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HEMATOLOGICAL EFFECTS OF DICLOFENAC SODIUM IN GOAT

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ABSTRACT

Diclofenac sodium is commonly used as an analgesic, anti-inflammatory and ant rheumatic agent. In addition to its beneficial effects diclofenac has also been reported to be associated with some adverse effects. In this study hematological effects of diclofenac sodium were studied. The drug was administered in six goats, in two phases with adequate wash out period of 21 days between each phase. In phase-1 diclofenac was administered in all animals with 2.5 mg/kg body weight and in phase-2 with 1 mg/kg dose rate. Blood parameters altered significantly only with the high dose (2.5 mg/kg) of diclofenac sodium. red blood cells was significant decrease ($P<0.05$) at 12 hours and hemoglobin (at 6 and 12 hours) was observed with the high dose of the drug. A significant increased $P<0.05$) was detected in WBCs count at 6, 12 and 24 hours post high dose of diclofenac. Granulocytes decreased significantly ($P<0.05$) at 6 and 12 hours post high dose administration. Monocytes and lymphocytes increased significantly ($P<0.05$) with high dose of diclofenac at 6,12,24,48 and at 12 and 24 hours respectively. Decreased ($P<0.05$) platelets count was marked at 12 and 24 hours post diclofenac administration. It has been concluded that high dose of diclofenac sodium significantly affect blood cell count and the effects are drug related and subside 72 hours post drug administration.

Keywords: Non steroidal anti – inflammatory drugs (NSAID), red blood cell, white blood cell, platelets, diclofenac sodium, goat.

INTRODUCTION

Inflammation and pain mostly occur in many diseases of goats and are associated with shoulder tenditis, rheumatic arthritis or bursitis, gouty arthritis, polymyalgia, rheumatica other painful condition of the joints and musculo skeletal system. Inflammation is also associated with osteoarthritis, fibromyolgia, muscular low back pain and muscular neck pain (Susan, 1998). To provide improvement in animal's well being and outcome, the condition of inflammation and pain is treated/managed by a variety of pharmacological agents of which NSAIDs is one of the important. Among NSAIDs Diclofenac is widely available veterinary drug. Diclofenac a phenyl-acetic acid is widely used in human medical and veterinary practice (Ramesh *et al*., 2002). Diclofenac sodium is an inhibitor of cyclooxygenase enzyme and acts by decreasing the free arachidonate level. (Goodman and Gilman, 2001).

Along with its therapeutic effects, several adverse effects of diclofenac have been described after therapeutically use in humans, rats and dogs. These include gastric problem (Ramesh *et al*., 2002; Manocha and Venkataraman, 2000), nonsteroidal drug colitis (Bjorkman, 1998 and Puspok *et al*., 2000), degenerative and inflammatory liver alterations (Bjorkman, 1998; Hackstein *et al*., 1998; Globisch *et al*., 2000; Manocha and Venkataraman, 2000).

Altered homeostasis has been reported by the use of diclofenac and its major effects are found in platelets functions (Power *et al*., 1990). Diclofenac has also the cause of immune hemolytic anemia (Meyer *et al*., 2003). Therapeutic doses of diclofenac in healthy young and elderly subjects were apparently not associated with a significant change in blood pressure (Dilger *et al*., 2002)

Diclofenac associated drug reaction remain a challenging animal health problem. Recent report indicates that diclofenac is responsible for the decline number of vulture population fed on the carcass of diclofenac treated animals. This limits the clinical use of diclofenac in animals and raises many questions regarding its safety potential and / or toxicity in livestock. This study has therefore been designed to investigate the hematological parameters in goat specie.

MATERIALS AND METHODS

Six healthy goats were first acclimatized to local environment, Tandojam, Sindh, Pakistan, in a period of two weeks. Control base line values were determined for targeted hematological parameters of blood of experimental goats.

Diclofenac sodium was administered I/M in six goats in two phases with adequate wash out period of 21 days between each phase. In phase-1 drug was administered in all animals with a dose of 2.5 mg/kg (highest therapeutic recommended dose), while in phase-2 dose rate of drug was 1 mg/kg of body weight. For

hematological analysis the blood samples were collected at 1, 2, 3, 6, 12, 24, 48, 72 and 96hrs post drug administration. Hematological parameters were Red Blood cell, hemoglobin, white blood cell, granulocytes monocytes lymphocytes and platelets were measured. The Blood parameters were analyzed by beckman hematology analyzer.

RESULT AND DISCUSSION

In this study significant decrease in RBCs count and Hb level was observed after I/M administration of high dose of diclofenac sodium. This significant ($P<0.05$) decrease in RBCs count was observed at 12 hours (table-I) and in case of hemoglobin at 6 and 12 (table-I). These findings have also been reported by others when they use diclofenac in humans and animals (Susana *et al*., 2002; Meyer *et al*., 2003 and Sachs *et al*., 2004) The decreased number of red blood cell described in humans is thought to be due to an inhibition of haematopoiesis. The possibly of anemia is also been suggested due to gastric bleeding in humans afer he use of diclofenc sodium (Susana *et al*., 2002. Another possibility of decreased RBCs and hemoglobin level is that, diclofenac decreases the heart rate and respiration rate, with this result the oxygen carrying capacity of the blood is decreased, as a result the RBCs and hemoglobin level is decreased.

Diclofenac showed a significant effect on white blood cells. Significant ($P<0.05$) increase in leukocytes count was observed at 6, 12 and 24 hours post maximum dose administration ((table-II). By the 96 hours the leukocyte values returned to the normal. These results are in the line of findings investigated by Hofer *et al*. (1996) and Philips *et al*. (1996). Leukotriene (LTB4) and 5-hydroxyeicosatetranoic acid (5-HETE) are strongly chemo attractants stimulating polymorph nuclear leukocytes movement (Paino *et al*., 2005).

In our study significant increase in lymphocytes and monocytes was also observed with high dose of diclofenac sodium. The significant ($P<0.05$) increase in monocytes was observed at 6, 12, 24 and 48 hours post drug administration (Appendix-IV) and lymphocytes were significantly increased ($P<0.05$) at 12 and 24 hours post dosage regimen (Appendix-V). Results are in agreement with the findings reported b (Garcia, 1994 and Perez *et al*., 1999). The agranulocytosis induced by aminopyrine or diclofenac may involve metabolic activation to reactive intermediates by hypochlorite formed by myeloperoxidase in activated neutrophils (Uetrecht *et al*., 1995 and Miyamoto *et al*., 1997). Susceptibility of the individual to aminopyrine or diclofenac could result from the large variation (200-fold) in the individuals' hepatic metabolism to these drugs (Agundez *et al*., 1995).

The decreased number of granulocytes with diclofenac was observed in this study. A significant ($P<0.05$) decrease in granulocytes was observed at 6 and 12 hours after administration of 2.5 mg/kg (b.w) of diclofenac sodium (Appendix-IV). Similar effects of diclofenac have also been observed by others in humans and different animals (Colomina and Garcia, 1989; Hunt and William, 1991 and Perez *et al*., 1999). Studies using in vitro systems have shown that NSAIDs alter the inflammatory response by inhibiting activation of neutrophils and thus resulting an inhibition of inflammatory cellular enzymes such as collagenase, elastase, hyaluronidase and others (Adam, 2001). It was reported that NSAIDs are able to inhibit he generaion of hypochlorous acid which suppress the oxidative functions of neutrophils in rats (Paino *et al*., 2005). Yoshida, *et al*., (2000) also reported that that proton pump inhibitors may have anti-inflammatory activity b attenuate neutrophil adherence to endothelial cells via inhibiting the expression of adhesion molecules.

Table I. Mean RBC (Mill/ul) and hemoglobin values (g/dl) of six goats obtained after administration of I/M diclofenac sodium at the dose rate of 2.5 and 1mg/kg (b.w).

	RBC values		hemoglobin values (g/dl)	
Time(hours)	**2.5 mg/kg (b.w) of diclofenac**	**1 mg/kg (b.w) of diclofenac**	**2.5 mg/kg (b.w) of diclofenac**	**1 mg/kg (b.w) of diclofenac**
	Mean/ S.E	Mean(S.E)	Mean (S.E)	Mean
C	5.61±0.15	5.58±0.16	8.83±0.21	8.85±0.19
1	5.50±0.16	5.43±0.14	8.68±0.17	8.71±0.17
2	5.41±0.17	5.23±0.17	8.4±0.17	8.53±0.17
3	5.16±0.22	5.11±0.19	8.05±0.08	8.33±0.14
6	4.66±0.19	4.81±0.20	7.10*±0.16	8.28±0.08
12	3.68±0.18*	4.61±0.18	7.15*± 0.12	8.23±0.09
24	4.91±0.17	5.00±0.18	8.15±0.09	8.41±0.09
48	5.11±0.22	5.21±0.21	8.31±0.12	8.53±0.11
72	5.26±0.23	5.28±0.21	8.43±0.17	8.63±0.14
96	5.46±0.32	5.46±0.20	8.66±0.23	8.86±0.17

(Significantly ($p<0.05$) different from control) *

Table II Mean WBC (×1000/ul) and granulocytes values of six goats obtained after administration of I/M diclofenac sodium at the dose rate of 2.5 and 1mg/kg (b.w).

	WBC values (×1000/ul)		granulocytes values (1000/ul)	
Time(hours)	2.5 mg/kg (b.w) of diclofenac	1 mg/kg (b.w) of diclofenac	2.5 mg/kg (b.w) of diclofenac	1 mg/kg (b.w) of diclofenac
	Mean S.E	Mean S.E	Mean S.E	Mean
C	8.30±0.45	8.28±0.45	2.20±0.09	2.16±0.21
1	8.71±0.46	8.58±0.52	2.05±0.11	1.98±0.21
2	9.36±0.44	8.83±0.51	1.83± 0.11	1.86±0.19
3	10.06±0.47	9.01±0.50	1.65±0.14	1.78±0.25
6	11.26*±0.41	9.25±0.53	1.21*±0.14	1.54±0.25
12	11.58*±0.48	8.98±0.50	1.24*±0.06	1.59±0.29
24	11.90* π±0.47	8.55 π±0.60	1.71±0.06	1.87±0.28
48	9.18±0.53	8.55±0.47	1.91±0.09	1.96±0.28
72	8.55±0.51	8.41±0.40	2.05±0.09	2.06±0.27
96	8.26±0.46	8.25±0.44	2.23±0.09	2.15±0.23

(Significantly ($p<0.05$) different from control) * (Significantly ($p<0.05$) different between values of 2.5mg/kg and 1mg/kg) π

Table III. Mean monocytes and lymphocytes values (1000/ul) of six goats obtained after administration of I/M diclofenac sodium at the dose rate of 2.5 and 1mg/kg (b.w).

	monocytes values (1000/ul)		lymphocytes values (1000/ul)	
Time(hours)	2.5 mg/kg (b.w) of diclofenac	1 mg/kg (b.w) of diclofenac	2.5 mg/kg (b.w) of diclofenac	1 mg/kg (b.w) of diclofenac
	Mean S.E	Mean S.E	Mean S.E	Mean S.E
C	2.83±0.21	2.81±0.27	7.30±1.00	7.40±0.95
1	2.95±0.23	2.90±0.26	7.53±0.90	7.42±0.78
2	3.05±0.17	2.98±0.27	7.83±0.84	7.50±0.64
3	3.28±0.18	3.15±0.27	8.25±0.70	7.45±0.77
6	3.74*±0.26	3.30±0.30	8.51±0.67	7.80±0.62
12	3.86*±0.28	3.30 ±0.33	9.10*±0.59	8.18±0.33
24	3.81*±0.25	3.31±0.33	9.15*±0.53	8.20±0.31
48	3.80*±0.23	3.23±0.30	8.05±0.49	7.60±0.58
72	3.21±0.22	3.06±0.28	7.60±0.66	7.29±0.47
96	2.81±0.23	2.83±0.24	7.26±0.81	7.46±0.13

(Significantly ($p<0.05$) different from control) *

Table IV. Mean platelets values (1000/ul) of six goats obtained after administration of I/M diclofenac sodium at the dose rate of 2.5 and 1mg/kg (b.w).

Time (hours)	2.5 mg/kg (b.w) of diclofenac		1 mg/kg (b.w) of diclofenac	
	Mean	S.E	Mean	S.E
C	437.00	±3.94	438.00	±4.00
1	435.00	±3.82	435.50	±3.70
2	432.00	±2.95	433.00	±3.38
3	429.00	±3.81	430.00	±3.72
6	427.00	±3.21	428.00	±3.43
12	395.33*	±3.60	420.00	±3.23
24	397.60*	±3.10	426.00	±3.66
48	429.00	±3.00	432.00	±3.63
72	432.00	±3.87	435.66	±3.52
96	436.00	±3.63	437.16	±3.68

(Significantly ($p<0.05$) different from control) *

In this study the platelets count decreases significantly after administration of 2.5 mg/kg (b.w) of diclofenac sodium. The effect was observed significantly ($P<0.05$) at 12 and 24 hours post dosage regimen

(Appendix- IIV). The study findings are in consistent with the results reported by George and Rahi, 1995 and Munsterhjelm *et al*., 2003; Alex *et al*., 2004 and Ahrens *et al*., 2006. It is accepted that the alteration of platelet function caused by NSAIDs is due to eciosanoid metabolism, mainly the inhibition of the enzyme cyclooxygenase (COX) (Alex *et al*., 2004). Diclofenac is a non selective anti-inflammatory drug and block both cyclooxygenase-1 and cyclooxygenase-2 (Susana *et al*., 2002), as a result platelets inhibition occurred. Thromboxnae (TXA2), which is mainly synthesized in platelets during COX-1 activity, causes platelets aggregation, vasoconstriction and smooth muscle proliferation (Ahrens *et al*., 2006). Hence diclofenac cause the inhibition of prostaglandin and thromboxane (Altaher *et al*., 2006) counteracts the effects consequential in inhibition of platelets aggregation and antiproliferative effects (Ahrens *et al*., 2006).

Conclusion: From the present study it is concluded that diclofenac sodium impair the Blood parameters but the effects are drug related and once the drug is cleared off from the body the organs retains their normal functions.

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