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"TOXICOLOGICAL EVALUATION FOLLOWING CONCURRENT ADMINISTRATION OF LEVOFLOXACIN AND KETOPROFEN IN SHEEP"

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Levofloxacin is third generation fluoroquinone having broad-spectrum antibacterial activity. Ketoprofen is non-steroidal anti-inflammatory drug commonly used in veterinary medicine. The present study was planned to determine blood biochemical profile following daily intravenous administration of levofloxacin (3 mg/kg) along with intramuscular administration of ketoprofen (3 mg/kg) for 5 days in sheep. Blood samples were collected daily for 5 days before and after the initiation of drugs administration and proceeded for determination of haematological (Hb, PCV, TEC, TLC and DLC) and biochemical parameters (AST, ALT, LDH, ACP, AKP, serum creatinine, serum bilirubine, BUN, serum total protein, serum albumin, serum globulin and blood glucose). Hematological and biochemical profile before and after the treatment revealed that levofloxacin did not have any significant impact on major blood parameters except BUN which are altered in disease or pathological conditions. Thus, the drug may be useful in combination of ketoprofen to treat bacterial diseases accompanied by febrile condition in sheep.

Key words: Toxicological evaluation, multiple intravenous administrations, levofloxacin, ketoprofen, sheep

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Levofloxacin (LFX) is a third-generation fluoroquinolone that has been widely used for the treatment of bacterial infections in human beings. It is active against Gram-negative organisms (including *Pseudomonas* species), Gram-positive organisms (including *Staphylococcus aureus*) and anaerobic bacteria (Davis and Bryson, 1994). The drug thus seems to be extremely useful in a variety of bacterial infections. Pharmacokinetic studies of levofloxacin have been evaluated in sheep (Patel, 2009; Patel et al., 2012[a], 2012[b]), goats (Goudah and Abo-El-Sooud, 2009) and other animals (Albarellos *et al.,* 2005, Ram *et al.,* 2008, Goudah *et al.,* 2008, Goudah, 2009).

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently recommended as an adjunct therapy with antibacterials to treat various bacterial infections accompanied by fever and other inflammatory conditions in animals. Ketoprofen is non-steroidal anti-inflammatory drug having anti-inflammatory, analgesic and anti-pyretic properties. It is widely used to lower body temperature in animals having fever, to relieve bacteremia and pain in all animals (Lees *et al*., 2004).

Safety data of levofloxacin on concomitant administration with anti-inflammatory drug like ketoprofen may clear the future of both drugs for the treatment of bacterial diseases accompanied by fever in minor animal species like sheep. Therefore, the present study was planned to evaluate blood biochemical profile following daily intravenous administration of levofloxacin (3 mg/kg) along with intramuscular administration of ketoprofen (3 mg/kg) for 5 days in sheep.

MATERIALS AND METHODS

Experimental animals and Design

The present study was conducted on six Patanwadi sheep reared at Instruction farm, Veterinary College, Anand Agricultural University, Anand, Gujarat. The animals were weighing between 23.5 and 30.0 kilograms at 2-3 years of age. They were kept under constant observation for two weeks prior to commencement of the experiment. During this period they were subjected to clinical examination in order to exclude the possibility of any disease. The animals were then housed in separate pen and were provided standard ration. Water was provided *ad libitum.* All necessary managemental procedures were adopted to keep the animals free from stress.

Animals were treated with daily intravenous administration of levofloxacin (Tavanic®, Aventis Pharmaceutical Ltd, Bangalore) through jugular vein puncture at the dose rate of 3.0 mg/kg body weight for 5 days along with intramuscular administration of ketoprofen (Neoprofen®, Vetnex Ranbaxy Fine Chemicals Limited, New Delhi, India) in deep gluteal muscle using a 20 G X 25 mm needle at the dose rate of 3 mg/kg of body weight for 5 days. Blood samples were collected before administration of the drug which served as control (day 0). After administration of drug, blood samples were collected at day 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} from jugular vein (before administration of drugs) into sterile tubes for haematological and serum biochemical analysis. Blood smear for determination of differential leukocyte count (DLC) were prepared from fresh blood at the time of blood collection (Schalm, 1967). Blood samples (3 mL) collected in test tubes with K_3EDTA were utilized for haematological evaluation, whereas blood samples (5 mL) collected in centrifuge tubes without K_3EDTA were allowed to clot at room temperature. Serum was harvested by centrifugation at 3000 rpm for 10 minutes (Eppendorf 5804 R, Germany) and analyzed for biochemical parameters.

Estimation of hematological and biochemical parameters

Haemoglobin (Hb), packed cell volume (PCV), total erythrocytes count (TEC) and total leukocyte count (TLC) were estimated using automated haematology analyzer (Medonic CA620 VET, Sweden). Differential leukocyte count (DLC) was carried out as per method described by Schalm, (1967). Serum from blood samples collected at predetermined time intervals was used to determine serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum acid phosphatase (ACP), serum alkaline phosphatase (AKP), serum lactate dehydrogenase (LDH), serum creatinine, serum bilirubin, blood urea nitrogen, total protein, albumin, globulin and blood glucose using standard assay kits (Merck specialties Ltd., Mumbai, India) with the help of clinical serum biochemistry analyser (Junior Selectra, Vital Scientific NV). Globulin level was determined by subtracting the albumin level from total protein level.

Statistical analysis

All the data have been presented as mean ± S.E. The data of hematological and biochemical parameters before and after repeated administration of the drug were analyzed using students' "t" test (Snedecor and Cochran, 1980).

RESULTS

Mean values of hematological and biochemical parameters determined before and after daily intravenous administration of levofloxacin (3 mg/kg) along with intramuscular administration of ketoprofen (3 mg/kg) for 5 days in sheep are presented in Table 1 and 2, respectively. All animals were not exhibited any local or systemic adverse reactions after daily intravenous and intramuscular administration of levofloxacin and ketoprofen, respectively in sheep. Animals did not show change in behavior and pain on palpation of joints during study period.

DISCUSSION

The mean value of Hb, PCV, TEC, TLC, and DLC estimated during treatment periods did not differ significantly ($p>0.05$) from corresponding control values (day 0). It indicates that repeated long term administrations of levofloxacin and ketoprofen in sheep were well tolerated. Mean values of biochemical parameters

except BUN following daily administrations of drugs were not altered significantly ($p<0.05$) compared to control (day 0).

Table 1. Hematological parameters (Mean ± S.E.) after daily intravenous administration of levofloxacin (3 mg/kg) along with ketoprofen administration (3 mg/kg, Intramuscular) for 5 days in sheep (n=6)

Hematological parameters	Day					
	0 (Control)	1	2	3	4	5
Haemoglobin (g/dL)	10.13 ± 0.13	10.27 ± 0.29	9.97 ± 0.21	9.88 ± 0.34	9.77 ± 0.20	10.10 ± 0.17
PCV (%)	31.67 ± 0.61	32.17 ± 0.54	31.83 ± 0.54	32.00 ± 0.73	32.83 ± 0.40	31.67 ± 0.61
TEC ($\times 10^6/\mu L$)	10.17 ± 0.57	10.50 ± 0.77	10.32 ± 0.74	10.26 ± 0.81	10.66 ± 0.61	10.53 ± 0.82
TLC ($\times 10^3$ per cmm)	8.58 ± 0.34	8.84 ± 0.42	8.78 ± 0.38	8.74 ± 0.51	9.20 ± 0.47	9.27 ± 0.38
Neutrophil (%)	38.50 ± 0.56	37.33 ± 0.56	38.17 ± 0.40	39.67 ± 0.49	37.50 ± 0.56	39.33 ± 0.84
Lymphocyte (%)	58.17 ± 0.70	59.00 ± 0.52	58.33 ± 0.42	57.00 ± 0.37	59.33 ± 0.76	58.17 ± 0.91
Basophil (%)	0.67 ± 0.33	0.83 ± 0.31	1.00 ± 0.26	1.00 ± 0.37	0.83 ± 0.31	0.67 ± 0.21
Eosinophil (%)	1.33 ± 0.33	1.50 ± 0.34	1.33 ± 0.21	1.17 ± 0.31	1.33 ± 0.33	1.00 ± 0.00
Monocyte (%)	1.33 ± 0.21	1.33 ± 0.21	1.17 ± 0.17	1.17 ± 0.17	1.00 ± 0.26	0.83 ± 0.31

All data were not significantly altered compared to control ($P> 0.05$)

Table 2. Biochemical parameters (Mean ± S.E.) after daily intravenous administration of levofloxacin (3 mg/kg) along with ketoprofen administration (3 mg/kg, Intramuscular) for 5 days in sheep (n=6)

Absence of reports of significant alteration in blood biochemical parameters in human following repeated administration of levofloxacin (Chien *et al.,* 1997) supports our findings. Similarly, Han *et al.,* (2003) have found no alteration in blood biochemistry following repeated oral administration of new fluoroquinolone, zabofloxacin at 10 mg/kg dose rate in dogs. However, Alicia et al. (2000) observed transient neurological signs and lameness in horses after oral administration of enrofloxacin at the dose rate of 15 and 25 mg/kg for 21 days, but no alterations have been found after 7 days treatment.

Significant alterations in biochemical parameters have not been observed following multiple intravenous administration of levofloxacin in sheep (Patel, 2009) which is in agreement results obtained in human (Chien *et al.,* 1997; Chow *et al.,* 2001) and poultry (Patel *et al.,* 2009) following repeated administration of levofloxacin. In the present study, the level of AST (124.32 ± 7.23 IU/L) was found higher at day 5 compared to the AST level (105.55 ± 2.74 IU/L) observed after daily intravenous administration of levofloxacin alone in sheep (Patel, 2009). Increase in total bilirubin, AST, WBC and lymphocytes have also been observed with new fluoroquinolone, fandofloxacin in rats (Kim *et al.,* 2003). AST is normally found in a diversity of tissues like liver, heart, kidney, muscle and heart. It is released into serum when any one of above mentioned tissues is damaged.

Convulsions in rats due to interaction between fenbufen and both enoxacin and ciprofloxacin have been observed (Kamali *et al.,* 1998). However details of interactions are still unclear. Hori *et al.* (2003) suggested that each quinolone has an individual drug interaction with each anti-inflammatory drug. In the present study, significant ($p<0.05$) increase in BUN level (50.38 ±

1.72 mg/dL) at day 5 has been observed compared to the BUN level (40.72 ± 2.37 mg/dL) found at day 0 (Control). However, it was observed that BUN level has not significantly altered following multiple intravenous administrations of levofloxacin in sheep (Patel, 2009).

Table 2. Biochemical parameters (Mean ± S.E.) after daily intravenous administration of levofloxacin (3 mg/kg) along with ketoprofen administration (3 mg/kg, Intramuscular) for 5 days in sheep (n=6)

Biochemical parameters	Day					
	0 (Control)	1	2	3	4	5
AST (IU/L)	110.59 ± 4.66	117.48 ± 5.02	116.23 ± 2.49	119.17 ± 3.86	124.88 ± 7.69	124.32 ± 7.23
ALT (IU/L)	30.03 ± 1.43	31.82 ± 2.01	31.98 ± 1.57	33.62 ± 2.16	34.13 ± 1.90	34.78 ± 3.21
ACP (IU/L)	1.07 ± 0.04	1.08 ± 0.05	1.18 ± 0.09	1.17 ± 0.08	1.20 ± 0.13	1.12 ± 0.05
AKP (IU/L)	222.30 ± 15.67	220.45 ± 9.34	213.27 ± 10.25	213.03 ± 9.25	203.97 ± 7.13	204.92 ± 6.51
LDH (IU/L)	330.84 ± 6.23	329.10 ± 3.49	330.49 ± 5.35	329.02 ± 5.34	334.22 ± 5.39	332.01 ± 6.66
Creatinine (mg/dl)	1.05 ± 0.05	1.04 ± 0.05	1.03 ± 0.03	1.00 ± 0.04	1.01 ± 0.04	1.02 ± 0.04
Total Bilirubin (mg/dl)	0.13 ± 0.04	0.16 ± 0.01	0.16 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.12 ± 0.01
BUN (mg/dl)	40.72 ± 2.37	43.62 ± 1.01	43.62 ± 1.14	46.45 ± 1.21	47.52 ± 2.68	50.38 ± 1.72*
Total Protein (g/dl)	6.78 ± 0.23	7.15 ± 0.20	7.05 ± 0.15	7.08 ± 0.09	6.90 ± 0.10	6.85 ± 0.12
Albumin (g/dl)	3.02 ± 0.05	3.10 ± 0.04	3.00 ± 0.04	2.92 ± 0.15	3.03 ± 0.04	3.03 ± 0.07
Globulin (g/dl)	3.77 ± 0.19	4.05 ± 0.21	4.05 ± 0.18	4.17 ± 0.21	3.87 ± 0.13	3.82 ± 0.16
Glucose (mg/dl)	42.73 ± 1.97	41.19 ± 1.08	38.51 ± 2.79	39.64 ± 1.80	45.70 ± 4.34	44.99 ± 4.65

* Significant compared to control (P< 0.05)

In addition, Gondaliya (2008) reported significant increase in BUN level following intramuscular administration of ketoprofen in sheep. Results obtained in the present study clearly indicate little alteration in patency of liver and renal function when ketoprofen is administered along with levofloxacin in sheep.

Major alterations in hematology and biochemical profile have not been observed after daily intravenous administration of levofloxacin along with intramuscular injection of ketoprofen in sheep; hence both drugs may be used for clinical use for the treatment of bacterial diseases caused by susceptible bacteria. However, liver and kidney functions should be monitored during long term use of levofloxacin and ketoprofen for the treatment of bacterial diseases accompanied with fever.

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