



Hematological and Biochemical Changes Following Double Dose Administration of Florfenicol in Goats

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Abstract | Florfenicol is a broad spectrum antibiotic used clinically in treatment of many diseases in domesticated animals. This study was conducted to investigate the effect of Florfenicol (20mg/Kg) injected twice intramuscularly with 48 hr. dosing interval on hematological and biochemical parameters in 5 baladi goats. The results revealed significant increase in total leukocytic count on 1st (P< 0.01), 7th and 14th (P<0.05) days post administration of the second dose. Lymphocytes and monocytes displayed significant increase on 1st (P<0.05) and 7th (P< 0.01) days post administration. No significant differences were observed between RBCs, PCV, Hb, MCH and MCHC values pre and post administration of florfenicol. Total protein and globulin serum levels significantly increased (P< 0.001) on 1st day post administration of the second dose. AST serum levels displayed significant increase (P<0.05) together with ALT serum level (P< 0.001) on 1st day post administration of Florfenicol. Bilirubin, G-GT, ALP, urea and uric acids showed non significant changes. creatinine serum level significantly increased (P< 0.001) on 7th day post administration of Florenicol. It is concluded that Florfenicol enhanced total leukocytes specially lymphocytes and monocytes while it had mild transient effects on liver and kidney functions.

Keywords | Florfenicol, Hematology, Biochemical, Immunity, Goats

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INTRODUCTION

Florfenicol is a broad spectrum antibiotic belonging to amphenicols, the family of agents that includes also thiamphenicol and chloramphenicol. Florfenicol is a C-3 fluorinated derivative of chloramphenicol so it is not susceptible to deactivation by acetyl transferase secreted by resistant bacteria which targets C-3 hydroxyl group in thiamphenicol and chloramphenicol (Sams, 1994; Kobal, 2004). Like other drugs in its class Florfenicol acts by inhibiting of protein synthesis by binding 50s ribosomal subunits of susceptible bacteria (Cannon *et al.*, 1990). Florfenicol showed high in vitro efficiency against Gram positive, Gram negative bacteria and many chloramphenicol resistant organisms like *Klebsiella*

pneumoniae, *E. coli* and *Salmonella typhi* (Lobell *et al.*, 1994).

One of the main structural modifications between Florfenicol and chloramphenicol is the substitution of the nitro group located in the chloramphenicol aromatic ring with sulfomethyl group. Such modification is related to the safety of Florfenicol and prevention of induced, non-dose related irreversible aplastic anemia associated with chloramphenicol. (Neu and Fu, 1980; Skolimowski *et al.*, 1983; Almajano *et al.*, 1998).

Florfenicol is clinically effective in treatment of many diseases in domesticated animals as bovine respiratory disease (Lockwood *et al.*, 1994; Haas *et al.*, 1995;

Quintavalla et al., 1997), pododermatitis in cows (Cosgrove, 1999), foot rot in ewes (Vandyke et al., 1999), Infectious bovine Keratoconjunctivitis (Angelos et al., 2000; Gokce et al., 2002), Puerperal uterine infection in dairy cow (Fernandes et al., 2000).

There are limited reports and observations of florfenicol use in goats (Wang et al., 2011) so; this study presents a complementary investigation of Florfenicol effects on hematological and biochemical parameters in this species.

MATERIALS AND METHODS

Drug and chemicals: Florfenicol (Nuflor)[®] was purchased from Merck animal health, USA. All Reagents used for in vitro analysis of liver and kidney function parameters were provided from Spinreact, Spain.

Animals: This study was conducted on Five Clinically healthy non lactating baladi goats weighting 13-16 Kg and aged 15-18 months obtained from Zagazig animal Market, Egypt. Goats were housed in hygienic stable and fed balanced commercial ration free from any medication for 30 days prior to study with free access to water. Clinical examination of goats regarding temperature, respiratory rate, heart rate, feed consumption and fecal consistency was performed once daily. The study was approved by the Ethical Committee for care and use of animals at Faculty of Veterinary Medicine, Zagazig University, Egypt.

Experimental design: Each goat was injected with florfenicol (20 mg/kg body weight) twice with a dosing interval 48 hour intramuscularly in the thigh according to the instruction of the manufacturer (Elitok et al., 2015). Two blood samples (each of 3 ml) were collected at the same time from jugular vein of each goat just before administration of the first dose of florfenicol and on the 1st, 7th and 14th days post administration of the second dose. The first sample was collected on heparinized tube for hematological studies while the second sample was collected on clean test tube without anticoagulant, left to clot and centrifuged for 3000 rpm/ 20 minutes. The obtained non hemolyzed sera were transferred into eppendorf tubes and kept at -20 C were analyzed after 24 hours of collection for estimation of the biochemical parameters.

HEMATOLOGICAL EXAMINATION

Erythrocytes (RBCs), packed cell volume (PCV), hemoglobin (Hb), Mean Corpuscular hemoglobin (MCH), Mean Corpuscular hemoglobin Concentration (MCHC) total and differential leukocytic count (TLC and DLC) were determined using an automatic cell counter (Hospitex Diagnostics Hemoscreen 18, Italy). RBCs and TLC were confirmed using the Improved Neubauer haemocytometer method.

SERUM BIOCHEMICAL ANALYSIS

Serum aspartate aminotransferase (AST), Alanine aminotransferase (ALT) (Murray, 1984), Total protein (Koller, 1984), Albumin (Webster, 1974), bilirubin (Levitt, 2014), gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP), Creatinine (Young, 1995), Urea (Kroll, 1999) and Uric acid (Schultz, 1984) were estimated. The serum globulin level was computed by subtracting the albumin from the total protein.

STATISTICAL ANALYSIS

Different variables were analyzed using Student's (t) test. All values were presented as means (\pm) standard error (SE) (Tamhane and Dunlop, 2000).

RESULTS

HEMATOLOGICAL CHANGES

The current investigation revealed that administration of florfenicol (20mg/kg) in baladi goats produced significant increase in total leukocytic count (TLC) on 1st (P< 0.01), 7th and 14th (P<0.05) days post administration of the second dose. Lymphocytes and monocytes MID displayed significant increase on 1st (P<0.05), 7th (P< 0.01) days post administration. Granulocytes showed non significant increase as shown in Table 1.

No significant differences were observed between RBCs, PCV, Hb, MCH and MCHC values pre and post administration of florfenicol during the experimental period as presented in Tables 1 and 2.

SERUM BIOCHEMICAL CHANGES

It has been found that administration of florenicol (20mg/kg) in goats produced significant increase in total protein serum level (P< 0.001) and significant increase in globulin serum level (P< 0.001) as compared to the control samples, significant decrease in A/G ratio with non significant changes in albumin serum level on 1st day post administration of the second dose. AST serum levels displayed significant increase (P<0.05) together with ALT serum level (P< 0.001) on 1st day post administration of the second dose. Total, direct and indirect bilirubin serum level showed non significant increase during the experimental period. Non-significant changes in G-GT and ALP were observed as shown in Table 3.

Administration of double dose of florenicol (20mg/kg) in goats produced non-significant changes in urea and uric acids during the experimental period with significant increase in creatinine serum level (P< 0.001) on 7th day post administration of the second dose as shown in Table 4.

Table 1: The effect of I/M administration of florfenicol double dose (20 mg/Kg. BW) on some hematological parameters (Total and differential Leukocytic counts) (Mean ±SE).

Parameters	Control	1 st day	7 th day	14 th day
TLC (x 10 ³ /μl)	13.9±0.39	18.78±0.92**	17.4±1.36*	17.12±0.89*
LYM (x 10 ³ /μl)	6.74±0.55	9.55±1.08*	8.76±0.11**	8.59±0.61
MID (x 10 ³ /μl)	0.95±0.06	1.27±0.09*	1.63±0.19**	1.29±0.22
GRA (x 10 ³ /μl)	6.21±0.96	7.97±1.74	6.99±1.2	7.24±0.89

TLC, total leukocytic count; LYM, lymphocytes; GRA, neutrophil, eosinophil and basophil; MID, monocytes and some eosinophil. *significantly different compared with control at probability: * P<0.05 ** P< 0.01 *** P< 0.001

Table 2: the effect of I/M administration of florfenicol double dose (20 mg/Kg. BW) on some hematological parameters (RBCs, PCV, Hb, RBCs indices) (Mean ±SE).

Parameters	control	1 st day	7 th day	14 th day
RBCs (10 ⁶ / μl)	16.79±0.65	16.42±0.70	17.61±0.83	17.38±0.39
PCV %	41.33±0.93	38.67±1.23	39.00±0.77	42.00± 0.89
Hb (gm/dl)	11.13±0.10	10.73±0.29	10.60±0.24	11.2± 0.15
MCV fl	24.80±1.46	23.64±0.92	22.79±1.46	24.19±0.57
MCH Pg	6.67±0.30	6.57±0.25	6.19±0.39	6.45±0.09
MCHC (%)	26.97±0.48	27.77±0.13	27.18±0.13	26.68 ±0.32

RBC total erythrocytic count; PCV packed cell volume; Hb hemoglobin; MCV mean corpuscular volume; MCH mean corpuscular hemoglobin; MCHC mean corpuscular hemoglobin concentration. *significantly different compared with control at probability: * P<0.05 ** P< 0.01 *** P< 0.001

Table 3: The effect of I/M administration of florfenicol double dose (20 mg/Kg. BW) on some biochemical parameters of liver function in goats. (Mean ±SE).

Parameters	Control	1 st day	7 th day	14 th day
Total bilirubin (mg/dl)	0.24±0.05	0.38±0.01	0.18±0.02	0.50±0.15
Direct bilirubin (mg/dl)	0.19±0.04	0.26±0.04	0.13±0.01	0.30±0.08
Indirect Bilirubin (mg/dl)	0.05±0.02	0.12±0.03	0.05±0.01	0.2±0.07
ALT (U/L)	20±1.34	36.33±2.1***	18.67±2.62	15±1.61
AST (U/L)	90.33±2.7	203.67±34.8*	82±7.64	76.6±2.11
GGT(U/L)	23.33±4.93	24±3.9	25.33±4.69	15.67±5.16
ALP (U/L)	243.67±16.17	187±12.03	211.67±13.49	186.33±13.06
Total protein (gm/dl)	6.2±0.16	6.9±0.08***	6.13±0.09	5.97±0.03
Albumin (gm/dl)	3.23±0.05	3.23±0.02	3.3±0.08	3.20±0.04
Globulin (gm/dl)	2.97±0.11	3.67±0.09***	2.83±0.02	2.77±0.05
A/G ratio	1.07±0.02	0.87±0.02***	1.17±0.02	1.16±0.04

ALT, Alanine aminotransferase ; AST, Aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, Alkaline phosphatase. *significantly different compared with control at probability: * P<0.05 ** P< 0.01 *** P< 0.001

Table 4: The effect of I/M administration of florfenicol double dose (20 mg/Kg. BW) on some biochemical parameters of kidney function in goats. (Mean ±SE).

Parameters	Control	1 st day	7 th day	14 th day
Urea (mg/dL)	35.17±5.74	25.5±3.09	30.97±1.21	39.4±2.6
Creatinine (mg/dL)	0.73±0.016	0.74±0.4	0.84±0.02***	0.77±0.01
Uric acid (mg/dL)	0.06±0.02	0.06±0.0	0.07 ±0.02	0.06 ±0.05

*significantly different compared with control at probability: * P<0.05 ** P< 0.01 *** P< 0.001

DISCUSSION

Clinically, no adverse effects were observed following

administration of double dose florfenicol in goats which remained in good health throughout the study period. This finding was consistent with other studies referred to safety

of florfenicol in cattles (Almajano et al., 1998), camels, sheep and goats receiving florfenicol (Ali et al., 2003). On contrary local inflammatory signs at site of injection, soft feces, decreased food and water intake were observed in goats and were attributed to high dose and frequency of administration (Shah et al., 2016).

The most significant hematological changes observed in the present study is a significant increase of total leukocytes, lymphocytes and MID with non significant increase in granulocytes. These findings may be correlated to our obtained results regarding significant increase of both plasma total protein and globulin level. The number of circulating lymphocytes may be reflected on serum globulin concentrations due to Immunoglobulin production (Shenton and Rebelatto, 2015). Several studies recorded different effects of florfenicol on cellular and humoral immunity (Breitzlaff et al., 1987; Khalifeh et al., 2009; Hassanin et al., 2014; Shiry et al., 2019). The increase in the percentage of thymocytes and absolute count of T lymphocytes in mesenteric lymph nodes were observed in non-immunized mice following oral administration of florenicol for 6 days with non-significant change in the number of total leukocytes and lymphocytes in blood (Lis et al., 2011). The lysozyme serum level increased as a result of increasing the number of granulocytes and monocytes (Kobayashi et al., 2003) So, significant increasing of those cells reported in the current work are fit with the elevated serum level of lysozyme and respiratory burst activity of phagocytic cells reported in rainbow trout fish following administration of florfenicol (Shiry et al., 2019). On contrary Florfenicol had suppressive effect on the leukocytic, lymphocytic and granulocytic counts in buffalo calves (Khodary and Risk, 2000), pigslets (Hu et al., 2014), goats (Shah et al., 2016) and it had no significant effect on differential leukocytic counts in horse (Mckellar and Varma, 1996) and trout (Lundén et al., 1999).

Non significant changes in RBCS, MCH and MCHC displayed in the current investigation are consistent with those previously reported for florenicol in horse (Mckellar and Varma, 1996) and goats (Shah et al., 2016). The safety of florenicol could be attributed to the presence of sulfomethyl group instead of nitro group responsible for aplastic anemia. Additionally, florfenicol maintained the normal level of GSH which played an important role in protection of RBCs from free radicals and oxidant agents while GSH level in RBCs is decreased following administration of chloramphenicol (Karadenizi et al., 2007). On contrary normocytic normochromic anemia was reported in buffalo calves (Khodary and Risk, 2000).

Biochemical investigation revealed transient significant increase in AST and ALT which is similar to the results obtained in trout (Er and Dik, 2014; Shiry et al., 2019).

The elevation of serum level of AST was mainly due damage in heart and lung tissue. However, ALT activity is more specific and more index for liver (Kaneko, 1980). Such increase in those tissue biomarkers level may be explained by the pharmacokinetic character and extensive tissue distribution ability of florfenicol as it reaches high concentrations in lung, muscle, heart and liver (Adams et al., 1987; Lobell et al., 1994; Afifi and El-Sooud, 1997; Ueda et al., 1995) which supposed to cause mild cellular injury and leakage of such indicative enzymes into the blood (Amacher, 1998). GGT and ALP displayed non-significant changes confirmed by the previous studies (Shah et al., 2016; Shiry et al., 2019).

The plasma globulin concentration represents many different proteins synthesized in liver, some of these proteins are acute phase protein acting as acute-phase reactants which rapidly and markedly increases after tissue injury and can contribute substantially to an increased total globulin concentration (Brito Galvao and Center, 2012). This may give an explanation for the significant increase in globulin and subsequently total protein observed in the current study. On contrary total protein, globulin displayed no change in trout (Er and Dik, 2014), horse (Mckellar and Varma, 1996), camel, goat and sheep (Ali et al., 2003).

The non significant increase of total bilirubin reported in the current study go in agreement with that observed in horse (Mckellar and Varma, 1996) and goats (Shah et al., 2016) and may be reflected to the temporary impairment of bilirubin conjugation with glucuronic acid in liver, subsequently increase level of unconjugated bilirubin instead of water soluble bilirubin diglucuronide.

Regarding the effect of florfenicol on kidney functions, the transient elevation of creatinine serum level observed in our study may be attributed to the excretion of about 64% of florfenicol in urine as a parent drug (Sams, 1994). Moreover, the highest concentration of florfenicol was reported in kidney (Adams et al., 1987; Afifi and El Sooud, 1997) which may be related to mild degenerative changes in kidney tubules affecting its ability for excretion of creatinine. Other kidney function indices urea and uric acids remained at normal levels which were matched that reported in horse, (Mckellar and Varma, 1996) and goats (Shah et al., 2016) received therapeutic dose of florfenicol.

Although the mild reversible effect of florfenicol on liver and kidney functions, it is of clinical importance to mention that the transient and statistically significant changes of biochemical parameters of liver and kidney functions obtained in this study were within reference ranges that documented previously in goats (Aiello et al., 2016).

It is concluded that therapeutic dose of florfenicol enhanced total leukocytes especially lymphocytes and monocytes in baladi goats and more studies may be needed for further investigation of its effect on immunity in goats.

AUTHORS CONTRIBUTION

All authors contributed equally .

CONFLICT OF INTEREST

There is no conflict of interest related to this publication.

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