Evaluation of hematological and biochemical effects of pefloxacin/ diclofenac interaction in goat

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Abstract: Co-administration of Nonsteroidal Anti-inflammatory Drugs (NSAIDs) and antimicrobials may occur frequently because of worldwide in medical as well as veterinary practices. Often it is either diminished therapeutic efficacy or increased toxicity of one or more of the administered drugs. In this study the effect of pefloxacin/ diclofenac interaction was investigated on twenty clinically healthy goats of balady breed with an average body weight (21-27 kg). Goats were injected i.m. with pefloxacin at dose rate of 5 mg/kg b.wt daily for 4 days consequently. Diclofenac sodium was injected i.m. at dose rate of 1 mg/kg b.wt daily for 4 days consequently to different groups of goats . Two blood samples were collected from each animal of all groups at 3rd day, then at 1st , 2nd , 3rd week post injection. The obtained results revealed that co-administration of diclofenac sodium and pefloxacin showed significant changes ($p < 0.05$) in total erythrothytic count (RBCs), haemoglobin (Hb) % and packed cell volume (PCV). no significant change in total leucocytic count . Drugs caused slight impairment of hepatic and renal functions of goats. The effect of both drugs was short-lived and most of the parameters went back to normal after 2 weeks post drug(s) administration.

[El-Ghoneimy A. A. and Shaheen H. M. **Evaluation of hematological and biochemical effects of pefloxacin/ diclofenac interaction in goat.** *Life Sci J* 2012;9(4):4847-4854] (ISSN:1097-8135). http://www.lifesciencesite.com. 729

Keywords: Hematological; Biochemical; Pefloxacin; Diclofenac; goat

1. 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 and antimicrobials are one of the important and large group (Aydin *et al*., 2003).

NSAID's are used for the symptomatic treatment and management of inflammation, fever and or pain associated with disease or injury of domestic livestock (George, 2003). Diclofenac is used in a variety of painful conditions like osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, renal colic, acute muscle pain, in dentistry and preoperatively to reduce postoperative pain. Beside its therapeutic effects some significant adverse effects are also associated with diclofenac in different species (Katzung, 1998; Bhagat *et al.,* 2003 ; Ahmad, 2008).

Co-administration of several drugs often results in unpredictable therapeutic outcome. Often it is either diminished therapeutic efficacy or increased toxicity of one or more of the administered drugs. Various pharmacokinetic interactions between antimicrobials and NSAIDs have been described (Chambers and Jawetz, 1997; Brooks *et al.,* 1998; Harry *et al.,* 1998; Barbosa *et al.,* 2001; Ahmed *et al.,* 2005).

Pefloxacin, is a synthetic antibacterial agent of the flouroquinolone group developed exclusively for veterinary use. It is mainly indicated for gastrointestinal, urogenital, skin and respiratory tract infections caused by gram-positive and gram-negative bacteria as well as mycoplasma in various domestic animal species (Moutafchieva and Yarkov, 2006).

Because of the published information on the pharmacology of both drugs in the goat is very limited, however, there is a need to investigate in this species their pharmacodynamics (PD), when administered both singly and in combination These data are required for determining dosage schedules for clinical use.

This study has therefore been designed to investigate the safety or potential toxicity of pefloxacin / diclofenac sodium usage in goats on some haematological and biochemical parameters that indicating general health condition in goat specie. This study will help to develop an opinion regarding the concern raises for its clinical use in animals.

2.Materials and Methods

Experimental animals

A total of twenty healthy adult female native Baladi goats with an average body weight of 21.6 ± 2.24 kg (mean ± SE) were obtained from the farm at South Valley University. The animals were housed in

hygienic stables and fed an antibiotic-free diet for at least 30 days before the study. For each treatment period, the animals were observed daily for general health. Clinical observations were made prior to injection and at 2, 10 and 24 hrs post-injection. The animals were maintained under optimal nutritional conditions and fed barseem, a drug-free concentrate, and water *ad libitum*.

Drugs and treatment

Peflodad® **(pefloxacin 10%)** was obtained from Dar Aldawa Veterinary and Agriculture Industrial Co. Ltd. Jordan. Each ml contains 100 mg of pefloxacin base.

Dicloflame ® **(diclofenac sodium 2.5%)** was obtained from Unipharma Elobour City, Cairo, Egypt. Each ml contains 25 mg of diclofenac sodium.

The goats were divided into 4 equal groups (each of 5):

Group 1. Goats were kept without any medication as control group.

Group 2. Pefloxacin was injected intramuscularly at a dose of 5 mg kg^{-1} (recommended dose) daily for 4 days consequently ,pefloxacin treated group.

Group 3. Diclofenac sodium was injected intramuscularly at a dose of 1 mg kg^{-1} (recommended dose) daily for 4 days consequently ,diclofenac sodium treated group.

Group 4. Both pefloxacin and diclofenac sodium were injected intramuscularly at the same dose rate for 4 days consequently, pefloxacin/diclofenac sodium treated group. The injection had occurred at two different sites.

Sample collection

Two blood samples were collected from each animal of all groups at 3rd day of injection then at 1st, 2nd, 3rd week post injection. The first blood sample was collected in test tubes containing EDTA for hematological studies. While the second blood samples were allowed to coagulate at 4 °C and were then centrifuged at 3000 × g for 15 min to separate the serum. The serum samples were frozen at −20°C.

Hematological Analysis

The hematological parameters, Red blood cells (RBCs) count, White blood cells (WBCs) counts, Hemoglobin (Hb%), Packed Cell Volume (PCV%) and Red blood cells indices including Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were estimated by using automatic cell counter (Exigo, Veterinary Hematology System, Boule Medical AB, Stockholm, Sweden.).

Biochemical Analysis

Biochemical parameters alanine transaminase (ALT) and aspartate transaminase (AST), total proteins, albumin, blood urea nitrogen, serum uric acid and serum creatinine . were measured. The Biochemical parameters were analyzed by commercially available kit methods. Globulins were estimated by electrophoretic analysis of serum protein.

Statistical analysis

The descriptive data are presented as the means ± SE. The statistical differences were calculated on the basis of two way test of ANOVA and $p < 0.05$ is considered as significant between the groups. The data were statistically analyzed by using (One way ANOVA test for variance analysis) (Student-Newman-Keuls) at $p < 0.05$ (Quinton *et al.,* 1995).

3. Results

All goats were clinically healthy throughout the experiment. None of the goats in all groups suffered from identifiable reactions following the administration of pefloxacin or diclofenac.

Hematological Analysis

Intramuscular administration of both diclofenac sodium at a dose rate of 1 mg kg^{-1} and pefloxacin at a dose of 5 mg kg^{-1} revealed significant effect on total erythrocytic count , haemoglobin % and packed cell volume (Table 1) .

A significant drop ($p < 0.05$) in total erythrocytic count at 3rd day post treatment. The total erythrocytic count was (8.05 ± 0.37) with respect to (9.41 ± 0.21 ; 9.57 ± 0.23 and 12.53 ± 0.56 in control goats respectively).

Hemoglobin % decreased significantly till the 1st week post drug administration. It was (8.04± 0.18) vs. (8.70 ± 0.11; 9.83 ± 0.10 and 10.20 ± 0.12 in control goats respectively).

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Packed cell volume (%) decreased significantly till the 1st week post drug administration. It was (26.67 ± 0.67) vs. (29.14 ± 0.50; 30.55 ± 0.88 and 34.67 ± 0.45 in control goats respectively).

By the 2nd week post drug administration, the total erythrocytic count, Hemoglobin and Packed cell volume values returned to normal levels in all groups.

No statistically relevant differences in total leucocytic count that remained within the same values between all groups (Table 3).

Biochemical Analysis

Intramuscular administration of both diclofenac sodium at a dose rate of 1 mg kg^{-1} and

pefloxacin at a dose of 5 mg kg^{-1} revealed significant effect on ALT, AST, serum total proteins, serum albumin , serum globulin, serum creatinine, serum uric acid and blood urea values.

A significant increase (p<0.05) in serum level of ALT was recorded at 72 hrs post treatment (Table 4).

AST increased significantly at 72 hrs and still examined by the end of the 1st week post treatment (36.0 ± 1.73) with respect to (31.0 ± 0.58; 25.0 ± 1.12 and 23.33 ± 0.88) in different groups (Table 4).

Total protein values was significantly dropped at 72 hrs post treatment and then gradually returned to base line values (Table 5).

Serum creatinine, uric acid and blood urea values increased significantly at 72 hrs post treatment. Both serum creatinine and blood urea were still examined by the end of the 1st week post treatment (Table 6).

Table 1. Effect of intramuscular injection of pefloxacin (5 mg kg^{-1}) or diclofenac sodium (1 mg kg^{-1}) and both drugs for 4 consecutive days on total erythrocytic count, hemoglobin concentration and Packed cell volume values of healthy goats (n=5).

Parameters / Groups	Erythrocytic count (10^6/mm)				Hemoglobin concentration (%)				Packed cell volume (%)			
	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week
Control	12.53 ± 0.56 a	11.97 ± 0.70 a	11.84 ± 0.31 a	11.80 ± 0.31 a	11.38 ± 0.09 a	10.20 ± 0.12 a	10.27 ±0.2 0 a	10.15 ± 0.19 a	34.22 ± 0.73 a	34.67 ± 0.45 a	33.15 ± 0.88 a	33.17 ± 1.20 a
Pefloxacin	9.57 ± 0.23 b	10.97 ± 0.75 ab	10.53 ± 0.82 a	10.75 ± 0.68 a	9.07 ± 0.20 b	9.83 ± 0.10 a	9.98 ± 0.17 a	9.96 ± 0.10 a	26.33 ± 0.53 b	30.55 ± 0.88 b	31.09 ± 0.70 a	31.80 ± 0.98 a
Diclofenac sod.	9.41 ± 0.21 b	9.83 ± 0.38 b	10.65 ±0.26a	10.65± 0.40 a	8.47 ± 0.13 b	8.70 ± 0.11b	9.95± 0.12a	9.93 ± 0.18 a	25.94± 0.33 b	29.14 ± 0.50 b	30.95 ± 0.57 a	31.67 ± 0.67 a
Pefloxacin + Diclofenac sod.	8.05 ± 0.37 c	9.42 ± 0.29 b	10.32± 0.29 a	10.47 ±0.33a	7.83 ± 0.08c	8.04± 0.18 c	9.76 ± 0.10a	9.76 ± 0.12 a	22.00 ± 0.58c	26.67 ± 0.67 c	30.67 ± 1.2 a	30.93 ± 0.68 a

Values are expressed as Mean ± SE.
The means which carry different letter in the same column were significantly different, (p < 0.05).

Table 2. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of healthy goats (n=5).

Parameters / Groups	Mean corpuscular volume (MCV)				Mean corpuscular hemoglobin (MCH)				Mean corpuscular hemoglobin concentration (MCHC)			
	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week
Control	27.31 ± 0.83 a	28.96 ± 1.98a	28.00± 0.12 a	28.11 ± 0.25a	9.08 ± 0.51 a	8.52 ± 0.69 a	8.67 ± 0.12 a	8.60 ± 0.48 a	32.18 ± 0.96 a	29.42 ± 0.98 a	30.98 ± 0.31 a	30.60 ± 1.77 a
Pefloxacin	27.51 ± 1.12 a	27.85 ± 1.24a	29.53± 1.57 a	29.58 ± 2.2 a	9.48± 0.23 a	8.99 ± 0.02 a	8.58 ± 0.63 a	9.27 ± 0.5 a	34.45 ± 0.31a	32.18± 0.83 a	32.10± 1.24 a	31.32 ± 0.65 a
Diclofenac sod.	27.57 ± 0.32 a	29.64 ± 0.65a	29.06 ± 0.28a	29.74 ± 0.5 a	9.00 ± 0.14 a	8.85 ± 0.26 a	9.11 ± 0.24 a	9.32 ± 0.23 a	32.65 ± 0.45 b	29.86 ± 0.23 a	32.15 ± 0.55 a	31.36 ± 0.49 a
Pefloxacin + Diclofenac sod.	27.33 ± 0.68 a	28.31 ± 0.23a	29.72 ± 0.35a	31.40 ± 0.62a	9. 72± 0.27 a	8.54 ± 0.12 a	9.46 ± 0.38 a	9.32 ± 0.34 a	35.59± 0.35 c	30.15 ± 0.25 a	31.82 ± 1.51 a	31.56 ± 0.7 a

Values are expressed as Mean ± SE.
The means which carry different letter in the same column were significantly different, (p < 0.05).

Table 3. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on total leucocytic count (cell/mm^3) of healthy goats (n=5).

Parameter / Groups	Total leucocytic count (cell/mm^3)			
	3rd day	1st Week	2nd Week	3rd Week
Control	7.83 ± 0.4 a	7.82 ± 0.27 a	7.63 ± 0.13 a	7.18 ± 0.42 a
Pefloxacin	7.54 ± 0.27 a	7.48 ± 0.12 a	7.25 ± 0.2 a	7.53 ± 0.23 a
Diclofenac sod.	7.63 ± 0.04 a	7.60 ± 0.14 a	7.57 ± 0.12 a	7.34 ± 0.01 a
Pefloxacin + Diclofenac sod.	7.49 ± 0.35 a	7.95 ± 0.34 a	7.80 ± 0.35 a	7.67 ± 0.32 a

Values are expressed as Mean ± SE.
The means which carry different letter in the same column were significantly different, (p < 0.05).

Table 4. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on serum alanine aminotransferase enzyme (ALT, IU/L) and serum asparatate aminotransferase enzyme (AST, IU/L) of healthy (n=5).

Parameters / Groups	Serum Alanine Aminotransferase (ALT)				Serum Asparatate Aminotransferase (AST)			
	3rd day	1st week	2nd week	3rd week	3rd day	1st week	2nd week	3rd week
Control	24.27 ± 0.87 ^{d}	24.78 ± 0.94 ^{a}	24.70 ± 0.92 ^{a}	24.01 ± 0.75 ^{a}	21.67 ± 0.80 ^{d}	23.33 ± 0.88 ^{c}	24.67 ± 1.11^{a}	20.0 ± 1.03 ^{a}
Pefloxacin	45.38 ± 0.65 ^{c}	25.54 ± 0.74 ^{a}	25.87 ± 0.76 ^{a}	24.09 ± 0.38 ^{a}	39.33 ± 1.19 ^{c}	25.0 ± 1.12 ^{c}	23.0 ± 1.03 ^{a}	24.33 ± 1.17 ^{a}
Diclofenac sod.	64.01 ± 0.77 ^{b}	27.40 ± 1.05 ^{a}	26.31 ± 0.81 ^{a}	25.13 ± 0.91 ^{a}	53.0 ± 0.58 ^{b}	31.0 ± 0.58 ^{b}	24.0 ± 1.10 ^{a}	25.0 ± 1.60 ^{a}
Pefloxacin + Diclofenac sod.	76.46 ± 0.93 ^{a}	27.90 ± 1.06 ^{a}	26.83 ± 1.04 ^{a}	25.48 ± 0.87 ^{a}	89.0 ± 1.31 ^{a}	36.0 ± 1.73 ^{a}	25.0 ± 1.06 ^{a}	28.0 ± 1.50 ^{a}

Values are expressed as Mean ± SE.

The means which carry different letter in the same column were significantly different, ($p < 0.05$).

Table 5. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on serum total proteins (g/dl), serum albumin (g/dl) and serum globulin (g/dl) levels of healthy goats (n=5).

Parameters / Groups	Serum total protein (g/dl)				Serum albumin (g/dl)				Serum globulin (g/dl)			
	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week
Control	6.94 ± 0.12^{a}	6.52 ± 0.21 ^{a}	6.79 ± 1.26 ^{a}	6.88 ± 0.15 ^{a}	4.92 ± 0.21 ^{a}	4.62 ± 0.26 ^{a}	4.56 ± 0.20 ^{a}	4.81 ± 0.17 ^{a}	2.02 ± 0.10 ^{a}	1.90 ± 0.21 ^{a}	2.23 ± 0.14 ^{a}	2.07 ± 0.25 ^{a}
Pefloxacin	6.48 ± 0.41^{a}	6.15 ± 0.33 ^{a}	6.18 ± 1.0 6^{a}	6.70 ± 0.21 ^{a}	4.56 ± 0.16 ^{a}	4.20 ± 0.24 ^{ab}	4.36 ± 0.16 ^{a}	4.77 ± 0.16 ^{a}	1.92 ± 0.11 ^{a}	1.95 ± 0.14 ^{a}	1.82 ± 0.22 ^{a}	1.93 ± 0.15 ^{a}
Diclofenac sod.	4.89 ± 0.31 ^{b}	5.34 ± 0.19 ^{b}	6.06 ± 0.18 ^{a}	6.48 ± 0.17 ^{a}	3.66 ± 0.11 ^{b}	3.50 0.15 ^{bc}	4.24 ± 0.12 ^{a}	4.68 ± 0.18 ^{a}	1.23 ± 0.24 ^{b}	1.84 ± 0.28 ^{a}	1.82 ± 0.10 ^{a}	1.80 ± 0.14 ^{a}
Pefloxacin + Diclofenac sod.	4.26 ± 0.18 ^{b}	4.89 ± 0.31 ^{b}	5.68 ± 0.17 ^{a}	6.30 ± 0.23 ^{a}	3.11 ± 0.13 ^{c}	3.21 ± 0.21 ^{c}	3.92 ± 0.22 ^{a}	4.65 ± 0.26 ^{a}	1.15 ± 0.15 ^{b}	1.68 ± 0.12 ^{a}	1.76± 0.13 ^{a}	1.65 ± 0.12 ^{a}

Values are expressed as Mean ± SE.

The means which carry different letter in the same column were significantly different, ($p < 0.05$).

Table 6. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on serum urea (mg/dl), serum uric acid (mg/dl) and serum creatinine (mg/dl) levels of healthy (n=5).

Parameters / Groups	Serum urea (mg/dl)				Serum uric acid (mg/dl)				Serum creatinine (mg/dl)			
	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week
Control	14.36 ± 0.18 ^{d}	14.42 ± 0.22 ^{c}	15.05 ± 0.21 ^{a}	14.92 ± 0.15 ^{a}	0.87 ± 0.16 ^{b}	0.87 ± 0.21 ^{a}	0.80 ± 0.20 ^{a}	0.83 ± 0.40 ^{a}	0.90 ± 0.12 ^{c}	0.91 ± 0.11 ^{c}	0.91 ± 0.19 ^{a}	0.94 ± 0.14 ^{a}
Pefloxacin	17.87 ± 0.25 ^{c}	14.94 ± 0.13 ^{c}	15.23 ± 0.24 ^{a}	15.02 ± 0.12 ^{a}	1.69 ± 0.21 ^{a}	0.89 ± 0.11 ^{a}	0.85 ± 0.31 ^{a}	0.87 ± 0.55 ^{a}	1.43 ± 0.15 ^{b}	1.27 ± 0.10 ^{bc}	1.04 ± 0.22 ^{a}	1.01 ± 0.11 ^{a}
Diclofenac sod.	26.05 ± 0.22 ^{b}	16.63 ± 0.18 ^{b}	15.44 ± 0.36 ^{a}	15.13 ± 0.11 ^{a}	1.82 ± 0.22 ^{a}	1.03 ± 0.31 ^{a}	0.90± 0.25 ^{a}	0.89 ± 0.24 ^{a}	1.68 ± 0.11 ^{b}	1.52 ± 0.12 ^{ab}	1.08 ± 0.30 ^{a}	1.07 ± 0.17 ^{a}
Pefloxacin + Diclofenac sod.	31.09 ± 0.11 ^{a}	19.22 ± 0.16 ^{a}	15.97 ± 0.29 ^{a}	15.45 ± 0.19 ^{a}	1.98 ± 0.32 ^{a}	1.10 ± 0.20 ^{a}	0.98 ± 0.41 ^{a}	0.95 ± 0.34 ^{a}	2.38 ± 0.13 ^{a}	1.76 ± 0.22 ^{a}	1.10 ± 0.21 ^{a}	1.13 ± 0.30 ^{a}

Values are expressed as Mean ± SE.

The means which carry different letter in the same column were significantly different, ($p < 0.05$).

4.Discussion

Fluoroquinolones are increasingly employed in medicine to eradicate the susceptible bacterial infection. Co-administration of several drugs often may result in unpredictable therapeutic outcome. The use of non-steroidal anti-inflammatory drugs (NSAIDs) are frequently recommended with antibacterials for the treatment of various bacterial infections accompanied by fever and other inflammatory conditions in the men and animals. It is widely recognized that co-administration of two drugs may affect the absorption, distribution, biotransformation and/or excretion of both agents (Patel *et al.*, 2011).

Following concurrent intramuscular administration of diclofenac and enrofloxacin in sheep, there was a significant rise in the plasma concentration of diclofenac, the peak value being 7.74 µg ml^{-1} observed at 0.5 h. The plasma concentration of diclofenac was maintained above therapeutic plasma level(3.43 µg ml^{-1}) for 7 hrs after drug administration. However, these differences were not statistically significant, which suggests that diclofenac did not influence the kinetics of enrofloxacin and ciprofloxacin (Kumar *et al.,* 2003). Also, intramuscular administration of moxifloxacin did not produce significant influence over the elimination processes of intramuscularly

administered tolfenamic acid in the male and female rats (Patel *et al.*, 2011).

On contrary, enrofloxacin inhibits hepatic microsomal cytochrome P450 monooxygenases (Shlosberg *et al.,* 1997). There is a possibility that concurrent administration of enrofloxacin resulted in decreased oxidation of diclofenac due to which the plasma levels of diclofenac were maintained for a longer period. Moreover, fluoroquinolones have previously been reported to decrease the toxicity of acetaminophen by preventing its biotransformation by inhibiting cytochrome P450 isozymes (Nakashi and Okuno,1990).

So, the result of the study indicates that diclofenac when administered with pefloxacin in goats, causes significant effect on some hematological, liver and kidney function parameters could be associated with diclofenac effect.

Intramuscular administration of both diclofenac sodium at a dose rate of 1 mg kg^{-1} and pefloxacin at a dose of 5 mg kg^{-1} revealed significant effect on red cell related parameters. This could attributed to diclofenac induced immune hemolysis produce a broad spectrum of anti-diclofenac/RBCs antibodies. 4'-OH- diclofenac seems to represent the most immunogenic metabolite. Serum could contain a mixture of antibodies that recognize several and distinguishable epitopes. These epitopes consist of different drug metabolites and a target protein on the RBC surface. So, diclofenac forms neoantigens with RBCs that may stimulate the production of autoantibodies and drug-dependent antibodies (Salama *et al.,*1996 ; Sachs *et al.,*2004).

These findings could explain the significant decrease in globulins levels in both diclofenac and diclofenac/pefloxacin treated goats in comparison to the other two groups. On the other side, the level of leucocytic count remain unchanged, so, this may approve that that hemolytic anemia induced by diclofenac may be due to cellular and humoral immunity as well. According to the association of hemolytic anemia with duration of

treatment we found that hemolytic anemia was accompanied with diclofenac treatment and was drug dependent as upon discontinuation of treatment we noticed correction of

hemoglobin concentration.

Despite the fact that diclofenac itself was effective in impairing ATP synthesis by mitochondria, there was evidence that toxicity was also related to the drug metabolism and was reduced by the addition of cytochrome p-450 inhibitors to the culture medium (Bort *et al.,1999*). Moreover, the key role of mitochondrial dysfunction in the pathogenesis of diclofenac-induced hepatocyte injury, as a result of the decrease of ATP and MPT, has also been recently reported (Masubuchi *et al.*, 2002). Since MPT is considered a major common mechanism for drug-induced hepatocyte necrosis and apoptosis (Higuchi *et al.,* 2001), it is very likely that apoptosis is involved in the adverse effect of diclofenac.

The result of the presented study indicates that intramuscular administration of both diclofenac sodium at a dose rate of 1 mg kg^{-1} and pefloxacin at a dose of 5 mg kg^{-1} revealed significant effect on liver and kidney in goats.

To determine the effect of diclofenac sodium on liver function was done to evaluate the functional status of liver. The results of this study are in the line of early reported findings (Aydin *et al.,* 2003; Schwaiger *et al.,* 2004; Ahmad *et al.,* 2012**)**.

Significant changes occurred in serum level of ALT, AST, total proteins, albumin and globulins in goats after diclofenac administration. These parameters are functional indicators of liver. Functional and structural alteration in liver leads to increased level of these enzymes in circulation (O'Connor *et al.,* 2003). AST, and ALT are present in liver cells. 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 (O'Connor *et al.,* 2003).

Also, it has been reported that the damage in hepatocytes induced by diclofenac sodium and could be associated with an idiosyncratic reaction (Kertz-Rommel and Boelsteri, 1993).

The two serum proteins measured to assess liver function are albumin and globulin that could reflect the hepatic cell condition (Talwar and Srivastava, 2003). Albumin, produced only in the liver, is the major plasma protein that circulates in the blood stream.

Albumin is also very important in the transportation of many substances such as drugs, lipids, hormones, and toxins that are bound to albumin in the bloodstream. Once the drug or other substance reaches the liver, it is detached from the albumin and made less toxic by conversion to a water-soluble form that can be excreted, as albumin largely account on acidic drugs (e.g. nonsteroidal antinflammatory drugs) on their transport in plasma, so may interfere with drug kinetic (Baggot, 2001).

The reduction in the total protein could be attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of cytochrome P-450 enzymes leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides may leading to fatty liver (Suresh Kumar *et al.,* 2007).

The correction of liver biochemical results from the 2nd week post treatment could explain that

the toxic effects of diclofenac to liver cells could be reversible and dose-dependent (Kayaalp *et al.,1998*). Despite the fact that diclofenac itself was effective in impairing ATP synthesis by mitochondria, there was evidence that toxicity was also related to the drug metabolism and was reduced by the addition of cytochrome p-450 inhibitors to the culture medium (Aydin *et al.,* 2003), so combination of diclofenac with fluoroquinolones could be useful in correction of toxic effect due to diclofenac sodium in goat.

Another possible factor for such correction of liver biochemical results is reported by Javed *et al.* (2009). glomerular filtration rate being the highest in goats than other ruminants so, urinary excretion of ciprofloxacin revealed that the excretion rate of the drug was highest in goats, followed by sheep, buffaloes, and cows.

Significant increase occurred in the uric acid after diclofenac sodium administration alone and in combination with pefloxacin. These findings are in agreement with the finding of Reddy *et al.* (2006).

Data of total plasma protein in our study is significantly decreased that accompanied with diclofenac administration this could attributed to increase in liver activity to protein catabolism which indicated by high levels of serum uric levels in the presented study (Talwar and Srivastava, 2003). Diclofenac is more cytotoxic to drug metabolizing cells than to non-metabolizing cell lines (Bort *et al.,* 1999).

Uric acid is freely filtered by the glomerulus and is reabsorbed in the early renal proximal convoluted tubule via uric acid transporter followed by secretion and finally, postsecretory reabsorption .Hyperuricemia may occur either of an overall decrease in secretion or an increase in uric acid production, or both. (Enomoto *et al.,* 2002).

Hyperuricemia ultimately may resulted from renal disease, that includes the interstitial renal disease, as well as tubular injury (Mazzali *et al.,* 2002). The mitochondrion is clearly a target of diclofenac-induced nephrotoxicity as demonstrated by the oxidative stress and massive DNA fragmentation reported from studies with diclofenac *in vivo* . A decrease in ATP synthesis was demonstrated in isolated kidney mitochondria with glutamate/malate (Lin Eng *et al.,* 2008).

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 O´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. Nephrotoxic effect of 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).

As diclofenac, like all other NSAIDS, prevents the synthesis of prostaglandin by inhibiting the enzyme cyclooxygenase in the cells of the body (Laurence, 2006) .The kidney is extremely active in the synthesis and metabolism of prostaglandins. These compounds participate in several processes in renal physiology including auto-regulation of renal blood flow, glomerular filtration, modulation of rennin release, tubular ion transport and water metabolism. It is not surprising that diminished prostaglandin synthesis may be initiating events in the patho-physiological process of diclofenac sodium induced renal dysfunction (Yasmeent *et al.,* 2007 .

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).

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 (Talwar and Srivastava,2003). Previous studies have shown that minor increase in serum creatinine can reflect a marked fall in glomerular filteration rate (Salomons *et al.,* 2003). Creatinine and blood urea is increased due to less glomerular filtration rate which is possibly impaired by diclofenac.

Goats treated with pefloxacin showed significant decrease in total erythrothytic (RBCs) count, haemoglobin (Hb) % and packed cell volume (PCV) at 3rd day and 1st week post treatment as compared to control group.

Dutta and Badhe (1999) mentioned that the rapid depression of bone marrow following use of ciprofloxacin suggests an idiosyncratic reaction. In one case, pancytopenia was observed only 2 days after use of ciprofloxacin, while in the other case, bleeding symptoms started 5 days after starting ciprofloxacin (even though the drug was stopped on the fourth day). Thus, it is possible that a single dose may cause bone marrow depression in rare instances.

Our results coincided with Amer and EL-Shaieb (1998) who recorded that intramuscular injection of rabbits with enrofloxacin at a dose level of 10 mg/kg b.wt. for five successive days induced a significant decrease in erythrocytic count, haemoglobin concentration, PCV, MCV, MCH and as compared to the control. Kar and Ghosh (2002) who stated that goats treated with consecutive graded dose levels of ciprofloxacin showed significant decrease in Hb (Haemoglobin) and PCV (Packed cell volume) as compared to the control. El-Ghoneimy *et al.*(2008) who reported that rats treated with

enrofloxacin for five days evoked a significant decrease in erythrocytic count and haemoglobin concentration (Hb%).

Goats treated with pefloxacin revealed a non significant change in total leucocytic count as compared to control group. These results are in agreement with El-Ghoneimy *et al.* (2008) who found that enrofloxacin treated rats for five successive days showed no significant changes in total leucocytic count.

Although fluoroquinolones are occasionally associated with mild, transient elevations in aminotransferase levels, serious acute liver injury is uncommon (Paterson *et al.,* 2012) .

These findings coincided with El-Ghoneimy *et al.* (2008) who found that enrofloxacin treated rats for five successive days provoked significant increase in serum ALT and AST enzymes level.

Animals treated with pefloxacin provoked a non significant change in serum total protein, albumin and globulin levels as compared with control group. These results are in accordance with Kar and Ghosh (2002) who recorded that treatment of rabbit with enrofloxacin daily for five successive days induced no significant change in plasma protein levels as compared with the control. El-Ghoneimy *et al.* (2008) who recorded that enrofloxacin treated rats for five successive days provoked non significant change in serum total protein, albumin and globulin levels as compared with control group. Shoorijeh *et al.* (2012) who reported that cats treated with enrofloxacin daily for 7 consecutive days showed no significance change in serum total protein levels as compared with the control groups.

Allergic nephropathy associated with quinolone antibiotics has been reported. The mechanism might be a hypersensitivity reaction. Norfloxacin has been incriminated as a cause of acute interstitial nephritis (AIN) as the histopathological finding. Ciprofloxacin-associated nephropathy has been reported in 28 cases, with AIN as the main histopathological finding. Clinicians should be aware of quinolone-associated AIN, which is a rare but potentially dangerous renal complication (Hadimeri *et al.,* 1997).

The increased blood urea, uric acid and creatinine levels post pefloxacin treatment was in harmony with the findings reported by Khodary and El-Sayed (1997) and El-Ghoneimy *et al.* (2008).

Finally, it could be concluded that combination of diclofenac with fluoroquinolones could be useful in correction of toxic effect due to diclofenac sodium in goat.

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