Sindh Univ. Res. Jour. (Sci. Ser.) Vol. 48 (2) 251-256 (2016)

SINDH UNIVERSITY RESEARCH JOURNAL (SCIENCE SERIES)

Short Term Administration of High Dosed Dexamethasone Altered Leukocyte Concentration in Blood of Goat

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Received 15th June 2015 and Revised 2nd August 2015

Abstract: Dexamethasone (DEX), a glucocorticoid, widely used as life saving drug in humans and animals. Its therapeutic use in veterinary clinics as growth promoter, anti-stress, anti-shock, anti-inflammatory and in metabolic disorders has been reported. Besides beneficial therapeutic effects, it exerts broad range of untoward effects especially on immune cells. Thus, present study was carried out with an objective to evaluate the effects of high dose of dexamethasone on immune cell population and on hematological parameters, i.e Erythrocyte Count, Hb, and PCV. To achieve objective of study, dexamethasone was administered intramuscularly at high dose rate of 4 mg/kg.BW for 5 days in six goats. Blood samples were collected before and on day 5, at 1, 6, 12, 24, 48, 72 and 96 hrs post drug administration and analyzed for Complete Blood Count (CBC). The results showed significant increase ($p<0.01$) in WBCs count at 1, 6, 12 hrs and ($p<0.05$) at 24 hrs. Granulocytes (Leukocyte Count) also increased significantly ($p<0.05$) at 6, 12 hrs and ($p<0.01$) at 24 and 48 hrs post administration in treatment group as compare to control group. Whereas, non-significant increase was found in RBCs count, PCV % and Hb at 12 hrs post dexamethasone administration. Thrombocyte count, was higher at 24 hrs, while, monocytes and lymphocytes were significantly decreased ($p<0.01$) at 6, 12, 24, 48 hrs in treatment group as compared to control. It was concluded from the present study that, the administration of dexamethasone at higher dose significantly causes immune suppressive as well as immunomodulant effects in goats. These effects subsided as the drug cleared from the body in goats.

Keywords: **G**lucocorticoides, Dexamethasone, Hematology, Immunity, goats

1. INTRODUCTION

Glucocorticoids (GCs) belong to Corticosteroids which develop immune response and provide protection against foreign pathogens and environmental stresses (Schimmer and Parker 2001). Dexamethasone is a corticosteroid available in different types of preparation for commercial use and is being widely used in veterinary practice due to broad spectral pharmacological actions. In veterinary clinics, DEX is used as anti-shock, anti-stress, and anti-inflammatory agent for the treatment of metabolic diseases (Capolongo *et al.,* 2006; Zhang *et al.,* 2006; Odore *et al.,* 2007). Dexamethasone produces its effects on animal body by different ways for example; it increases the activity of adrenal cortex following the activation of the hypothalamic–pituitary–adrenal (HPA) axis, has been revealed to weaken the immune system and this could impact on animal health (Raussi *et al.,* 2006). It has been observed that leukocytosis, eosinopenia, lymphopenia, neutrophilia, and monocytosis developes in Cattle and veal calves following treatment with DEX (Anderson *et al.,* 1999; Thanasak *et al.,* 2004). Immuno-modulant effect of DEX is evident from the expression of circulating leukocytes and vascular endothelial cells (Farsky *et al.,* 1995; Pitzalis *et al.,* 2002). Especially, endogenous and exogenous DEX up-regulate the circulating neutrophils in human and other model systems, among bovine neutrophils showed increased sensitivity to the steroids (Barton *et al.,* 2000; Seekamp *et al.,* 2001; Weber *et al.,* 2001). Several unwanted or adverse effects of DEX have been reported by researchers. Chronic administration of glucocorticoids, results in Cushing's syndrome or associated with increased risk of cardiovascular disease (Souverein *et al.,* 2004; Solomon *et al.,* 2006). DEX inhibits various components of the acute phase response; particularly increase in body temperature. It also produces transient hypertension, increases systolic blood pressure and decreases resting heart rate (Daniel *et al.,* 2005). Moreover, increases blood pressure, in very low birth weight neonates on administration of low-dose dexamethasone (Shahab *et al.,* 2006). Effects of DEX on hemogram shown that it stimulates erythropoiesis both *in vivo* (Amylon *et al.,* 1986; King *et al.,* 1988) and *in vitro*. (Moya *et al.,* 1993). It also affects white blood cells, segmented neutrophils, lymphocytes, basophils, and eosinophils in neonates. (Barak *et al.,* 1992) and increases platelet counts in infants (Bourchier and Weston 1991). Furthermore, it induces an increase in granulocyte counts, paralleled by decreased lymphocyte and monocyte counts (Winter and Van, 1991; Nakagawa *et al.,* 1998; Schuld *et al.,* 2001).

Since dexamthasone has dose dependent effects on animal body, its indiscriminate short or long term use may produce severe adverse effect on animal health. A

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little information is available on its use in small ruminants especially in goat species. Therefore, assuming the importance and widespread use of dexamethasone in veterinary clinics in our conditions and its adverse effects reported in some studies, emphasized to plan this study to evaluate any adverse effects of its short term use with higher dose on complete blood count (CBC) in goat.

2. MATERIALS AND METHODS

Six healthy adult goats of mixed breed weighing 20 kg average BW were used in this study. All the studied animals were approved by Sindh Agriculture University, Tandojam, Pakistan, animal Care and Use Committee and were kept at Livestock Experimental Station of Faculty of Animal Husbandry and Veterinary Sciences. All the experimental animals were dewormed and vaccinated against contagious pleuropneumonia and enterotoxaemia and then acclimatized to a local environment for a period of one month. Standard feeding regimen was followed during the experiment. Dexamethasone (Dexafar: Farvet) was administered intramuscularly (I/M) at the gluteal muscle region in six goats once daily for 5 days at the high dose rate of 4 mg/kg.BW. Blood samples were collected at day 0 (Control), and on day 5 at 1, 6, 12, 24, 48, 72 and 96 hrs post dexamethasone administration. 5ml of whole blood from each goat was collected through jugular vein into Heparinized Vaccutainers (BDH, UK). Samples were then brought to postgraduate laboratory, Department of Veterinary Physiology and Pharmacology. Blood samples were analyzed for Complete Blood Count (CBC) by automated Hematology Analyzer, Backman Coulter Counter, (Germany). Values of White Blood Cells (WBC) and Red Blood Cells (RBC) count, Packed cell volume (PCV %), hemoglobin, granulocytes, lymphocytes, monocytes and platelets were recorded. Data were analyzed using the statistical software (Graph Pad Prism USA) by analysis of variance (ANOVA) and the differences were considered significant at ($p<0.05$ and $p<0.01$). The Tukey Karmer multiple comparison test was applied to compare the means.

3. RESULTS

Data **(Table 1)** indicate that dexamethasone caused a transient increase in RBCs count at 6 to 12 hrs post administration followed by decrease in count that reached to normal (control level) at 96 hrs post administration. Similar trend appeared in PCV % that slightly increased from 1~~hr~~ to 24hr post administration, highest percentage of PCV was recorded in blood samples collected at 24hr. Concentration of hemoglobin also increased upto 12hr and then declined to normal. However, there was a slight increase in RBC count, PCV %, and Hb Concentration but statistical analysis of data showed non-significant ($p\geq0.05$) difference in between control and post treatment values.

The data depicted in **(Table 2)** showed that WBCs count was significantly increased ($p<0.01$) at 1, 6, 12 hrs and ($p<0.05$) at 24 hrs post dexamethasone administration. Afterwards, WBCs count returned towards the base line values at 72-96 hrs. Dexamethasone administration significantly increased the granulocytes ($p<0.05$) at 6, 12 hrs and at 24 and 48 hrs ($p<0.01$). Their concentration declined to base line at 96 hrs following drug administration. Thrombocyte (platelets) count increased at 24 hrs after administration; however, this effect was non-significant ($p\geq0.05$) in between control and post administration samples. Significant decrease ($p<0.01$) in the lymphocytes count was observed at 6, 12, 24, 48 hrs and at 1 and 72 hrs ($p<0.05$) post administration following gradual increase in count that reached upto to the control level at 96 hrs post dexamethasone administration. Monocytes were also significantly decreased ($p<0.01$) at 6, 12, 24, 48 hrs following dexamethasone administration and subsequently, returned towards the base line level at 96 hrs.

Table-1 Effect of short term I/M administration of dexamethasone on the concentration of RBCs, Hemoglobin and PCV in blood of goats (mean ± SE).

Time (h)	RBCs (Million/cmm)	PCV (%)	Hemoglobin (g/dl)
C	0.75±0.60	34.56±1.28	7.73±0.51
1	0.81±0.49	35.76±1.37	7.79±0.42
6	0.85±0.69	35.78±1.36	7.81±0.40
12	0.85±0.74	35.83±1.38	7.85±0.49
24	0.83±0.53	35.96±1.19	7.83±0.52
48	0.77±0.76	35.36±1.07	7.80±0.42
72	0.76±0.40	34.86±1.25	7.78±0.30
96	0.75±0.59	34.33±1.29	7.76±0.40

Different from 0 at *p<0.05 and **p<0.01.

Table-2 Effect of short term I/M administration of dexamethasone on the concentration of WBCs and Platelets in blood of goats (mean ± SE).

Time (h)	WBCs (1000/cmm)	Lymphocytes (1000/cmm)	Monocytes (1000/cmm)	Granulocytes (1000/cmm)	Platelets cell Count (1000/cmm)
C	16.91±0.29	9.8±0.23	2.65±1.89	4.6±0.44	2263.5±2.56
1	30.80±1.96**	8.65±0.27*	2.16±1.47	5.9±0.39	2329.0±29.86
6	28.55±2.14**	7.68±0.15**	1.73±1.76**	6.4±0.38*	2346.8±14.92
12	27.36±2.89**	7.00±0.18**	1.30±1.93**	6.6±0.42*	2384.6±18.93
24	23.78±1.98*	6.50±0.17**	1.01±0.92**	8.2±0.33**	2386.1±24.50
48	18.70±1.04	7.30±0.16**	1.68±0.61**	7.3±0.26**	2275.5±20.19
72	16.20±0.86	8.30±0.10*	2.11±2.04	5.98±0.28	2245.1±82.85
96	16.51±0.32	9.4±0.18	2.48±1.52	4.9±0.36	2240.6±22.49

Different from 0 at *p<0.05 and **p<0.01.

4. DISCUSSION

Corticosterioids, produced from adrenal cortex of adrenal gland, plays an important role in the regulation of many important function of the body. They are known to manipulate the function of major systems of the body, including the musculoskeletal, nervous, cardiovascular, and immune systems. Haemotological indices reflects any positive or negative change in systems of body influenced by various factors. Synthetic glucocorticoid i.e: corticosterioid, also exert important effect on body. Commercially prepared corticosteroid i.e: dexamethasone is known to be life saving medicine. It has anti-inflammatory, immnomodulent and immune suppressive effects. These effects could be determined by haematological indicators. Hence, we investigated to see whether DEX alters the blood parameters in our local goat breeds. In present study we examined the effects of short term intramuscular administration of high dose of dexamethasone on some hematological parameters in goat. We observed that dexamethasone significantly increased (p<0.05) total leukocyte and granulocytes. Researchers have reported different causes of increase in Leukocytes following glucocorticoid administration. It has been reported that the GC administration results in an increased polymorphonuclear leukocytes (PMNs) in blood due to the increased rate of entrance from bone marrow and a decreased rate of removal from the vascular compartment (Holland *et al.,* 2003). In another study, it is stated that GC inhibits the expression of adhesion molecules on the endothelial cells particularly endothelial adhesion molecules (ie, ELAM-1) and intercellular adhesion molecules (ie, ICAM-1), thereby, interferes with the movement of leukocytes from the vasculature into inflamed tissues (Sousa, 2005). It has also been speculated that elevated WBC count is linked with hypertension because the inflammation stimulate the sympathetic nervous system (Judith and Whitworth**,** 2004). Hence, there is a relation between cardiac effect and increased WBCs count that they are increased with cortisol treatment. Findings of the present study are in agreement with similar findings in camel (Wasfi *et al.,* 1990), in dolphin (Reidarson and McBrain 1999), in human (Barak *et al.,* 1992; Zachman *et al.,* 1988; Ching *et al.,* 1999), in cattle (Anderson *et al.,* 1999), and in lambs (Pena *et al.,* 2004). The current study have revealed immunosuppressive effect of dexamethasone in goat that is evident from significant decrease in the lymphocytes cells population post administration as shown in Table. 2. Studies conducted on camel (Wasfi *et al.,* 1990), in human (Ching *et al.,* 1999), in dolphins (Reidarson and McBrain, 1999), in cattle (Anderson *et al.,* 1999), in lambs (Pena *et al.,* 2004) and in quails (Aengwanich and Chinrasri 2003) have also affirmed such effect. Decrease in lymphocyte population could be due to either rapid apoptosis (cell death) of lymphoid cells or activated T lymphocytes are susceptible to glucocorticoid-induced apoptosis (Sousa, 2005). Immunosuppressive effect of glucocorticoid is essential in protection from an immune response that may result in tissue grafte rejection or fetal allograft rejection. High doses of glucocorticoids inhibit immunoglobulin synthesis, kill B cells, and decrease production of components of the complement system. Holland *et al.,* 2003).

Dexamethasone treatment significantly decreased monocytes (Table-2). Results of the present study are in agreement with previous study, where it was reported that a single dose of cortisol results in a 90% decrease in monocytes that occurred 4 to 6 h following treatment and persisted for about 24 h (Holland *et al.,* 2003). Significant increase in the granulocytes cell count was observed post drug administration (Table-2). These findings have also been reported in humans (Nakagawa *et al.,* 1998, Winter and Van 1991, Schuld *et al.,* 2001).

Glucocorticoid-induced granulocytosis attributed to increased release of PMNs from bone marrow, delayed apoptosis, and reduced egression of PMNs into tissues and there by shifted to the circulating pool, with a minor contribution from bone marrow release (Nakagawa *et al.,* 1998). We found non-significant increase in RBCs count, PCV% and Hb following I/M administration of dexamethasone. Increase in transient increase in RBCs in systemic circulation could be due to the effect of glucocorticoid on hemopoetic cells or contraction of its reservoirs to release cells in circulation. It has been stated that corticosteroids increases hemoglobin and red cell count of blood, by retarding erythrophagocytosis (endocytosis of red blood cells). Similar findings have also been reported in camel (Wasfi *et al.,* 1990) in human (King *et al*, .1988) and in lambs (Pena *et al.,* 2004). Another reason for increase RBC, HBb and PCV % following dexamethasone administration is due to increased cardiac output and respiration rate (Holland *et al.,* 2003). The increase in cardiac output occurred due to tissue hypoxia that results in the stimulation of erythropoiesis process (Holland *et al.,* 2003). Thus, it can be speculated that the dexamethasone could be used in acute condition where body tissues are hypoxic.

6. CONCLUSION

It was concluded from the current study that the intramuscular administration of dexamethasone at the high dose rate of 4 mg/kg BW for 5 days increased leukocytes, however, it decreases monocytes and lymphocytes and thereby suppresses the immune system of goats. Hence, because of its immune suppressive effects it could be used cautiously in goat specie, otherwise, goat will be more exposed to infection.

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