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# EVALUATION OF BIOCHEMICAL EFFECTS OF DICLOFENAC SODIUM IN GOATS

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## ABSTRACT

Diclofenac sodium is one of the most commonly using Non steroidal anti – inflammatory drugs (NSAID) worldwide in medical as well as veterinary practices. Use of anti-inflammatory drugs may affect liver function which may or may not be reversible in various livestock breeds. In this study effect of diclofenac sodium on Alanin transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALK), serum creatinine, serum uric acid, blood urea and total protein of liver and kidney of local dairy goats has been evaluated at Sindh Agriculture University, Tandojam since 2007. The drug was administered in six goats in two phases with adequate wash out period of 21 days between each phase. Dose rates, 2.5mg/kg (b.w) and 1 mg/kg (b.w), of diclofenac was administered in Phase-1 and Phase-2 respectively. For biochemical analysis the blood samples were collected at different intervals up to 96 hrs post drug administration. Significant change ($p<0.05$) with high dose was documented at 2, 3, 6, 12, 24 48 hrs in blood serum level of ALT, AST, ALK.PO4, creatinine, uric acid, and blood urea respectively. Where as highly significant change ($p<0.01$) was monitored at 6, 12, 24, 48 hrs in ALT and AST, ALK.PO4, and blood urea respectively. Significant increase in serum level of Alanin transaminase, Aspartate transaminase and Alkaline phosphatase was noticed at 12 and 24 hrs with low dose of diclofenac respectively. No significant change in serum creatinine and uric acid was observed but blood urea significantly increased at 48 hrs with low dose. No change was examined in total serum protein with both the doses. The effect of diclofenac was short-lived and most of the parameters went back to normal after 72hrs of drug administration.

**Key words:** Non steroidal anti – inflammatory drugs (NSAID), Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase ($ALK.PO_4$), diclofenac sodium, goat, inflammation.

## INTRODUCTION

Inflammation and pain mostly occurs in many diseases of goats. To provide improvement in animals well being and outcome, the condition of inflammation and pain is treated /managed by a variety of pharmacological agents of which NSAID's is one of the important and large group (Aydin *et al*., 2003). Among NSAID's diclofenac sodium is widely available veterinary drug. It is used for the symptomatic treatment and management of inflammation, fever and or pain associated with disease or injury of domestic livestock (Oaks *et al*., 2004). Diclofenac is an inhibitor of cyclooxygenase enzyme and is decreased in leukocyte intracellular free arachidonate level (Goodman and Gillman, 1995). The exact mechanism is not known but it is probably related to the decrease in the fatty acid entering the cell or released from the cell (Goodman and Gillman, 1995). Several studies have indicated that NSAIDs can generally prevent prostaglandin synthesis from arachidonic acid by inhibiting the activity of the prostaglandin synthesizing enzyme, cyclooxygenase. Prostaglandins are formed from dietary essential fatty acids (principally arachidonic acid) esterified to phospholipids and in some instances to triglycerides. In addition to its beneficial effects, diclofenac has also reported from world wide studies to be associated with adverse effects (Willkens., 1985 and Carson *et al.*, 1990). There are evidences that diclofenac produce adverse effects on liver and kidney (Aydin *et al*., 2003).

This study has therefore been designed to investigate the effects of diclofenac sodium on biochemical parameters that indicating the functional activity of organ of liver and kidney in goat specie. This study will help to develop an opinion regarding the concern raises for its clinical use in animals.

## MATERIALS AND METHODS

Six healthy local dairy goats were first acclimatized to local environment, Tandojam, Sindh, Pakistan, in a period of two weeks. Control base line values were determined for targeted biochemical parameters of blood serum of experimental goats. Diclofenac sodium was administered 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 rate of 2.5 mg/kg of body weight (the highest therapeutic recommended dose), while in phase-2 dose rate of drug was 1 mg/kg of body weight. For biochemical analysis the blood samples were collected at 1, 2, 3, 6, 12, 24, 48, 72 and 96 hrs post drug administration. Biochemical parameters ALT, AST,

ALK.PO4, serum uric acid, serum creatinine, blood urea and total protein, were measured. The Biochemical parameters were analyzed by commercially available kit methods.

## RESULTS AND DISCUSSION

Intramuscular administration of diclofenac sodium at a dose rate of 2.5mg/kg revealed significant effect on ALT, AST, ALK.PO4, serum creatinine, serum uric acid and blood urea, whereas 1 mg/kg of diclofenac sodium also bring significant effect on some biochemical parameters. A significant increase ($p<0.05$) in serum level of ALT was recorded at 2, 3, 6, 48 hrs, was observed at 12 and 24 hrs (Table 1). Significant increase in ALT was monitored at 12 hours with low dose of drug. By the 96 hrs post drug administration, the ALT values returned to normal. AST increased significantly at 6 and 48 hrs and highly significant at 12 and 24 hours with high dose of diclofenac, a significant increase at low dose in AST was examined only at 12 hrs. Increased alkaline phosphatase with high dose was reported at 3 hours and was highly significant at 6, 12, 24 and 48 hrs (Table 1). With low dose, increase in alkaline phosphatase was observed at 24 hrs. Serum creatinine and uric acid increased significantly with high dose at 12, 24, 48, 72 and 12, 24, 48 hours respectively. No significant changes were observed with dose of 1 mg/kg. A significant increased level was detected in blood urea at 6 hours and was highly significant at 12, 24 and 48 hours post high dose of diclofenac sodium. On the other hand at low dose the blood urea was significantly increased at 48 hours. The blood urea then gradually returned to base line values. Total Protein values remained the same in both of the Diclofenac sodium doses.

The result of the study indicates that diclofenac when administered in goats, causes significant effect on liver and kidney. To determine the effect of diclofenac sodium on liver, LFT (Liver function test) was done to evaluate the functional status of liver. The results of this study are in the line of findings reported by Aydin *et al*., 2003, Schwaiger *et al*., 2004, Helfgott, *et al*., 1990. Significant changes occurred in serum level of ALT, AST, and ALK.PO4 in goats after diclofenac administration. These enzymes are functional indicators of liver. Functional and structural alteration in liver leads to increased level of these enzymes in circulation. (Zaragoza *et al*., 1995, Brass, 1993, Breen *et al*., 1986, Connor *et al*., 2003). AST, ALT and Alkaline phosphatase are present in liver cells. All these enzymes are intracellular and are being located in mitochondria or cytoplasm or both and when cell's function altered, damaged or destroyed, the enzyme escapes into the blood (Doxey, 1971; Connor *et al*., 2003).

Several investigators report diclofenac ability to jeopardize critical mitochondrial functions (Jonnes, 1992 and Ku *et al*., 1993), including mitochondrial permeability transition and membrane potential (Sand storm, 1994 and Forrest *et al*., 1994) which may suggest that this organelle participated during the cell injury process. The hypothesis of in vitro mitochondrial toxicity, triggered by relatively low concentration of diclofenac, has been confirmed by damaging mitochondria response in humans and in animals receiving high doses (Lin *et al*., 2006). It is therefore possible (but not proven) that in susceptible patient's preexisrting mitochondrial defects may set the stage and sensitize these individuals to the pretoxicant effect of diclofenac (Boelsterli, 2003). Accordingly, a number of approaches of cultured hepatocytes from various species indicate that high concentration of diclofenac (in the absence of albumin added to the culture medium) were able to induce acute cell Sinjury (Rommel and Boelsterli, 1993, Jurima *et al*., 1994, Ponsoda *et al*., 1995: Bort *et al*., 1998: Masubuchi *et al*., 1998 and Masubuchi *et al*., 2000.

Significant increase occurred in the uric acid after diclofenac sodium administration. These findings are in agreement with the finding of Reddy *et al*., 2006 and Jelic *et al*., 1985. Metabolic activity is increased in liver due to diclofenac administration. With this the purine is metabolized to uric acid in the liver cells. When the metabolic activity due to diclofenac is stimulated the liver cell is affected /damaged and in turns the uric acid is increased in the blood (Connor *et al*., 2003). Uric acid is freely filtered by the glomerulus and is reabsorbed in the early renal proximal convoluted tubule vi a uric acid transporter followed by secretion and finaly postsecretory reabsorption (Grantham and Chonko, 1986 and Enomoto *et al*., 2002) it is reported that hyperuricemia may occur either of an overall decrease in secretion or an increase in uric acid production, or both. Hyperuricemia ultimately may result in renal disease, that includes the interstitial renal disease, as well as tubular injury (Mazzali *et al*., 2002).

In our study the increased blood urea is observed with diclofenac. The results are in agreement with the findings reported by Aydin *et al*., 2003 and Connor *et al*., 2003. It has been observed that diclofenac also influences the functions of kidney along with the liver. Urea is formed in the liver and represents the principal end product of protein catabolism and is excreted by the kidney. Diclofenac probably causes a decrease in the rate of excretion of urea nitrogen that may produce an increase in the concentration of urea (Aydin *et al*., 2003). It was reported that diclofenac induced deoxy ribonucleic acid (DNA) fragmentation may have been a consequence of intracellular calcium elevation. Increased in either cytosolic or intranuclear calcium can cause activation of proteases and /or phopholipases. These enzymes attack

**Table 1: Alanine Transaminase and Aspartate Transaminase values of goats administered with diclofenac sodium.**

| Time(hours) | Alanine Transaminase | | | | Aspartate Transaminase | | | |
|---|---|---|---|---|---|---|---|---|
| | 2.5 mg/kg (b.w) of diclofenac sodium | | 1 mg/kg (b.w) of diclofenac sodium | | 2.5 mg/kg (b.w) of diclofenac sodium | | 1 mg/kg (b.w) of diclofenac sodium | |
| | Mean | S.E | Mean | S.E | Mean | S.E | Mean | S.E |
| C | 12.33 | ±0.80 | 12.50 | ±0.76 | 9.33 | ±0.49 | 10.00 | ±0.49 |
| 1 | 13.66 | ±0.84 | 13.16 | ±0.70 | 11.00 | ±0.51 | 11.32 | ±0.42 |
| 2 | 16.50* | ±0.76 | 14.16 | ±0.79 | 13.66 | ±0.92 | 12.21 | ±0.51 |
| 3 | 18.83* | ±1.40 | 15.20 | ±0.80 | 15.50* | ±1.24 | 13.16 | ±0.40 |
| 6 | 19.10* π | ±0.77 | 15.66 π | ±0.88 | 16.83* | ±1.98 | 13.25 | ±0.49 |
| 12 | 23.00** π | ±0.87 | 16.89*π | ±0.76 | 20.33** π | ±0.79 | 15.10*π | ±0.36 |
| 24 | 22.00** π | ±0.49 | 15.16 π | ±0.74 | 19.60** π π | ±0.84 | 13.42 π π | ±0.25 |
| 48 | 16.50* | ±1.86 | 15.05 | ±0.84 | 15.76* | ±1.74 | 12.33 | ±0.42 |
| 72 | 15.83 | ±0.60 | 13.66 | ±0.49 | 13.46 | ±0.86 | 11.16 | ±0.30 |
| 96 | 12.00 | ±0.85 | 12.16 | ±0.60 | 9.58 | ±0.55 | 9.61 | ±1.49 |

Significantly different from control (* p<0.05, ** p<0.01); Significantly different among doses, π p<0.05, ππ p<0.01)

**Table 2. Akaline Phosphatase and Serum Creatinine values (mg/dl) values of goats administered with diclofenac sodium.**

| Time(hours) | Akaline Phosphatase | | | | Serum Creatinine | | | |
|---|---|---|---|---|---|---|---|---|
| | 2.5 mg/kg (b.w) of diclofenac sodium | | 1 mg/kg (b.w) of diclofenac sodium | | 2.5 mg/kg (b.w) of diclofenac sodium | | 1 mg/kg (b.w) of diclofenac sodium | |
| | Mean | S.E | Mean | S.E | Mean | S.E | Mean | S.E |
| C | 182.50 | ±0.76 | 182.00 | ±0.66 | 1.47 | ±0.23 | 1.47 | ±0.01 |
| 1 | 184.33 | ±0.71 | 183.33 | ±0.69 | 1.54 | ±0.02 | 1.48 | ±0.01 |
| 2 | 186.16 | ±0.79 | 184.00 | ±0.74 | 1.59 | ±0.50 | 1.48 | ±0.01 |
| 3 | 201.50* π | ±1.26 | 185.16 π | ±0.60 | 1.65 | ±0.04 | 1.49 | ±0.10 |
| 6 | 221.33**π π | ±0.80 | 186.50π π | ±0.76 | 1.70 | ±0.04 | 1.51 | ±0.30 |
| 12 | 229.16** π π | ±0.98 | 189.60 π π | ±1.40 | 1.95* | ±0.29 | 1.54 | ±0.23 |
| 24 | 232.50** π π | ±0.99 | 194.60* π π | ±1.86 | 2.03* π | ±0.36 | 1.58 π | ±0.41 |
| 48 | 211.33** π π | ±0.96 | 186.66 π π | ±1.64 | 2.1* π | ±0.12 | 1.61 π | ±0.31 |
| 72 | 189.33 | ±2.45 | 184.83 | ±1.19 | 1.9* | ±0.42 | 1.55 | ±0.71 |
| 96 | 181.16 | ±0.83 | 182.66 | ±0.70 | 1.51 | ±0.02 | 1.48 | ±0.13 |

Significantly different from control (* p<0.05, ** p<0.01); Significantly different among doses, π p<0.05, ππ p<0.01)

**Table 3. Serum Uric acid and Blood Urea values of goats administered with diclofenac sodium.**

| Time(hours) | Serum Uric acid (mg/dl) | | | | (mg/dl) | | | |
|---|---|---|---|---|---|---|---|---|
| | 2.5 mg/kg (b.w) of diclofenac sodium | | 1 mg/kg (b.w) of diclofenac sodium | | 2.5 mg/kg (b.w) of diclofenac sodium | | 1 mg/kg (b.w) of diclofenac sodium | |
| | Mean | S.E | Mean | S.E | Mean | S.E | Mean | S.E |
| C | 0.80 | ±0.04 | 0.80 | ±0.03 | 23.83 | ±0.6 | 23.66 | ±0.80 |
| 1 | 0.83 | ±0.04 | 0.81 | ±0.73 | 24.33 | ±0.49 | 24.16 | ±0.60 |
| 2 | 0.88 | ±0.06 | 0.82 | ±0.03 | 25.16 | ±0.69 | 24.83 | ±0.65 |
| 3 | 0.94 | ±0.05 | 0.83 | ±0.07 | 27.50 | ±0.91 | 26.00 | ±0.63 |
| 6 | 0.98 | ±0.03 | 0.85 | ±0.35 | 30.83* | ±1.60 | 27 .00 | ±0.63 |
| 12 | 1.20* | ±0.05 | 0.89 | ±0.09 | 36.66** π | ±0.98 | 27.83 π | ±0.70 |
| 24 | 1.29* π | ±0.23 | 0.87 π | ±0.31 | 40.66** π π | ±1.30 | 28.5π π | ±0.67 |
| 48 | 1.20* | ±0.04 | 0.85 | ±0.47 | 43** π π | ±0.96 | 29.83* π π | ±0.83 |
| 72 | 1.13 | ±0.17 | 0.84 | ±0.05 | 28.00 | ±1.82 | 25.66 | ±0.71 |
| 96 | 0.79 | ±0.04 | 0.80 | ±0.09 | 23.58 | ±0.63 | 23.75 | ±0.61 |

Significantly different from control (* p<0.05, ** p<0.01); Significantly different among doses, π p<0.05, ππ p<0.01)

**Table 4. Total Protein values (mg/dl) of six goats obtained after intra muscular (I/M) administration of diclofenac sodium at the dose rate of 2.5 and 1mg/kg (b.w).**

| Time(hours) | 2.5 mg/kg (b.w) of diclofenac sodium | | 1 mg/kg (b.w) of diclofenac sodium | |
|---|---|---|---|---|
| | Mean | S.E | Mean | S.E |
| C | 5.54 | ±0.13 | 5.55 | ±0.13 |
| 1 | 5.16 | ±0.09 | 5.36 | ±0.12 |
| 2 | 4.95 | ±0.08 | 5.23 | ±0.16 |
| 3 | 4.86 | ±0.10 | 5.15 | ±0.18 |
| 6 | 4.82 | ±0.11 | 5.08 | ±0.20 |
| 12 | 4.79 | ±0.10 | 5.03 | ±0.21 |
| 24 | 4.70 | ±0.08 | 4.99 | ±0.23 |
| 48 | 4.72 | ±0.07 | 5.04 | ±0.21 |
| 72 | 4.80 | ±0.09 | 5.14 | ±0.17 |
| 96 | 5.35 | ±0.10 | 5.53 | ±0.24 |

vital macromolecular targets, such as polymerases and plasma membranes, and cause irreversible cell injury. Increase in BUN observed after diclofenac administration justifies participation of these events (Hickey *et al*., 2001). The increased level of serum Creatinine has been observed in the study. Same observation is also reported by Aydin *et al*., 2003 and Reddy *et al*., 2006. The Creatinine is non protein nitrogenous substance formed during muscle metabolism from creatin and phosphocreatin. It is excreted by Glomerular filtrations. As with urea, the rate of excretion is influenced by Glomerular filtration rate (GFR), and any abnormalities that decrease GFR will result in an increase serum Creatinine (Doxey, 1971). Previous studies have shown that minor increase in serum creatinine can reflect a marked fall in glomerular filteration rate (Salomon *et al*., 2003). Creatinine and blood urea is increased due to less glomerular filtration rate which is possibly impaired by diclofenac. No significant change occurred in total protein. These results are also reported by Reddy *et al*., 2006 and Jelic *et al*., 1985.

**Conclusion:** From the present study it is concluded that diclofenac sodium impair the hepatic and renal functions of goats, 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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