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# Research Article
# Evaluation of Therapeutic and High Doses of Florfenicol on Some Hematological Indexes in Goat

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## Abstract

**Objective:** The aim of current study was to evaluate the therapeutic and high doses of florfenicol (FFC) on hematological values in goats. **Methodology:** Goats were intramuscularly induced with therapeutic (20 mg kg $^{-1}$ b.wt.) and high doses (40 and 60 mg kg $^{-1}$ b.wt.) of florfenicol for 3 days with 24 h interval. The blood samples were drawn at different timings from 0-120 h after drug administration and were assayed for the selected hematological parameters. **Results:** The therapeutic dose of FFC produced no effect on erythrocytes, hemoglobin, packed cell volume and leukocytes indices at all timing points, whereas decreased the lymphocyte count ($p<0.05$) at 24 h. The high dosages of FFC produced significant effect on erythrocytes, hemoglobin, packed cell volume, leukocytes and lymphocyte indices. In comparison with control, erythrocytic count and hemoglobin level ($p<0.05$) decreased from 48-72 h. Packed cell volume was observed to be reduced ($p<0.05$ and $p<0.01$) for 72 h. The leukocytic count ($p<0.05$) diminished from 24-72 h. The lymphocytic count decreased ($p<0.05$ and $p<0.01$) for 96 h. Clinically, local discomfort, reactions and pain at site of injection and some digestion disturbance were also noticed with high doses of florfenicol during experiment. **Conclusion:** It was concluded that both the therapeutic and high doses of florfenicol significantly affects the lymphocytes. The high doses of FFC produced anemic and immunosuppressive effects of drug.

Key words: Antibiotic, florfenicol, high dose, therapeutic dose, hematologic, immunosuppression, anemic, goat

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Chloramphenicol is broad spectrum antimicrobial drug, was used to treating the variety of infectious diseases. Due to its toxic effects on various biological systems importantly on hematopoietic and immune systems, it is eradicated or limited only for certain specific bacterial diseases in veterinary clinical practices[1]. It was a critical necessity to discover some new analogs which would have the same effectiveness in treatment but have less toxicity. Florfenicol (FFC) is now used in veterinary practice, which is safer and has less toxic profile[2] and also extensively used in aquaculture. The FFC, a synthetic fluorinated derivative of chloramphenicol and thiamphenicol, possess the same qualities of the parent substance but is less accountable to produce severe adverse effects[3]. Because of that it seems to be the right drug to substitute chloramphenicol. Due to reduced metabolism of FFC in the body and for that reason different high doses of FFC were investigated both at laboratory and clinical levels to combat the infections[1]. The recommended therapeutic dose (20 mg) of FFC practiced to treat the infections[4,5] and recent study confirmed that some bacterial species are susceptible with FFC[6]. But useful constant serum and tissue concentration of drug with therapeutic dose not achieved for long period. For that reason, high doses of FFC were still under investigation to combat the infections. Recently studies confirmed about high doses of FFC regarding to dose dependent reversible side effects (anemia, immunosuppressive, leukocytopenia and thrombocytopenia) in diverse species such as in human[7], fish[8], camel[9], alpaca[10], pig[11], mice[12] and in chicken[13,14]. To avoid unfavorable effects during therapy with high doses, laboratorial assessment of hematology should be executed to achieve clinically useful constant serum and tissue concentration for long period. In our environment such study has not been investigated before in farm animals especially in goats. Goat being important local species has been selected for current study. There is little information regarding various high doses of FFC with their effect on hematological profile in small ruminants such as goat. This prompted us for investigating high doses in goat. The current study was designed to evaluate the effects therapeutic and high doses of FFC on some hematological indices in goats. This study can be helpful for veterinary practitioners for safely effective treatment in goats considering proper dosing management.

## MATERIALS AND METHODS

Six adult healthy goats (mix breed and sex) with average weight of 25 kg were selected. Animals were managed at livestock experimental farm, Sindh Agriculture University, Tando Jam Pakistan. Goats were ear tagged with appropriate numbering from 1-6 for identification mark. Green fodder, concentrates and water were given *ad libidium*. All animals were permitted for acclimatization upto 21 days proceeding to experiment during this, animals were dewormetized. Firstly control base line values of hematological parameters established. The blood samples were collected aseptically from jugular vein. The heparinized test tubes were arranged for whole blood. The blood samples were brought to the Post Graduate Laboratory of Department of Physiology/Pharmacology, Sindh Agriculture University Tandojam Pakistan for investigation of hematological values. Hemoglobin (Hb) level was done by acid-hematin method, erythrocytes (Red blood cells, RBC's) and leukocytes (White blood cells, WBC's) quantified via haemocytometer method, Packed Cell Volume (PCV) measured via microhaematocrit method and lymphocytes through Differential Leukocyte Count (DLC).

**Drug administration and sample collection:** FFC (Naflor©) purchased from Nawan laboratory (Karachi, Pakistan) was administered intramuscularly with different dosage regimes. During phase-I, FFC therapeutic dose of 20 mg $kg^{-1}$ b.wt., was administered. Later in phase-II 40 mg $kg^{-1}$ dose and finally in phase-III 60 mg $kg^{-1}$ dose administered. Each dose was administered for 3 days with intervals of 24 h.

Blood samples were collected at 0, 24, 48, 72, 96 and 120 h post dosage regime of florfenical induction in each consecutive phase. About 0 indicated as Control (C) treatment without induction of drug. The crossover design with 21 days washout period amongst the treatments. Clinically, sign and symptom during experiment was also observed.

**Data evaluation:** Data obtained were presented as Means±SEM and analyzed through one way ANOVA employing student edition statistical software and least significant difference LSD was applied to specify significant difference in means between florfenicol treated doses and control base line values used at different timings. The significance level was set at $p<0.05$.

## RESULTS

Mean values of erthrocytic count after administration of different doses of FFC at different timings shown (Fig. 1). In comparison with control, therapeutic dose (20 mg $kg^{-1}$ b.wt.) non-significantly altered in erythrocytic count. However, both high doses significantly ($p<0.05$) decrease in erythrocytic count at 48 and 72 h.

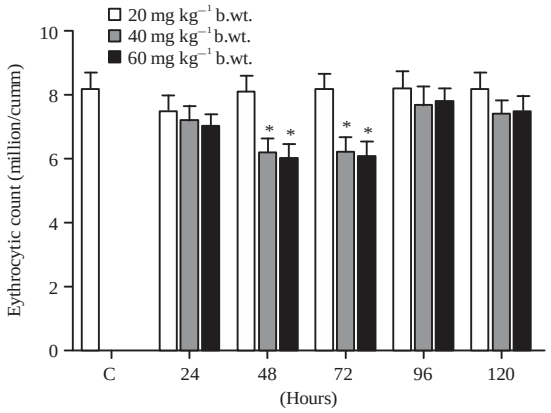


Fig. 1: Mean erthrocytic count (million/cumm) of 6 goats obtained after I/M administration of florfenicol (FFC) at the dose rate of 20, 40 and 60 mg $kg^{-1}$ b.wt. Significantly different (*p<0.05) from control values

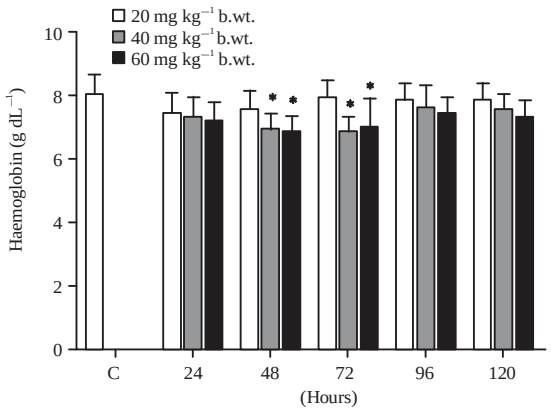


Fig. 2: Mean hemoglobin (g $dL^{-1}$) level of 6 goats obtained after I/M administration of florfenicol (FFC) at the dose rate of 20, 40 and 60 mg $kg^{-1}$ b.wt. Significantly different (*p<0.05) from control values

Hemoglobin mean values after induction of various doses at different timing points publicized (Fig. 2). As compared to control, therapeutic dose non-significantly decreased the hemoglobin level. Though, hemoglobin level was altered (p<0.05) from 48-72 h with both dosage regimes.

Mean values of Packed Cell Volume (PCV) are mentioned in Fig. 3 after dosage regimes at various timings. The PCV non-significantly decreased with therapeutic dose treatment. However, the animals received high doses of FFC showed significantly (p<0.01 and p<0.05) decreased in PCV volume at 24, 48 and 72 h when compared with control base line value.

Mean pre-treatment of white blood cell count was publicized in Fig. 4. With therapeutic dose of FFC non-significant decreased in leukocytes count were noticed.

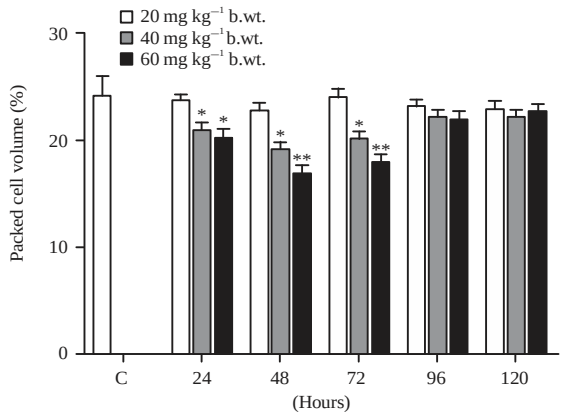


Fig. 3: Mean packed cell volume (%) of 6 goats obtained after I/M administration of florfenicol (FFC) at the dose rate of 20, 40 and 60 mg $kg^{-1}$ b.wt. Significantly different (*p<0.05 and **p<0.01) from control values

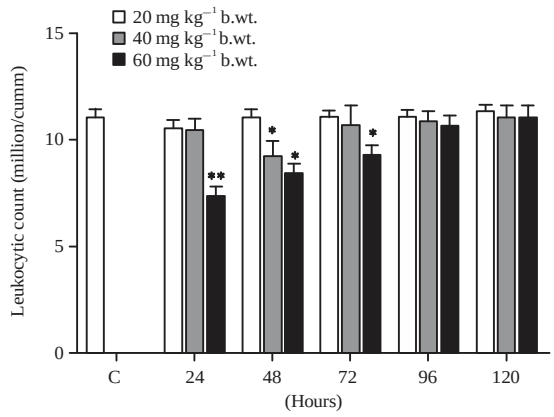


Fig. 4: Mean leukocytic count (1000/cumm) of 6 goats obtained after I/M administration of florfenicol (FFC) at the dose rate of 20, 40 and 60 mg $kg^{-1}$ b.wt. Significantly different (*p<0.05 and **p<0.01) from control values

In contrast to control, significantly decreased in leukocytic count was observed at different times after administration of FFC with high doses (40 and 60 mg $kg^{-1}$ b.wt.).

In comparison with control base line values the therapeutic dose produced decrease effect on lymphocytic count (p<0.05) at 24 h post medication. At high dose of 60 mg $kg^{-1}$ the lymphocytic count was decreased (p<0.01) upto 72 h and p<0.05 at 96 h of post drug administration. Whereas, 40 mg high dose almost p<0.05 diminished the lymphocyte values at 24, 72 and 96 h and p<0.01 at 48 h (Fig. 5).

**Clinical signs:** During administration of high doses of FFC some clinical reactions were also observed, such as

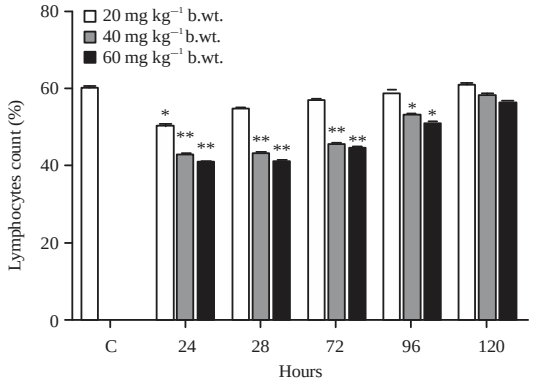


Fig. 5: Mean lymphocytes count (%) of 6 goats obtained after I/M administration of florfenicol (FFC) at the dose rate of 20, 40 and 60 mg $kg^{-1}$ b.wt. Significantly different (*p<0.05 and **p<0.01) from control values

irritation, pain feeling, swelling at site of injection, soft feces and decreased feed and water intake. The impacts were supposed to be dose related, as all animals recovered from this response after withdrawal of drug.

## DISCUSSION

The FFC is widely used in livestock and poultry to deal systemic infections therapeutically, prophylactically and as a feed additive. The recommended therapeutic dose of florfenicol in animals is 20 mg $kg^{-1}$ b.wt., with this dose major adverse effects were uncommon[15]. While, using high dose therapy is of great importance to get useful serum and tissues concentration of drug for long time without frequent dosing. But before using high dose therapy some adverse effects of FFC on major tissues and organs must be considered. This prompted us to investigating the laboratorial evaluation of hematological indices. In current study erythrocytes, hemoglobin and packed cell volume decreased with induction of FFC high doses. The high doses of FFC decreased the erthroyctic count might be interfering with various mechanisms were involved in hematopoietic toxicity, such as cytolysis, genotoxicity and mitochondrial protein synthesis inhibition[7]. It is reported that amphenicol antimicrobials interference with heme synthesis process in erythroid mitochondria in mammals[16]. The erythropoietin play major role in stimulation of RBC's production from hematopoietic stem cells in the bone marrow. The FFC might be suppressed the functional erythropoietin system and evoked morphological abnormality in bone marrow cells leads to formation of round vacuoles in erythrocytes, which may results in elevated plasma iron binding protein and decreased iron incorporation in hemoglobin[17]. It is also reported that chloramphenicol group drugs including FFC even at high doses cause reversible and irreversible bone marrow atrophy and suppression in animals and human due to mitochondrial injury. It is possible that FFC at high doses may cause some dose dependent, reversible bone marrow suppression[11]. Majority of hematopoietic tissues are found in kidney and spleen so FFC in high concentration causes minimal to mild decreased hematopoietic tissue of kidney and spleen, which in turn decreases proliferation/increased destruction of hematopoietic tissues[8-11]. The declined in hemoglobin and packed cell volume is also related with decreased erythrocytic population as well. The results of this study are in agreement with previous studies in various species[9-11]. Total leukocytic count was observed to be decreased with both high dosage regimes of FFC. The outcomes of current study are supported by other researchers who also mentioned similar decreased effects of FFC on leukocytes[9,10,18]. The high doses of FFC decreased the leukocytic count might be interfering with various mechanisms were involved in hematopoietic toxicity, such as cytolysis, immune mediated destruction and genotoxicity[7]. High doses of FFC are supposed to be associated with inhibition of enzymes involved in leukocytes maturation process in bone marrow and also interference with phagocytosis and chemotaxis activity of the leukocytes[19,20]. The FFC induced impairment of myeloid cells (associated with repopulate peripheral white blood cells and lymphoid cells) also showed decreased number of circulating leukocytes by interfering with cytologic and histologic vaculation, degeneration, necrosis, maturation arrest, reduced cellularity and impairment of cellular energy production[17]. Lymphocytic count was declined with therapeutic and high doses of FFC. Lymphocytes (T and B cells) produced mainly in various lymphogenesis tissues, especially from lymph nodes, spleen, thymus and bone marrow. These tissues constitute the immune system, importantly play role in cell mediated and humoral immunity. Hypoplasias of bone marrow decrease the immunity in chicken. It was analyzed that FFC may cause hypoplasia of bone marrow that resulted a drop in lymphocytic count[14]. High doses of FFC are supposed to cause significant effects particularly on lymphoid cells and organs and initiates temporarily decreased weight of spleen, mesenteric lymph node, thymus and bursa in dose dependent manner finally leads to an immunosuppressive effect on humoral and cell mediated immunities[18,21]. During present study also dose dependents effects were observed. The outcomes of present study in accordance with studies reported in various species, such as in fish[19], buffalo calves[20]

and in mice[21]. During experiment, it was observed that FFC produced pain and irritation response, which lasted up to 30 min after dosage administration. The 2-pyrrolidone is most common vehicle used in FFC formulations which causing local irritation of varying degree at site of injection. Even few animals showed excessive response, exhibited by their running and jumping reaction probably because of severe pain feeling. The oil based FFC formulations were used definitely causes irritation and pain response due to its prolong action and delaying release of active drug from this base. Previous studies also reported such clinical signs when oil based FFC formulations used along with 2-pyrrolidone (vehicle) in[10,22,23] numerous animal species.

## CONCLUSION

In conclusion, high dosage regimes of FFC produced reversible immunosuppression and anemic effect in dose dependent manner and a dose of 40 mg clinically can be used, but laboratory follow up must be considered during treatment period especially in debilitate animals such as goat. The current findings can be helpful for veterinary clinician for selecting proper dosing regimen especially with high doses for treating infectious diseases.

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## REFERENCES

1. Picco, E.J., D.C. Diaz, S.E. Valtorta and J.C. Boggio, 2001. Chronotoxicology of florfenicol. Chronobiol. Int., 18: 567-572.
2. Sams, R.A., 1995. Florfenicol: Chemistry and metabolism of a novel broad-spectrum antbiotic. Tieraerztliche Umschau, 50: 703-707.
3. Switala, M., R. Hrynyk, A. Smutkiewicz, K. Jaworski and P. Pawlowski *et al*., 2007. Pharmacokinetics of florfenicol, thiamphenicol and chloramphenicol in Turkeys. J. Vet. Pharmacol. Ther., 30: 145-150.
4. Elitok, O.M., B. Elitok, S. Konak and H.H. Demirel, 2015. Clinical efficacy of florfenicol on caprine pasteurellosis. Small Rumin. Res., 125: 142-145.
5. Pentecost, R.L., A.J. Niehaus, N.A. Werle and J. Lakritz, 2013. Pharmacokinetics of florfenicol after intravenous and intramuscular dosing in llamas. Res. Vet. Sci., 95: 594-599.
6. Wei, C.F., J.H. Shien, S.K. Chang and C.C. Chou, 2016. Florfenicol as a modulator enhancing antimicrobial activity: Example using combination with thiamphenicol against Pasteurella multocida. Frontiers Microbiol., Vol. 7. 10.3389/fmicb.2016.00389.
7. Kuter, D.J. and G.S. Tillotson, 2001. Hematologic effects of antimicrobials: Focus on the oxazolidinone linezolid. Pharmacotherapy: J. Hum. Pharmacol. Drug Therapy, 21: 1010-1013.
8. Gaikowski, M.P., J.C. Wolf, R.G. Endris and W.H. Gingerich, 2003. Safety of Aquaflor (florfenicol, 50% type A medicated article), administered in feed to channel catfish, Ictalurus punctatus. Toxicol. Pathol., 31: 689-697.
9. Al-Nazawi, M.H. and A.M. Homeida, 2005. Pharmacokinetics and tolerance of chloramphenicol and florfenicol in camels. J. Camel Pract. Res., Vol. 12.
10. Holmes, K., D. Bedenice and M.G. Papich, 2012. Florfenicol pharmacokinetics in healthy adult alpacas after subcutaneous and intramuscular injection. J. Vet. Pharmacol. Therapeutics, 35: 382-388.
11. Hu, D., T. Zhang, Z. Zhang, G. Wang and F. Wang *et al*., 2014. Toxicity to the hematopoietic and lymphoid organs of piglets treated with a therapeutic dose of florfenicol. Vet. Immunol. Immunopathol., 162: 122-131.
12. Guan, S., J. Lu, X. Shen, W. Qian, J. Liu and X. Deng, 2011. Florfenicol impairs the immune responses to vaccination against foot-and-mouth disease in mice. Immunopharmacol. Immunotoxicol., 33: 609-613.
13. Azeez, O., O. Iji and A. Akinrinde, 2015. Prolonged use of chloramphenicol induces aplastic anemia and oxidative stress in broiler chicken. FASEB J., Vol. 29.
14. Hassanin, O., F. Abdallah and A. Awad, 2014. Effects of florfenicol on the immune responses and the interferon-inducible genes in broiler chickens under the impact of E. coli infection. Vet. Res. Commun., 38: 51-58.
15. Chrząstek, K., J.P. Madej, E. Mytnik and A. Wieliczko, 2011. The influence of antibiotics on B-cell number, percentage and distribution in the bursa of Fabricius of newly hatched chicks. Poult. Sci., 90: 2723-2729.
16. Abou-Khalil, S., W.H. Abou-Khalil, P.L. Whitney and A.A. Yunis, 1987. Importance of the mitochondrial amino acid pool in the sensitivity of erythroid cells to chloramphenicol: Role of glycine and serine. Pharmacology, 35: 308-316.
17. Ferrari, V. and E. Pajola, 1981. Types of Haemopoietic Inhibition by Chloramphenicol and Thiamphenicol. In: Safety Problems Related to Chloramphenicol and Thiamphenicol Therapy, Najean, Y., G. Tognoni and A.A. Yunis (Eds.). Raven Press, New York, pp: 43-59.

18. Lis, M., M. Szczypka, A. Suszko, M. Switała and B. Obminska-Mrukowicz, 2011. The effects of florfenicol on lymphocyte subsets and humoral immune response in mice. Polish J. Vet. Sci., 14: 191-198.
19. Sieroslawska, A., M. Studnicka, A.K. Siwicki, A. Bownik, A. Rymuszka and J. Sionka, 1998. Antibiotics and cell-mediated immunity in fish-*in vitro* study. Acta Vet. Brno, 67: 329-334.
20. Hasaan, H. and M. Nasser, 2003. Immunosuppressive role of florfenicol in buffalo calves. Vet. Med. J Giza., 51: 41-48.
21. Liu, K.Y., X.X. Li, H. Teng, S.B. Li and J.Y. Guo *et al*., 2009. Toxicity of florfenicol to immune organs and its effect on physical and chemical characteristics of muscle in mice. J. Anhui Sci. Technol. Univ., 3: 6-10.
22. Hariharan, H., 2007. Antimicrobial therapy in veterinary medicine. Can. Vet. J., Vol. 48.
23. Thiry, J., S. Rubion, P. Sarasola, M. Bonnier, M. Hartmann and V. de Haas, 2011. Efficacy and safety of a new 450 mg $mL^{-1}$ florfenicol formulation administered intramuscularly in the treatment of bacterial bovine respiratory disease. Vet. Record, Vol. 169. 10.1136/vr.d5498.