Effect of flunixin on the disposition kinetics of florfenicol in goats

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Abstract

The disposition kinetic of florfenicol either alone or concurrently with flunixin meglumine was studied following single intravenous and intramuscular injections in dairy goats. Five clinically healthy lactating goats were injected with florfenicol in a dose of 20 mg kg^{-1}b.wt. intravenously and intramuscularly with one month period in between. The same protocol was repeated after one month but flunixin (2.5 mg kg^{-1}b.wt.) was concurrently administered with florfenicol. Samples of blood for serum, and samples of urine, milk and ruminal juice were taken from each goat at specific time intervals. Florfenicol concentrations were determined in all samples using HPLC. Following i.v injection of florfenicol and flunixin, there was a significant increase in Cp^{O}, k_{12}, k_{21} and a significant decrease in MRT, $t_{0.5\alpha}$, Vd_{ss}, V_c, Vd_{area} and Vd_B of florfenicol compared to florfenicol when administered alone. After intramuscular injection, there was a significant increase in K_{ab}, C_{max}, AUC, MRT and F % and a significant decrease in T_{max} and t0.5ab values of florfenicol following injection of florfenicol with flunixin. Moreover, flunixin meglumine alters the protein binding tendency of florfenicol and affect its rate of excretion in urine, milk and ruminal juice. Significant variation in the disposition of florfenicol when concurrently administered with florfenicol was reported and should be considered when the two drugs are used concurrently.

Keywords: florfenicol, flunixin meglumine, HPLC, pharmacokinetic, goats

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1-Introduction

Florfenicol is a broad – spectrum antibiotic belonging to the family of agents that include thiamphenicol and chloramphenicol. The three compounds have the same antibacterial mechanism and spectrum and act by inhibiting bacterial protein synthesis by binding to 50S and 70S subunits in the ribosome. Florfenicol is proposed for treatment of bovine respiratory disease and shipping fever (Carbon et al. 1981). Florfenicol is a fluorinated derivative of thiamphenicol this may allow florfenicol to be less susceptible to deactivation by bacterial acetylation. Neither of these two compounds contains the nitro groups. Thus aplastic anemia is not associated with their administration (Sams 1995). Flunixin meglumine, a non-steroidal anti-inflammatory drug has been licensed and widely used in ruminants (Landoni et al. 1995). Florfenicol has been used with flunixin for treatment of undifferentiated fever in feedlot calves (Hannon et al. 2009, Van et al. 2009) and bovine respiratory disease in juvenile calves (Thiry et al. 2014). As a result of the presence of more than one drug in a single preparation, a pharmacokinetic interaction could occur at any step of drug disposition (absorption, distribution, metabolism, and elimination}. The consequences of pharmacokinetic interactions are either accumulation of the drug leading to toxicity or lowering of plasma concentrations resulting in reduced efficacy (Loiseau 1998; Dumka and Singh 2014). Flunixin meglumine (FLU) altered the disposition of sulfamethazine when concurrently administered in swine. It also altered the disposition of enrofloxacin when concurrently administered in dogs (Ogino et al. 2005). The objective of this study was to evaluate the pharmacokinetic properties of florfenicol when concurrently administered with flunixin meglumine in healthy lactating goats. The effect of flunixin elimination of florfenicol in urine, milk and ruminal juice is also investigated.

2-Material and methods

Florfenicol Nuflor®, Schering-plough Union, NJ, USA was obtained in the form of injectable solution 300 mg/ml. Flunixin meglumine Finadyne®, Schering-plough Union, NJ, USA was obtained in the form of injectable solution 50 mg/ml.

2.1. Animals

Five clinically healthy lactating goats of 2-2.5 years old and 20-25 kg weight each were used. Animals were kept indoors under good hygienic conditions, fed on antibiotic-free diet and water ad libitum. Thorough milking of each goat was done twice daily. This experiment was carried out according to the guidelines of the Institutional Animal Care and Use Committee, Cairo University.

2.2. Experimental design

Florfenicol was dissolved in an organic solvent dimethylformamide (DMF), a widely used solubilizing agent and injected into the right jugular vein at a dose of 20 mg kg^{-1}

b.wt. One month later; animals were injected with florfenicol in the same dose intramuscularly (IM) into the left gluteal muscle with massage.

In separate experiment and after one month, each of the five goats was injected with florfenicol 20 mg kg^{-1} b.wt. and flunixin 2.5 mg kg^{-1}b.wt. intravenous (IV) as one bolus. One month later, the same dose of florfenicol and flunixin was injected IM into the left gluteal muscle with massage

2.3. Sampling of Biological Fluids

Blood samples were collected from the left jugular vein just before and at 5, 10, 20 and 30 minutes, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72 hours after drug administration. The time intervals for sampling were the same in all experiments. Blood samples were taken in clean sterile centrifuge tubes, allowed to clot at room temperature and sera were separated by centrifugation at 3000 r.p.m. for 15 minutes and stored at -20°C until assessment .

Urine samples were collected by using rubber balloon catheters Folatex No. 14, (Sewoon Medical Co. LTD) fixed inside the bladder and allowed to flow its urine content at times of sampling. The bladder was emptied before drug administration. Urine samples were collected before and at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96 hours post-injection. Urine samples taken at 0.25 hour were discarded. The pH of each urine sample was measured directly after collection. All urine samples were stored at -20°C until used for assaying. At the end of sampling, the urinary bladder was irrigated with 10 ml of potassium permanganate 1:5000 as an antiseptic.

Milk samples were collected by hand milking. Complete evacuation of the udder was done before injection and after each sampling. Samples were taken at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96 hours following drug administration. Milk samples taken at 0.25 h were discarded.

Ruminal juice samples were obtained by using a stomach tube connected to a vacuum pump. Samples were taken before and after 1, 2, 4, 6, 8 hours post administration .

2.4. Analytical procedures:

The concentration of florfenicol in serum, urine, milk and ruminal juice was determined by using reversed phase high-performance liquid chromatography (Shimadzu modular HPLC system, Japan). Separation of the injected compound was achieved by a Novapak C18 column 10 cm x 5 mm. I.D, 10 um particle size, fitted with C18 guard column. The mobile phase used for elution of florfenicol consisted of acetonitrile: water (40:60, Vol/Vol).

2.5. Preparation of standard curves

A stock sol. was prepared by dissolving 1 mg of RM 085 florfenicol standard (Sch 24048-1 in 1 ml acetonitrile Fisher Scientific International Company, UK) to give a concentrated standard solution of 1 mg ml^{-1}. Standard concentrations of 50 to 0.025 $\mu g.ml^{-1}$ in serums, 200 to 0.1 $\mu g.ml^{-1}$ in urine, 20 to 0.1 $\mu g.ml^{-1}$ in milk and 10 to 0.1 $\mu g.ml^{-1}$ in rumen juice were prepared. Standard curves were derived by plotting florfenicol concentration versus the peak area from HPLC.

2.6. Samples preparation

Protein was precipitated from the standard and samples by addition of acetonitrile 1:1 Vol. /Vol. in a test tube. The tube was centrifuged at 3000 r.p.m. for 15 min. The supernatant was collected, filtrated through sample filter (Simple pure NY 0.45µm) and 20 µl was injected into the HPLC.

2.7. Estimation of protein binding percent of the tested drug:

After precipitating the protein bound part in serum, the free unbound drug is the part which is only measured by HPLC. Differences in the curve area between the solution of the tested antibiotic alone or combination in a buffer acetonitrile and that of serum of goats at the same concentrations were used to calculate protein binding percent of the tested drugs according to the following formula:

$$\text{Protein binding \%} = \frac{\text{AUC of the drug in the buffer} - \text{AUC of the drug in serum}}{\text{AUC of the drug in buffer}} \times 100$$

2.8. Pharmacokinetic and statistical analysis

A computerized curve - stripping program R strip (Micro Math Scientific Software, Saltlake city, UT, USA) was used for data analysis for each animal. Following IV injection, the disposition curves of the tested drugs which express the decline in serum drug concentration as a function of time was best described by two compartmental open model bio exponential expression.

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

Where Cp is the concentration of drugs in serum at time t, A and B are the intercept of the distribution and elimination phases expressed as mg.ml-1; α and β are the distribution and elimination rate constants expressed in units of reciprocal time h^{-1}. The volume of distribution was calculated mathematically (Baggot 1978).

Following intramuscular administration, the curves of the drugs vs time were analyzed to determine peak concentration C_{max}, time of peak concentration Tmax. This program also calculated non-compartmental analysis of statistical moment theory. The elimination half-life t0.5el was calculated as ln2/β. The area under the concentration-time curves AUC from zero to infinity was calculated by trapezoidal rule (Øie 1983). Mean Residence time (MRT) for florfenicol was calculated as

AUMC/ AUC where AUMC is the area under first-moment curve and AUC the area under the curve. The systemic bioavailability (F%) was calculated as $AUC_{i.m}$/ $AUC_{i.v}$ x 100 where AUC= A/ α +B/ β. The body clearance Cl_{tot} of drug was calculated as $Cl_{tot} = K_{el} X V_c = L.kg^{-1}.h^{-1}$. Results are presented as mean ± SEM. Differences between means were tested for significance by using student "t" test .

3. Results

Following IV injection of florfenicol, its serum levels decreased gradually till it reaches the minimum concentration at 12 hours post-injection. No florfenicol could be detected in serum 24 hours following IV injection of florfenicol alone or concurrently with flunixin. Higher serum concentrations of florfenicol were observed when the florfenicol was co-administered with flunixin as compared to that when florfenicol was given alone till 4 hours post-injection (Fig. 1) .

The initial serum concentration of florfenicol at time zero (Cp^o) was significantly higher when florfenicol was given with flunixin (31.3 ± 0.97 $\mu g.ml^{-1}$) as compared to that for florfenicol when given alone (25.7 ± 1.4$\mu g.ml^{-1}$). The calculated over all tissue to serum ratio k_{12}/k_{21} were 1.31 and 1.04 for both treatments, respectively indicating rapid entry to the peripheral compartment. The rate of transfer of florfenicol from peripheral to the central compartment was slower (k_{21}; 1.3 ± 0.15 h^{-1}) than that occurs when it was co-administered with flunixin (K_{21}; 2.5 ± 0.2 h^{-1}). Moreover, the MRT, $t_{0.5\alpha}$, Vd_{ss}, V_c, Vd_{area}, and Vd_B values were significantly lower when florfenicol was injected concurrently with flunixin as compared to that when florfenicol was injected alone (Table 1). Following IM injection, florfenicol was rapidly absorbed from its site of injection as it could be detected in serum 5 minutes post-injection and indicated by a short half-life $t_{0.5}$; 0.58± 0.04 h. Florfenicol reached to a maximum concentration of 2.2±0.3 and 3.8± 0.24 $\mu g.ml^{-1}$ at 1.8 ± 0.06 and 1.62 ± 0.05 hours and decreased to its lowest level at 48 hours and 24 h post-injection when given alone or concurrently with flunixin respectively (Table 2 and Fig. 2). Statistical analysis revealed a significant increase in K_{ab}, C_{max}, AUC, MRT and F % and a significant decrease in T_{max} and $t_{0.5ab}$ values following injection of florfenicol with flunixin as compared to the respective values when florfenicol was injected alone. Florfenicol was detected at high concentration in urine as compared to its concentration in serum when injected either IV or IM alone or in combination with flunixin. Following IV or IM injection of florfenicol, there was no significant difference in florfenicol urine concentration either injected alone or in combination with flunixin during the first 12 hours after injection. Florfenicol concentration in urine was higher when co-administered with flunixin starting at 12h post injection and could be detected up to 48 h in comparison to florfenicol which was detected only at 24 h post-injection when given alone (Table 3). Following IV or IM injection of florfenicol alone or in combination with flunixin it was excreted in milk at a low concentration. Significantly lower concentrations were reported when florfenicol was injected with flunixin either IV or IM as compared with those when florfenicol was given alone. The maximum concentration of florfenicol in milk was achieved 6 h post IM injection. No florfenicol was reported in milk 8 h and 12 h post IV and IM. injection, respectively (Table 4). Following IV injection of florfenicol alone or in

combination with flunixin it was excreted at a low concentration in the ruminal fluid at 1 h post-injection and disappears after 4 hours post-injection. Florfenicol concentration in ruminal juice was higher when injected concurrently with flunixin. Florfenicol was not detected in ruminal juice at all times post IM injection (Table 5(. The in vitro protein binding percent of florfenicol was 52.45 ± 5.3% of florfenicol and 36.6 ± 2.66 % of florfenicol when co-administered with flunixin (Table 6).

4. Discussion

The present results indicate that the disappearance of florfenicol from the plasma of goats after IV injection either alone or co-administered with flunixin, follows a two-compartment open model with rapid distribution and elimination half-lives. These findings are in agreement with results previously reported in calves (Varma et al. 1986; de Craene et al. 1997), goats (Atef et al. 2001) and in dogs (Birdane and Birdane 2015). However, a tri-exponential term was applied to describe the disposition of florfenicol from the serum after IV administration (Varma et al. 1986; Bretzlaff et al. 1987; Lobell et al. 1994; Soback et al. 1995). Furthermore, a non-compartmental analysis was also applied (Pentecost et al. 2013). These differences are unlikely to be of clinical importance .

The initial distribution phase of florfenicol either injected alone or concurrently with flunixin was very rapid with ($t_{0.5\alpha}$ of 0.21±0.016 and 0.13±0.012 h, respectively), indicating a rapid distribution to peripheral tissues. This rapid distribution is further substantiated by the high value of K_{12}; 1.7 ± 0.15 h^{-1} with lower value of K_{21}; 1.3 ± 0.15 h^{-1} indicating a rapid transfer of the drug between peripheral and central compartment. The elimination half-life $t_{0.5\beta}$ of florfenicol was 3.3 ± 0.32 h indicating a rapid elimination. The elimination half-life ($t_{0.5\beta}$) of florfenicol in the present study are nearly similar to other ruminants; 1.9 min in veal calves (Varma et al. 1986), 176 min in lactating cows (Soback et al. 1995) and in dogs, 185.4 min (Birdane and Birdane 2015). Lower values were reported in equines; 108 min (McKellar and Varma 1996) and rabbits 92.4 min (Abd El-Aty, Goudah et al. 2004). The present observations disagree with that reported in sheep 18.83h (Jianzhong et al. 2004). The total body clearance Cltot appeared to be relatively slow (0.38 ± 0.02 $L.kg^{-1}.h^{-1}$). However, similar values were reported in rabbits, 0.34 $L.kg^{-1}h^{-1}$ (Abd El-Aty et al. 2004) and dogs, 0.37 $L.kg^{-1}.h^{-1}$ (Birdane and Birdane 2015). Higher values were reported in veal calves 14 $L.kg^{-1}h^{-1}$ (Varma et al. 1986, Adams et al. 1987). Small Cl_{tot} value of florfenicol in animals indicates low metabolic clearance that due to the replacement of – OH in chloramphenicol and thiamphenicol by – F in florfenicol structure, thereby preventing the conjugation with glucuronic acid and delaying its excretion (Bretzlaff et al. 1987). The volume of distribution at steady state Vd_{ss} is an accurate indication for the diffusion of the drug in the body tissues (Galinsky and Svensson 1995). The pharmacokinetic interpretation of serum florfenicol concentration data revealed the high distribution of the drug in the body of goats (Atef et al. 2001). The present values of Vd_{ss} are higher than those reported in veal calves, 0.75 $L.kg^{-1}$ (Varma et al. 1986), cattle, 0.76 $L.kg^{-1}$ (Lobell et al. 1994), lactating cows, 0.35 $L.kg^{-1}$ (Soback et al. 1995), rabbits. 0.57 $L.kg^{-1}$ (Abd El-Aty et al. 2004), sheep, 1.86 $L.kg^{-1}$ (Jianzhong et al. 2004), dogs, 1.19 $L.kg^{-1}$ (Birdane and

Birdane 2015) and in llamas (Pentecost et al. 2013). Higher values were reported 4.99 $L.kg^{-1}$ in chickens (Shen et al. 2002).

The plasma florfenicol concentration vs curve obtained following IM injection emphasize that florfenicol was rapidly absorbed with C_{max} of 2.2 ± 0.3 $\mu g.ml^{-1}$ achieved early at T_{max} of 1.8 ± 0.06 h. This result was nearly similar to that previously reported in goats 2.38$\mu g.ml^{-1}$ at 1.57 h (Atef et al. 2001), dogs, 3.05 $\mu g.ml^{-1}$ (Birdane and Birdanc 2015) and in turkcy, 1.02 h (Watteyn et al. 2018) but less than thosc recorded in sheep 4.13 $\mu g.ml^{-1}$ at 1.45h (Jianzhong et al. 2004) and alpacas (4.31±3.03 $\mu g.ml^{-1}$) (Holmes et al. 2012). The MRT and the terminal half-life after IM injection (4.9 ± 0.3 h and 4.0 ± 0.31 h, respectively) were slightly higher than that after IV injection (4.2 ± 0.38 h and 3.3± 0.32 h) indicating absorption rate-dependent elimination (Abd El-Aty et al. 2004). The systemic bioavailability (F %) of florfenicol in goats after IM injection was 41.65 ± 4.15%. This value was similar to that recorded in lactating cows, 38% (Lobell et al. 1994, Soback et al. 1995) and dogs, 44.70 % (Birdane and Birdane 2015). The variability in absorption from the IM site might be due to differences in regional blood flow from different muscle tissues. It should be noted that no adverse reaction was observed at the site of administration that could alter the absorption of the drug .

The protein binding of florfenicol was 52.45 ± 5.3% which is higher than that reported either in non-lactating cows, 18%-19% (Bretzlaff et al. 1987) or in veal calves, 22% to 26% (Adams et al. 1987). This value indicates that the binding of the drug to serum proteins was moderate. It was noticed that after injection of florfenicol with flunixin, florfenicol serum concentration at all times intervals till 12-hour post-injection was higher than that of florfenicol given alone. A similar effect of flunixin meglumine with enrofloxacin was previously reported in ICR mice (Ogino and Arai 2007). The higher serum concentrations of florfenicol observed when florfenicol was given with flunixin as compared to that when florfenicol was given alone could be attributed to displacement of florfenicol from protein binding sites due to the presence of flunixin and consequently increased free florfenicol available for determination (Anton and Rodriguez 1973). A similar pattern of interaction between other anti-inflammatory drugs and antibiotics was previously suggested (Carbon et al. 1981; Dumka and Singh 2014). The increased the bioavailability (F%) of florfenicol from 41.65 % to 54.52 % 12.87% denoting a moderate absorption from the site of injection as reflected by a slight increase in the rate of absorption and short half-life of absorption

The obtained results showed that flunixin meglumine decreased the protein binding percent of florfenicol. A value of 52.45 % of florfenicol alone was bound to serum proteins, whereas 36.6 % when florfenicol was given with flunixin. This is probably by displacement of flrofenicol to the protein sites since flunixin exhibits a high

degree of plasma protein binding (approximately 99%) (Odensvik and Johansson 1995). The decreased protein binding of florfenicol, when combined with flunixin could explain the high concentration of free flunixin in plasma and consequently higher AUC and extended MRT following IM injection. Florfenicol continues to be excreted in urine up to 24 hours post IV injection of florfenicol alone. It has been reported that approximately 50% of a 22 $mg.kg^{-1}$ intravenous dose is eliminated unchanged in the urine within 30 hours (Varma, Adams et al. 1986). Relatively high concentrations were found in urine of calves (Adams et al. 1987).

Florfenicol concentration in urine when co-administered with flunixin was higher than its concentration when injected alone at least starting from 8 hours and up to 48 hours. This could be attributed to the reported decreased protein binding percent that allows free drug to be excreted in urine. On the other hand florfenicol has a pka 10.7 while flunixin is a weak acid (pKa 5.82) (Johansson and Anlér 1988) which facilitates the urinary excretion of florfenicol on the bases of decreased back diffusion from renal tubules into the blood. This means that, in the treatment of urinary tract infections, co-administration of flunixin with florfenicol has the advantage of extended excretion of florfenicol at concentrations higher than the MIC for susceptible pathogens in goats (0.5 $\mu g.ml^{-1}$) (Luthman and Jacobsson 1982) .

The present findings revealed that florfenicol concentrations in milk were much higher than those when injected concurrently with flunixin either injected IV or IM. In this respect, our results regarding the level of florfenicol in milk following its injection alone was inconsistent with those obtained by (Soback et al. 1995). The high excretion of florfenicol in milk could be explained on the basis of its high degree of ionization in milk (PKa value; 10.73) with consequent less back diffusion to blood pH 7.3. In addition, the high lipophilicity of florfenicol help its diffusability through lipid layers of blood-milk barriers and its passage is mainly through large pores. This explanation was previously confirmed by (Ziv and Sulman 1974) in cows and ewes. The maximum concentration of florfenicol in milk after IV (11.5 ± 0.178 $\mu g.ml^{-1}$) was achieved after 30 min. similar concentration (13.2 ± 1.9 $\mu g.ml^{-1}$) was previously reported in lactating goats but achieved after 1h (Lavy et al. 1991). The maximum concentration of florfenicol in milk after IM (2.24 ± 0.19 $\mu g.ml^{-1}$) was achieved after 2 h. This was nearly twice its concentration (1.7 ± 0.4 $\mu g.ml^{-1}$) in cows after subcutaneous administration and but it was achieved after longer T_{max} (12h) (Kawalek et al. 2016), probably because of difference of the route of administration.

Florfenicol concentration in milk following its injection combined with flunixin was significantly lower than that following its injection alone. This finding is confirmed by the lower volume of distribution of florfenicol in its combined form with flunixin observed in the present study. A similar lower volume of distribution of sulphadimidine, when combined with flunixin meglumine (el-Banna 1999) and of

various antibiotics when combined with other anti-inflammatory drugs, were reported (Firth et al. 1990). The harmful residue of the florfenicol eliminated in milk is completely cleared 10 hours post-injection of this antibiotic. However, florfenicol concentration in urine after IV was higher than its concentration when co-administered with flunixin than when injected alone after the first eight hours. This makes co-administration of flunixin with florfenicol is significantly superior to florfenicol. Similar observation was recorded in the treatment of bovine respiratory tract infection (Thiry et al. 2014). The ruminal juice concentration of florfenicol following IV injection of florfenicol alone or its combination was much low and could not be traced after IM injection. This finding could be explained similarly with milk depending on the acidic pH of ruminal juice with subsequent increase back diffusion of the drug. The low concentration of florfenicol in the ruminal fluid is advantageous that it is likely to adversely affect the ruminal microflora. In conclusion: Significant alterations in the pharmacokinetic disposition of florfenicol in goats were reported when concurrently administered with flunixin.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Kinetic parameters of florfenicol (20 mg. kg.$^{-1}$ b.wt.) either alone or concurrently with flunixin meglumine following IV injection in goats (Mean ± SE, n=5).

Parameters	Units	Florfenicol	Florfenicol + flunixin
Cp°	µg.ml^{1-}	25.7 ± 1.4	31.3 ± 0.97**
A	µg.ml^{1-}	18.06 ± 0.6	16.98 ± 0.73
ά	h^{1-}	3.33± 0.29	5.33± .53**
$t_{0.5ά}$	h	0.21±0.016	0.13±0.012**
B	µg.ml^{1-}	8.6 ± 0.57	14.37±0.89***
β	h^{1-}	0.22±0.017	0.28 ± 0.02*
$t_{0.5β}$	h	3.3 ± 0.32	2.49 ± 0.13*
K_{12}	h^{1-}	1.7 ± 0.15	2.6 ± 0.29*
K_{21}	h^{1-}	1.3 ± 0.15	2.5 ± 0.2**
K_{el}	h^{1-}	0.57±0.056	0.59 ± 0.047
$K_{12/21}$	Ratio	1.31	1.04
Vc	L.kg^{1-}	0.79±0.044	0.64 ± 0.02*
Vd_{area}	L.kg^{-1}	2.06±0.093	1.3 ± .072***
Vd_{ss}	L.kg^{1-}	2.22 ± 0.09	1.97 ± 0.06*
Vd_{B}	L.kg^{1-}	2.5 ± 0.07	1.4 ± 0.08***
CL_{tot}	L.kg^{1-}.h^{1-}	0.38 ± 0.02	0.35 ± 0.03
AUC	µg.ml^{1-}.h^{1-}	47.5 ± 2.64	51.38 ± 3.5
AUMC	µg.ml^{1-}.h^{2-}	207± 31.9	169.14 ± 15.93
MRT	h	4.28± 0.38	3.37 ± 0.21*

* P < 0.05, * * P < 0.01, *** P < 0.001

Table 2. Kinetic parameters of florfenicol (20 mg. kg.$^{-1}$ b.wt) either alone or concurrently with flunixin meglumine following IM injection in goats (Mean ± SE, n=5).

Parameters	Units	Florfenicol	Florfenicol + Flunixin
A	g.ml^{-1}	4.1± 0.8	6.0 ± 0.45*
K_{ab}	h $^{-1}$	1.2± 0.04	1.46 ± 0.01*
$t_{0.5ab}$	h	0.58± 0.04	0.47 ± 0.03*
B	g.ml^{-1}	4.0± 0.8	6.0 ± 0.32*
K_{el}	h^{1-}	0.2 ± 0.02	0.23 ± 0.02
$t_{0.5kel}$	h	4.0± 0.3	3.51 ± 0.28
C_{max}	g.ml^{-1}	2.2± 0.3	3.8 ± 0.24**
T_{max}	h	1.8 ± 0.06	1.62 ± 0.05*
MRT	h	4.9 ± 0.31	5.75 ± 0.16*
AUC	g.ml.h $^{-1}$	18.8± 0.75	26.34 ± 1.25***
F	%	41.65± 4.15	54.52 ± 3.83 **

* P < 0.05, * * P < 0.01, *** P < 0.001

Table 3. Florfenicol concentrations (µg.ml-1) in urine following a single IV and IM injection of florfenicol (20 mg. kg.-¹ b.wt) either alone or concurrently with flunixin meglumine in goats (Mean ± SE, n=5).

Time	IV		IM	
	Florfenicol	Florfenicol + flunixin	Florfenicol	Florfenicol + flunixin
Pre-injection	00	00	00	00
30 min	153.89 ± 6.72	136.55 ± 5.42	31.62 ± 1.75	26.63 ± 4.15
1 h	124.34 ± 6.39	112.74 ± 4.06	52.56 ± 1.0	48.9 ± 3.9
2 h	107.72 ± 7.83	96.58 ± 5.53	77.24 ± 1.71	80.86 ± 4.9
4 h	90.95 ± 7.82	76.8 ± 1.6	56.5 ± 2.24	52.3 ± 1.7
6 h	77.44 ± 6.88	61.98 ± 6.9	36.2 ± 2.7	39.88 ± 1.56
8 h	47.64 ± 6.19	51.51 ± 6.1	26.36 ± 2.7	26.35 ± 2.5
10 h	23.23 ± 3.29	30.58 ± 2.88	20.36 ± 2.8	21.57 ± 4.2
12 h	12.45 ± 0.88	22.84±2.64**	11.76 ± 1.23	14.0 ± 2.6
24 h	0.64 ± 0.03	3.9 ± 0.54***	3.34 ± 0.74	5.61 ± 0.25*
48 h	00	1.53 ±0.65***	00	2.04 ±0.32*
72 h	00	00	00	00

* P < 0.05, * * P < 0.01, *** P < 0.001

Table 4. Concentrations of florfenicol (µg.ml^{-1}) in milk following a single IV and IM injection of florfenicol (20 mg. kg.$^{-1}$ b.wt) either alone or concurrently with flunixin meglumine in goats (Mean ± SE, n=5).

Time of sampling	IV		IM	
	Florfenicol	Florfenicol + flunixin	Florfenicol	Florfenicol + flunixin
Pre-injection	00	00	00	00
30 min	11.5 ± 0.78	7.88 ± 0.13**	0.55 ± 0.08	0.13 ± 0.03***
1 h	7.33 ± 0.64	1.77 ± 0.4***	1.16 ± 0.09	0.25± 0.05***
2 h	3.87 ± 0.48	1.26 ± 0.07**	2.24 ± 0.19	0.54 ±0.07***
4 h	0.73 ± 0.11	0.41 ±0.04*	1.67 ± 0.06	1.12 ±0.08***
6 h	0.16 ± 0.05	0.11 ±0 .01*	1.34 ± 0.12	1.45 ± 0.13
8 h	00	00	0.9 ± 0.09	1.1 ± 0.09
10 h	00	00	0.29 ± 0.03	0.39 ± 0.05
12 h	00	00	00	00

* P < 0.05, * * P < 0.01, *** P < 0.001

Table 5. Concentrations of florfenicol ($\mu g.ml^{-1}$) in ruminal fluids following a single IV and IM injection of florfenicol (20 mg. kg^{-1} b.wt) either alone or concurrently with flunixin meglumine in goats (Mean ± S.E, n=5).

Time of sampling	IV		IM	
	Florfenicol	Florfenicol + flunixin	Florfenicol	Florfenicol + flunixin
30 min	00	00	00	00
1 h	0.63 ± 0.05	1.43 ± 0.07***	00	00
2 h	1.14 ± 0.12	1.58 ± 0.09**	00	00
4 h	00	00	00	00

* P < 0.05, * * P < 0.01, *** P < 0.001

Table 6. *In-vitro* protein binding percent of florfenicol and florfenicol/flunixin in normal goat's serum.

Standard concentration µg/ml	Average protein binding % of florfenicol	Average protein binding %of florfenicol in combination
10.0	36.0	35.0
5.0	54.0	40.0
2.5	53.0	45.0
1.0	66.8	30.0
0.5	52.25	33.0
Mean	52.45 ± 5.3	36.6 ± 2.66*

* P < 0.05

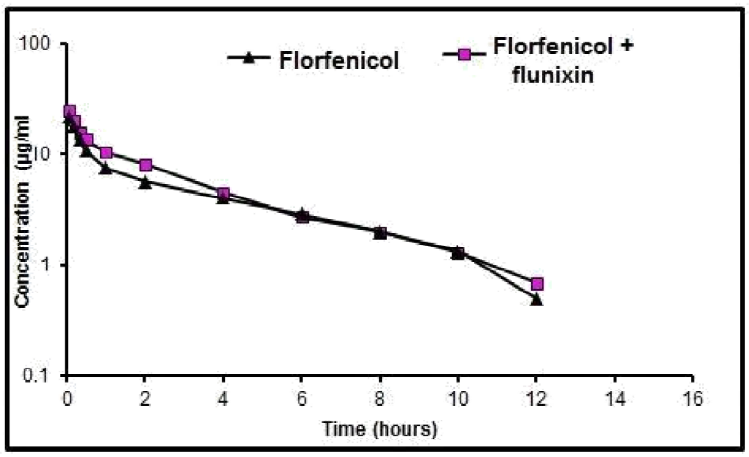

Figure 1: Semi logarithmic graph depicting the time concentration course of florfenicol (20 mg. kg^{-1}b.wt) flunixin meglumine (2.5 mg. kg^{-1}b.wt) combination in serum of goats after a single intravenous injection.

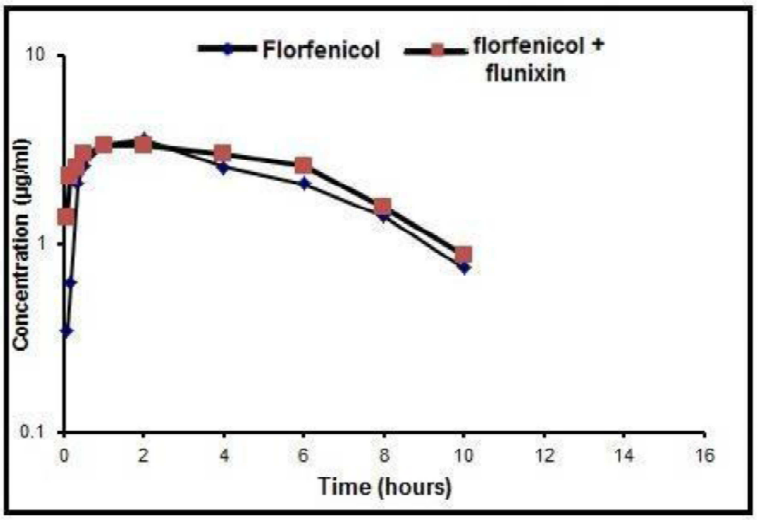

Figure 2: Semi logarithmic graph depicting the time concentration course of florfenicol (20 mg. kg^{-1}b.wt) flunixin meglumine (2.5 mg. kg^{-1}b.wt) combination in serum of goats after a single intramuscular injection.