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Effect of Florfenicol on Hematology, Cardiac Enzymes and its Residues in Broiler Chickens by HPLC

Ahmed A. Said[1], Sameh M. El-Nabtity[1], Abdulla A.M. Selim[2], Mai A. Fadel[2*]

[1]Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

[2]Animal Health Research Institute, Dokki, Giza, Egypt

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Abstract

The current study was conducted on multiple oral doses (40 mg/kg for 3 successive days) of florfenicol (FF) to determine its effect on some hematological parameters, cardiac enzymes and its residues in blood and tissues (liver, muscles and kidneys) of broiler chickens. Seventy broiler chickens were used and divided into two groups, each consisted of 35 birds. The first group was left as a control, while the second was given FF for 3 successive days. The FF residues in tissues were determined using reversed phase-high performance liquid chromatography (RP-HPLC) with ultraviolet (UV) detector at 223 nm. Results indicated a widespread distribution of FF in most of the tested tissues. All tissue samples were considered FF free on the 9th day after the last oral dose except liver. Florfenicol administration elicited a significant decrease in all blood parameters (hemoglobin concentration (Hb), red blood cell count (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and lymphocytes) from 1st up to 7th day except packed cell volume (PCV) which showed no significant change on 1st day but decreased on 3rd, 5th and 7th day after stopping medication. Moreover, monocytopenia was observed on the 5th and 7th day and white blood cells (WBCs) showed the same effect on the 5th day, while heterophiles revealed highly elevation. All cardiac enzymes (aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase MB (CK-MB) and Troponin I) were highly elevated. In conclusion, broiler chicken meat can be consumed safely after 9 days post FF treatment.

Keywords: Florfenicol, Broiler chicken, HPLC Residues, Hematological parameters, Cardiac enzymes

Introduction

Florfenicol (FF) is a bacteriostatic antibiotic that inhibits protein synthesis by binding ribosomal subunits of susceptible bacteria, consequently inhibiting the peptidyl transferase and then preventing the transfer of amino acids to growing peptide chains and subsequent protein formation [1]. Florfenicol has a fluorine atom instead of the hydroxyl group located at C-3 in the structure of chloramphenicol and thiamphenicol. It is used for treating bovine respiratory disease, in addition, FF may be considered a bactericidal against some *Mannheimia (Pasteurella) hemolytica* and *Pasteurella multocida* when it is administered to achieve minimum inhibitory concentrations (MICs) [2]. The minimum bactericidal concentrations (MBCs) are very close to the MICs.

Following the administration of FF (30 mg/kg BW) in broiler chickens via intravenous (IV), intramuscular (IM) and oral routes, the highest drug residues were found in the kidneys, bile, lungs, muscles, intestine, heart, liver, spleen and plasma, while the lowest concentrations were found in brain, bone marrow and fat [3]. No FF residues were detected in tissues and plasma after 72 h except in the bile which disappeared after 96 h [3].

The microbiological Acceptable Daily Intake (ADI) is 3 µg/kg BW, i.e. 180 µg per person and a toxicological ADI of 10 µg/kg BW, i.e. 600 µg per person had previously been reported by the Committee for Veterinary Medicinal Products [4]. The presence of FF residues in broiler meat and

***Corresponding author e-mail: (dr.mai87@yahoo.com), Animal Health Research Institute, Dokki, Giza, Egypt.**

liver causes antibiotic resistance and allergy to consumers [5].

The withdrawal times and maximum residue limits of drugs should be determined to prevent the formation of residues in meat of animals [6]. Florfenicol maximal residue limits were 100, 750 and 2500 μg/kg for muscles, kidneys and liver, respectively, according to the European Union [7]. The purpose of this study was to determine FF residues in tissues of broiler chickens and its withdrawal time. In addition, its effect on hematological parameters and cardiac enzymes (creatine kinase MB, Troponin I, aspartate aminotransferase and lactate dehydrogenase) was evaluated.

Material and Methods

Birds and experimental design

Florfenicol was obtained from ATCO pharma for pharmaceutical industry, made in Egypt as a suspension in dark plastic bottle containing 1000 mL (100 mg/mL). The drug was diluted in drinking water prior to administration at a dose of 40 mg/kg BW [4]. The molecular formula is: C12-H14-Cl2-F-N-O4-S. Seventy Healthy Hubbard broiler chickens of 4 weeks age and 1200 g weight were used.

Chickens were obtained from a private poultry farm in Cairo. The birds were housed in batteries in post graduate research laboratory at Animal Health Research Institute, Dokki, Giza, Egypt. Thirty-five chickens were used as control. These birds were used for preparation of blank and spiked samples for method validation. The other thirty-five birds were given FF directly into the crop at a dose of 40 mg/kg BW once daily for 3 consecutive days. Five chickens were sacrificed on 1st, 3rd, 5th, 7th, 9th,14th and 21st day following the last oral dose. Samples from liver, muscle and kidney were taken for quantitative determination of FF residues.

On the1st, 3rd, 5th and 7th day following the last oral dose, blood samples with EDTA (2 mL) from the wing vein were collected for hematological studies. While, on the 1st,3rd and 5th day another blood samples (3 mL) were collected for serum separation from each chicken following the last oral dose for estimation of cardiac enzymes in the serum (Creatine kinase-MB (CK-MB), Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST) and Troponin I).

Analytical procedures

Preparation of samples for analysis

At the time of assay, frozen chicken tissue samples (-20ºC) were partially thawed at room temperature (25ºC) for 30 min and were minced and homogenized in the mincer for 1 min and the samples were then analyzed by HPLC at the Central Laboratory, Faculty of Veterinary Medicine, Zagazig University.

Drug residues extraction

Extraction of the drug residues from the samples was carried out according to Wang *et al.* [8]. Five grams of the ground sample were weighed in 40 mL centrifuge tube. Five milliliters PBS and 20 mL ethyl acetate were added, and the mixture was mixed by a vortex and then centrifuged at 1500 xg for 20 min. The supernatant was transferred to a clean centrifugal tube. The extraction step was repeated and the extracts were combined and evaporated to dryness at 60°C under a gentle stream of nitrogen. The residue was dissolved in 3 ml of mobile phase consisting of acetonitrile/ water (27/73, v/v) solution and 2 mL hexane, and then it was mixed. After centrifugation at 4000 xg for 10 min, the hexane layer was discarded. The water-base phase was filtered through filter paper (0.45 μm × 25mm). The resulting solution was injected into the HPLC system.

Liquid chromatography operating conditions

Injection volume, 20 μL; flow rate, 1 mL/min; wave length, 223 nm; column temperature, ambient; stop time, 20 min; post time, 1 min; mobile phase consisting of acetonitrile/water (27/73, v/v).

Quantification

Quantification of residues in the samples was obtained and calculated from the area

under curves extrapolated automatically by the software (ChemStation, Germany).

Validation method

It is the evaluation process used to ensure that the performance characteristics of an analytical procedure are to demonstrate that it is suitable for its intended purpose.

System Precision: It was conducted using five replicates of the caffiene standard solution with acceptance criteria of Relative Standard Deviation (RSD) ≤ 1% according to the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH).

Linearity and range: Linearity was performed by preparing a minimum of five different concentrations of drug standard and defined by the squared correlation coefficient, which should be 0.99 (r^2) according to ICH.

Precision method: It was conducted using five replicates of tilmicosin standard solutions with acceptance criteria of RSD ≤ 1% according to ICH.

Selectivity and specificity: Verification of selectivity was conducted by evaluating the spiked standard response following extraction from different chicken tissues. Regarding the acceptance criteria, there is no interference between the pure standard and peaks of any impurities or extracted solvents according to ICH.

Accuracy and recovery: The tissue samples of chickens were spiked by adding known quantities of tilmicosin. Those samples were analyzed against standard solutions of the corresponding concentrations. The method was accurate according to the calculated test results from the % recovery.

Limit of detection (LOD): It is the concentration which gives signal to noise ratio 3:1 according to ICH.

Limit of quantification (LOQ): It is the concentration which gives signal to noise ratio 10:1 according to ICH.

Ruggedness: It was conducted by the analysis of the same samples under different conditions, such as different personnel, different times..etc. Acceptance criteria: pooled RSD is not more than 6% in every change item.

Robustness: It was determined by observing how an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Acceptance criteria: pooled RSD is not more than 6% in every change item.

Statistical Analysis

The obtained results for hematological parameters and cardiac enzymes are represented as mean ± standard error (S.E.) and results with $P \leq 0.01$ were considered significantly different and $P \leq 0.001$ with higher significance. The results were statistically analyzed using Student′s t-test. SPSS version 21, IBM Corp., Chicago, IL, USA was used for all analyses.

Results

Method validation

The HPLC system was found precise as the RSD of 5 replicates of the caffiene standard solution was 0.001%. High correlation coefficient was obtained indicating linearity (r^2=0.99996). The method for FF separation is precise as the RSD of 8 replicates of the FF standard solution was 0.4999%. There was no interference between the pure standard and peaks of any impurities or extracted solvents. The retention time (R.T.) of FF was 10.02 minutes (Figure1.1). The percentage recovery of FF spiked samples ranged from 97.8-100 %. The LOD for FF was 0.002 µg/mL, while the LOQ was 0.0048 µg/mL. The pooled RSD for FF was 3%.

Table 1: The concentrations of FF standard (µg/mL) and their corresponding peak response

RT	Level	Amount (µg/mL)	Area
10.02	1	0.005	3.312
	2	0.010	6.819
	3	0.020	13.522
	4	0.050	33.34
	5	0.100	66.326
	6	0.200	140.81
	7	0.500	362.66
	8	1.000	718.78

*RT=Retention Time

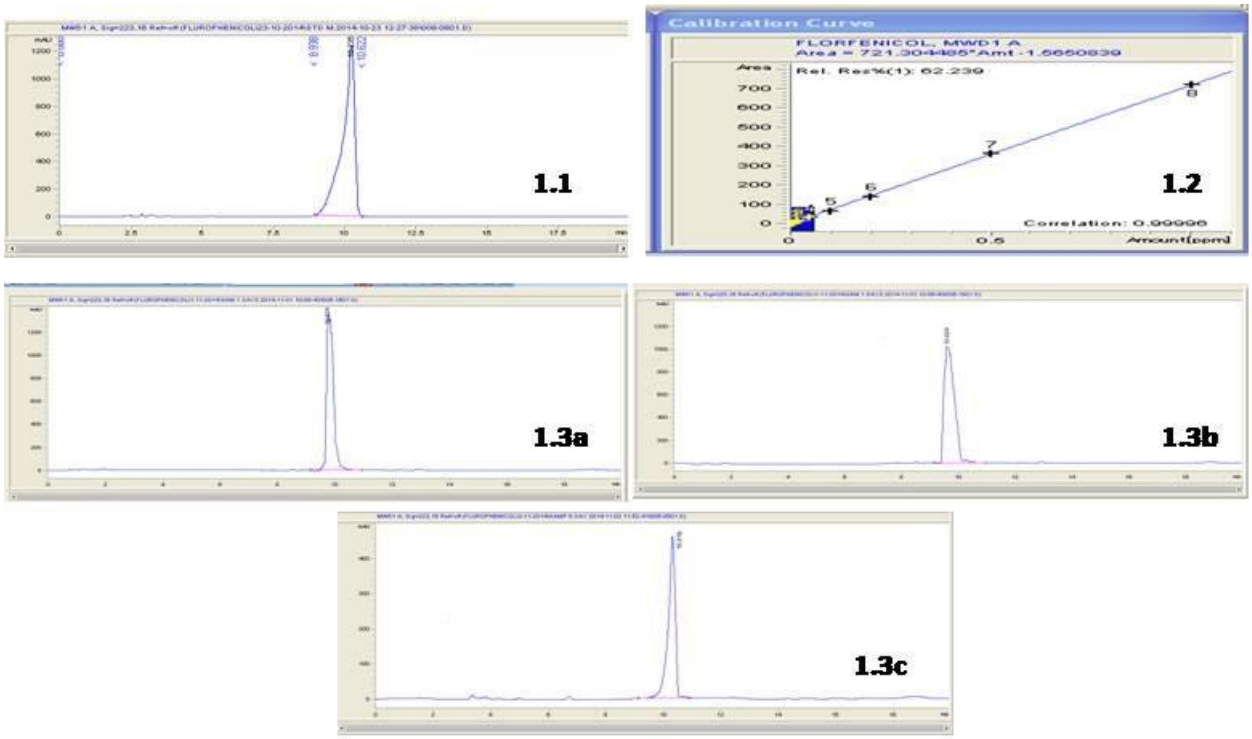

Figure 1: 1.1: Chromatograms of FF standard (1 µg/mL). 1.2: Standard curve of FF. 1.3: Chromatograms of FF extract of broiler liver (a), kidneys (b) and muscles (c) at 1st day following last oral dose.

Standard curve preparation

Florfenicol standard concentrations of 0.005 ,0.01 ,0.02 ,0.05 ,0.1 ,0.2,0.5 and 1 µg/mL and their corresponding peak responses are illustrated in Table (1) and Figure (1.2). The calibration curve was calculated by linear regression equation method as y = 721.304485x – 1.5650839 where y symbol indicated the area under peak and x symbol indicated concentrations of FF. Linearity existed witin the range of 0.005 and 1 µg/mL with a correlation coefficient (r^2 = 0. 99996).

Tissue residues

Tissue distribution of FF is represented in Table (2) and Figure (1.3 a,b,c). All control samples were free from any residues. The data represented emphasized a widespread distribution of the drug in tested tissues (liver, kidneys and muscles). Florfenicol concentrations were 2020 ±0.17, 1198±0.037 and 640±0.025 µg/kg on the 1st day post last oral dosage in liver, muscles and kidneys, respectively. Florfenicol remained within the detectable limit till the 7th day in most tested tissues, while till the 9th day in liver after drug administration (Table 2).

Haematological and biochemical results

Administration of FF resulted in a significant decrease in hematological parameters (Hb, RBCs, MCV, MCHC and lymphocytes) from 1st day up to 7th day except PCV which showed no significant change on 1st day but decreased on 3rd, 5th and 7th day after stopping medication, while heterophils count was highly increased. On the other hand, insignificant changes were observed in WBCs count except on the 5th day, it showed a significant decrease. Eosinophiles count showed insignificant changes from the 1st day up to the 7th day. There were insignificant changes in monocytes count on the 1st and 3rd day, but on the 5th and 7th days; FF depressed monocytes level. All tested cardiac enzymes (AST, LDH, CK-MB and Troponin I) showed highly significant elevation in their levels with FF administration (Table 3 and 4).

Table 2: The concentrations of FF in broiler chickens tissues at various intervals after treatment (40 mg/kg BW once daily for 3 consecutive days) (n=5)

Tissue	The concentration (µg/kg) mean ± SE					
	1st day	3rd day	5th day	7th day	9th day	14th day
Liver	2020 ± 0.17	659± 0.049	380±0.01	170 ±0.02	50 ±0.002	ND
Kidney	1198 ± 0.037	547 ± 0.027	138 ± 0.005	38±0.017	ND	ND
Muscle	640 ± 0.025	300±0.02	120 ±0.01	64±0.007	ND	ND

***ND=Notdetected**
****This table describes the withdrawal time of the drug from the tissues**

Discussion

In the present study, FF residues were detected in highest concentration in liver, kidneys and muscles on the 1st day post oral administration (40 mg/kg BW). These results agreed with Afifi and Abo el-Sooud [3] who detected the highest tissue concentrations of FF in the kidneys, muscles and liver following oral administration of 30 mg/kg BW in broilers.

On the other hand, EL-Banna *et al.* [9] reported that all tissues of slaughtered healthy and infected birds could be considered FF free except liver which had a concentration of 110 ±0.01 µg/kg on the 7th day after stopping the drug administration.

A withdrawal period > 6 days in healthy chickens and > 7 days in infected ones is satisfactory. Also, Anadón *et al.* [7] mentioned that the withdrawal time of FF was 6 days which is necessary to ensure that the residues were less than the maximal residue limits or tolerance established by the European Union. The present study revealed that the tissue residues persisted in liver till the 9th day which was (50 ±0.002 µg/kg) and (60 ±0.007 and 38 ± 0.017 µg/kg) in muscles and kidneys, respectively, on the 7th day of discontinuation of the drug medication. In our study, the results of the examined organs were lower than the recommended MRL on the 1st day post treatment for liver, on the 7th day for muscles and on the 3rd day for kidneys.

Table 3: Effect of FF (40 mg/kg BW for 3 consecutive days) on hematological parameters of broiler chickens

Time	Gr	Hb	RBCSx10^6	PCV	MCV	MCH	MCHC	WBCS	Eosinophil	Heterophiles	Lymphocyte	Monocytes
1st day	G1	12.9±0.22	3.1±0.03	34.46±0.3	111.22±1.12	41.62±0.65	37.42±0.42	8.4±0.18	0.69±0.01	24.9±0.11	72.18±0.05	2.23±0.07
	G2	11.52±0.03*	2.66±0.02**	34.36±0.1	129.2±0.45**	43.32±0.33	33.53±0.15**	8.66±0.09	0.62±0.01	29.18±0.09**	67.92±0.15**	2.28±0.06
3rd day	G1	13.14±0.04	2.96±0.05	34.04±0.25	115.15±1.22	44.47±0.62	38.61±0.28	8.88±0.24	0.65±0.01	25.1±0.2	71.9±0.21	2.35±0.04
	G2	11.38±0.07**	2.56±0.02**	32.42±0.09*	126.67±0.58**	44.46±0.29	35.1±0.22**	8.96±0.1	0.56±0.02	32.08±0.05**	65.24±0.05**	2.12±0.04
5th day	G1	13.04±0.07	3.1±0.02	34.08±0.19	109.96±0.72	42.08±0.39	38.27±0.29	8.88±0.13	0.67±0.01	25.32±0.34	71.7±0.28	2.31±0.04
	G2	9.86±0.09**	2.16±0.02**	30.68±0.18**	142.06±0.51**	45.67±0.51*	32.14±0.26**	7.94±0.1*	0.68±0.01	32.52±0.21**	64.8±0.22**	2 ±0.04*
7th day	G1	13.04±0.09	3.12±0.03	34.22±0.26	109.74±0.95	41.81±0.26	38.12±0.27	8.6±0.23	0.67±0.01	25.02±0.14	71.42±0.11	2.29±0.02
	G2	8.72±0.08**	2.08±0.03**	24.84±0.22**	119.47±0.46**	41.95±0.42	35.11±0.28**	7.62±0.14	0.62±0.01	33.46±0.19**	63.96±0.17**	1.96±0.03**

G1=Control *significant P<0.01 Hb= Hemoglobin MCH= mean corpuscular hemoglobin
G2=Florfenicol **significant P<0.001 MCV=mean corpuscular volume WBCs=white blood cells
RBCs=redbloodCells PCV= packed cell volume MCHC= mean corpuscular hemoglobin concentration

These results are concurrent with Anadón *et al*. [7] who reported that after multiple oral doses (40 mg/kg BW for 3 consecutive days). Concentrations of FF in kidney and liver were 119.34 ± 31.81 and 817.34 ±91.65 µg/kg, respectively, and florfenicol-amine (FFA) (60.67 ± 13.05 and 48.50 ±13.07 µg/kg, respectively) persisted for 7 days. Also, Zhou *et al.* [10] mentioned that the total residues of FF and FFA were lower than the MRLs (100 µg/kg) on the 7th withdrawal day and lower than the lowest LOD (1.5 µg/kg) at 11th withdrawal day.

The residues and total residues of FF and FFA in chicken muscle were all positively correlated with FF orally administered doses. Also, our results agree with Khalil *et al*. [11] who reported that liver FFC level was below the MRLs in treated groups during the studied time, but not compatible with them as the muscle tissue FFC level was higher than the MRLs on 3rd day post administration in their study. The level was then depleted to be below the MRLs on 5th day in the 30 mg/kg BW treated group and on 7th day in the 60 mg/kg BW treated group [11]. The differences could be due to different doses applied to broiler chicken in their experiment.

In another study, the high concentration of FF in kidneys, liver, thigh and breast muscles after 2 and 4 days of the last dose was reported, while, moderate concentration of FF after 6 days were detected [10]. Low concentration of FF in kidney and liver was only detectable on 8th day after last dose.

The previous results were in agreement with our results and with Nasim *et al*. [5] who reported the presence of FF residues in broiler meat and liver and the obtained results revealed that the mean residual concentrations of FF in broiler meat and liver were 311.42±186.56 and 2585.44±1759.71 µg/kg, respectively. While, Reda *et al.* [13] studied FF residues in fish muscles. The results showed that FF residues of 0.04 µg/g were detected in muscles after 15 days of feeding cessation. Their results were lower than the MRLs of FF (1µg/g) according to Commission Regulation [14].

El-shewy and Ibrahim [15] supported our results as they reported the highest concentration of Nuflor® in kidneys, liver and breast muscles. Also, Chang *et al*. [16] reported that the drug was quickly absorbed and widely distributed with tissue penetration factors significantly different between leg and breast muscles. Higher FF concentration in the brain, lung and kidneys, while, at least 12 h longer resident times in kidneys, heart and spleen for Taiwan Native chicken after a single oral dose of FF at 30 mg/kg was reported [15]. Moreover, our results were in agreement with EMA [4] in which broiler chickens received FF according to the recommended regimen via drinking water at concentrations equivalent to 17 to 30 mg/kg BW/day for 3 days.

Seven days after the end of the treatment, the concentrations of FF amine were below the limit of quantification for liver (less than 461 µg/kg) and could be still measured in kidneys (136 µg/kg).

Table 4: Effect of FF (40 mg/kg BW for 3 consecutive days) on heart enzymes of broiler chickens

Time	Group	LDH (U/L)	CK.MB (U/L)	AST (U/L)	Troponin I (ng/ml)
	G1	67.4±0.66	129±0.5	15.52±0.11	5.8±0.26
1st day	**G2**	95.8±0.52**	244.4±0.66**	19.48±0.13**	25± 0.22**
	G1	67.8±0.52	127.2±0.57	15.42±0.08	8.2±0.41
3rd day	**G2**	110.6±0.76**	258.2±0.57**	28.06±0.15**	38.6±0.53**
	G1	63±0.67	128.4±0.36	15.36±0.09	9 ±0.39
5th day	**G2**	138.4±0.66**	490.6±0.76**	22.82±0.08**	43.6±0.53**

G1=Control G2=Florfenicol *significant P<0.01 **significant P<0.001

In the present work, the hematological parameters, leucocytic count and cardiac enzymes in broilers were evaluated. Our results are concurrent with Elsenhwy *et al*. [12] who found that administration of FF to broiler chicks decreased WBCs counts. Also, Holmes *et al*. [17] stated that repeated S.C administration of FF (40 mg/kg) in alpacas was associated with a significant reduction in WBCs. El-shewy and Ibrahim [15] agreed with our study as FF lowered RBCs, WBCs, Hb, PCV, MCH and MCHC, while their study revealed an increase in MCV which antagonist with our study.

Our results are in agreement with the study which had been done by Elsenhwy *et al.* [12] who reported that FF decreased the number of RBCs, Hb, PCV and lymphocytes in broiler chicks. Also, our results are in agreement with Nuflor injectable solution product information [18] in which hemoglobin and red blood cells for some treatment groups had some statistically significantly lower values than the control group on various days of the study.

Soltan *et al*. [19] reported that fish fed on diet supplemented with FF (7.5 mg/kg) showed an increase in Hb concentration, RBCs count and insignificant decrease in the count of WBCs, while, fish fed on diet with 22.5 mg/kg FF recorded the lowest value in Hb, WBCs and RBCs compared with the control group.

In the present work, esinophiles showed insignificant changes from the 1st day up to the 7th day. These results disagreed with Elsenhwy *et al.* [12] who mentioned that FF decreased oesinophiles in broiler chicks. The obtained results demonstrated a highly significant increase in heterophils count from the 1st day up to the 7th day which disagreed with Lis *et al***.** [20] and Elsenhwy *et al.* [12] who reported that multiple exposures to FF decreased temporarily segmented neutrophils in mice and heterophils in broiler chickens, respectively.

On the other hand, Schering-Plough Animal Health [21] confirmed our results as absolute heterophils count for some treatment groups had significantly higher values compared to the control group on various days of the study. In the current study, lymphocytes demonstrated highly significant decrease from the 1st day up to the 7th day. In contrary, Schering-Plough Animal Health [21] documented that WBCs counts and lymphocytes had significantly higher values compared to the control group. There were insignificant changes in monocytes on the 1st and 3rd day, but on the 5th and 7th day, there was significant decrease (Table 3). These results agreed with Lis *et al.* [20].

Concerning the cardiac enzymes, marked increase in all parameters (LDH, CK-MB, AST and troponin) enzymes on the 1st, 3rd and 5th days (Table 4). Our results are in agreement with Ayse and Dik [22] who detected an increase of AST value in healthy fish after FF administration. Meanwhile, Holmes *et al.* [17] reported that FF administration in alpecas animal showed profound elevation in AST. The results of this study were supported by Nuflor injectable solution product information [18], Freedom of Information Summary [23] where FF was reported to mildly increase aspartate aminotransferase and lactase

dehydrogenase [LDH]. On the other hand, the results of El-shewy and Ibrahim [15] disagreed with our results as Nuflor® decreased serum levels of ALT and AST in all treated groups.

Conclusion

The obtained results clearly demonstrated that liver is the target tissue for FF residues in broiler chickens. All tissue samples were considered FF free on the 9th day after the last oral dose except liver where residues disappeared on 14th day after the last oral dose. Residues in muscles, liver and kidneys were below the MRLs on the 7th day after cessation of FF administration. The consumers can safely eat muscles, liver and kidney on the 7th day after cessation of FF administration. In addition, FF had adverse effects on hematological parameters. Finally, all tested cardiac enzymes (AST, LDH, CK-MB and Troponin I) showed highly elevation in their values with administration of FF (40 mg/kg BW once daily for 3 consecutive days).

Conflict of interest

None of the authors have any conflict of interest to declare

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الملخص العربي

تأثير الفلورفينكول علي صورة الدم وانزيمات القلب وبقاياه الدوائية فى دجاج التسمين باستخدام الجهاز الكروماتوجرافي العالي الكفاءة

احمد عبده سعيد[١]، سامح محمد النبتيتى[١]، عبدالله عبد الظاهر محمد سليم[٢] و مي عبد المنعم فاضل[٢]*

[١]قسم الفارمكولوجيا- كلية الطب البيطرى-جامعة الزقازيق-مصر

[٢]معهد بحوث صحة الحيوان- الدقى- الجيزة-مصر

استهدفت هذه الدراسة قياس بقايا الفلورفينكول فى أنسجة دجاج التسمين المختلفة (الكبد، الكلى و العضلات) وتأثيره علي انزيمات القلب وصورة مكونات الدم بعد استخدام عدد ٧٠ دجاجة لاجراء التجربة. وقد تم تقسيم الدجاج الي مجموعتين متساويتين(٣٥ دجاجة) لكل مجموعة،الأولي استخدمت كمجموعة ضابطة.المجموعة الثانية تم اعطائها الفلورفينكول عن طريق الفم بجرعة ٤٠ مجم/ كجم لمدة ٣ أيام متتالية ؛ وقد تم القاء الضوء على مدة سحب الأدوية من الأنسجة المختلفة لتصبح امنة للاستهلاك الأدمى عن طريق ذبح عدد ٥ دجاجات عند اليوم الأول، الثالث، الخامس، السابع، التاسع، الرابع عشر و الحادى و العشرين بعد آخر جرعة و أخذ الأنسجة المختلفة (الكبد، الكلى، العضلات) ثم فحصها و قياس مستوى هذه المضادات فى عينات الأنسجة المختلفة وذلك بواسطة جهاز الفصل الكروماتوجرافى السائل العالى الأداء و من هذه النتائج نستنتج انتشار الدواء انتشارا واسعا فى أنسجة دجاج التسمين المختلفة السابق ذكرها.و قد تلاحظ وجود الفلورفينكول فى الأنسجة المختلفة حتى اليوم السابع(الكلي والعضلات) من إعطاء الجرعة النهائية أما الكبد استمر وجود الدواء حتى اليوم التاسع من إعطاء الجرعة النهائية.من ناحية اخري تمت دراسة تأثير الدواء علي مكونات صورة الدم وانزيمات القلب حيث لوحظ نقص في جميع مكونات الدم مثل الهيموجلوبين وكرات الدم الحمراء والخلايا الليمفاوية ما عدا حجم الخلايا المضغوطة التي لم يحدث لها أي تغيير ملحوظ .كما أثر الدواء علي انزيمات القلب في السحبات من اليوم الأول والثالث والخامس الي ارتفاعها بشكل ملحوظ. من خلال النتائج يجب ان يتم إستهلاك الدواجن التي تم علاجهابالفلورفينكول بأمان بعد تسعة ايام من علاجها.

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