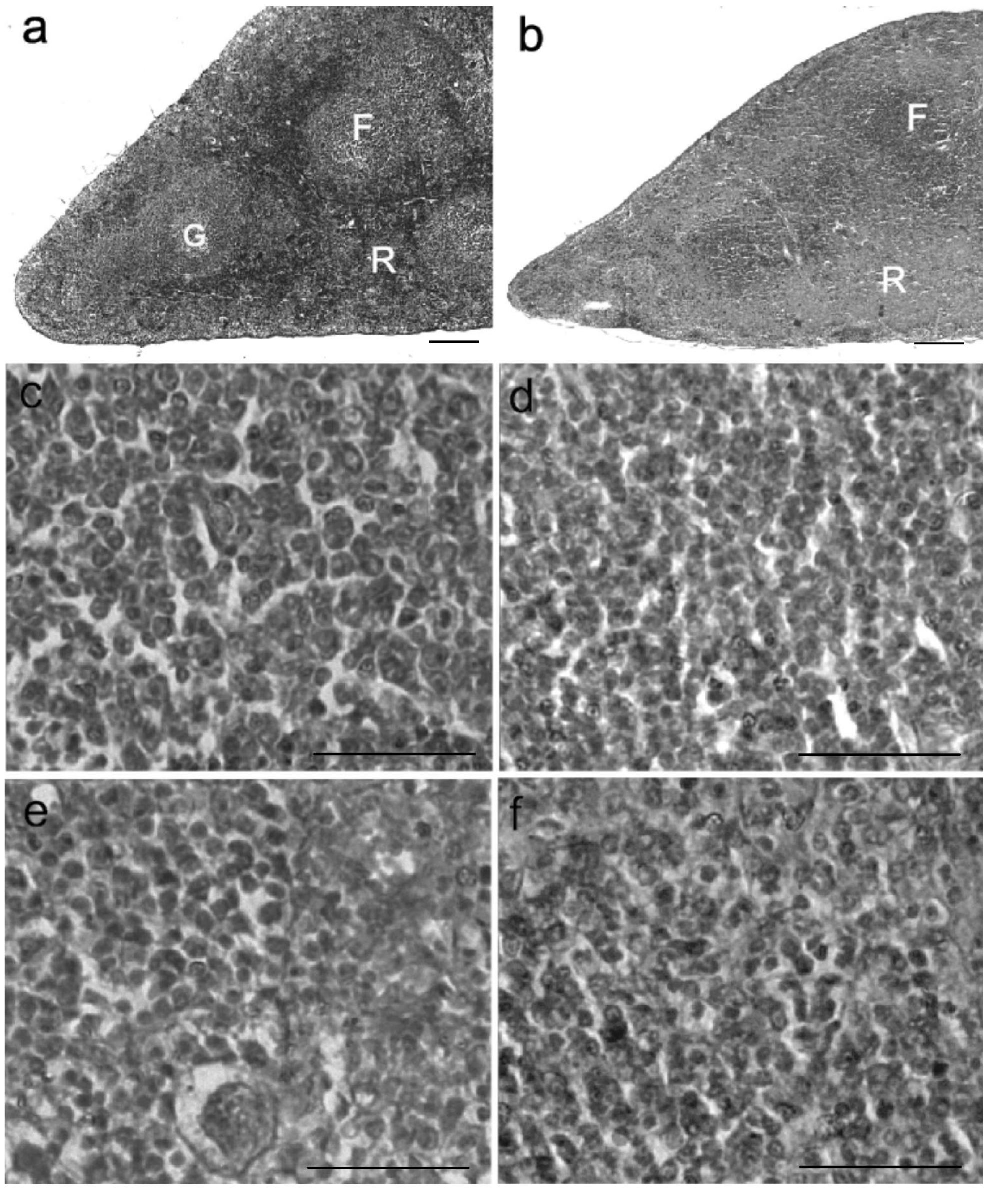


Fig. 4 Histology of spleen from SAL group (a, c, e) and DEX group (b, d, f) mice. (a) Spleen of adult mice showing red pulp (R) and white pulp with well-developed follicles (F) and germinal centers (G). (b) Spleen of DEX group mice showing less differentiated red and white pulp, reduced white pulp area and disorganized follicular arrangement. Cellular size and density in white pulp (follicles) area seems to be decreased (d, f) compared to SAL group (c, e). Sections are stained with hematoxylin and eosin, original magnification ×50 (a, b), ×500 (c-f). Bar 40μm (a, b), 20μm (c-f).

IV. DISCUSSION

Adrenal glucocorticoids (GCs) are one of the most important regulatory hormones in the human body (Vandewalle et al., 2018). 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, Giles et al., 2018). 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 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 are also suggestive of the change in organ functions.

In conclusion the findings of the present study indicates that in vivo treatment of mice with dexamethasone affects the weight and histopathology of main lymphoid organs thymus and spleen. Hence long-term use of dexamethasone may be a risk factor for the development of immune related disorders.

REFERENCES

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

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

[3]. Amber J Giles, Marsha-Kay N D Hutchinson, Heather M Sonnemann, Jinkyu Jung, Peter E Fecci, Nivedita M Ratnam, Wei Zhang, Hua Song, Rolanda Bailey, Dionne Davis, Caitlin M Reid, Deric M Park, Mark R Gilbert., 2018: Dexamethasone-induced immunosuppression: mechanisms and implications for immunotherapy. J. Immunother Cncer, 11;6(1):51.

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

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

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

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

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

[12]. 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.

[13]. 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.

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

[15]. 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.

[16]. 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.

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

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

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

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

[21]. 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.

[22]. 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

[23]. 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.

[24]. 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.

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

[26]. 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.

[27]. 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.

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

[29]. 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.

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

[31]. 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.