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RESIDUAL STUDIES OF FLORFENICOL IN BROILER CHICKEN

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A B S T R A C T

Florfenicol, (a structural analogue of thiamphenicol) is of great value in veterinary treatment of infectious diseases. This study was designed to investigate the toxicological effects of florfenicol on different organs, liver and kidney activities, histopathological changes with regard to the residues of Florfenicol in organs and tissue. In this study 160 one day old COBB broiler chicks divided into 4 groups each group contain 40 birds. First group (G1) received 120 mg/kg b.wt, second group (G2) received 60 mg/kg b.wt, while third group (G3) received 30 mg/kg b.wt, Florfenicol which given orally in drinking water once/a day 4 times /week for 6 weeks while forth group (G4) kept as control. The obtained results were reduction in weights of liver, heart, lung, brain and proventriculus, with significant increase in weights of kidney and gizzard in G1 and G2 respectively. Increase of Creatinine, AST and ALT in broiler chicken of G1and G2 with non-significant effect on G3 comparable to G4 (control) were recorded. High concentration of florfenicol in kidney, liver, spleen, lung, heart, thigh and breast muscle 2 days and 4 days after last dose were measured while moderate concentration of florfenicol after 6-dayes were detected. Low concentration of Florfenicol in kidney and liver only detectable at 8-dayes after last dose.

Keywords: florfenicol, residues, broiler chicken.

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1. INTRODUCTION

There is a wide use of antimicrobial drugs either to treat or prevent bacterial infectious diseases in poultry. In addition antimicrobial drugs are used as feed additives to enhance growth and feeding efficiency of food animals. (20). Florfenicol, a structural analogue of thiamphenicol is of great value in veterinary treatment of infectious diseases. The mechanism of antibacterial activity of florfenicol is the same as that of thiamphenicol and chloramphenicol, inhibiting bacterial protein synthesis at the ribosome (7). Although it acts at the same site as chloramphenicol and thiamphenicol, the pharmacological composition of florfenicol makes it more resistant to deactivation by bacteria. (28). Florfenicol also differs from chloramphenicol and thiamphenicol in that it does not cause a dose-related, reversible bone marrow suppression or irreversible aplastic anemia in people. A withdrwal period of florfenicol was >6 ds in healthy chickens and >7 ds in infected ones is satisfactory (13). The highest concentration of florfenicol was present in kidney, liver, spleen, breast muscle and thigh muscle (12) (4) it was necessary to control the residues of TAP and FF in animal food in order to ensure health and safety of consumer. It was established that High performance liquid chromatography (HPLC) The FF

residue quantity in all tissues was lower than the 5th day after withdrawal. Therefore, the WDT of primary form drug FF was about five days. But because FF had other metabolites residues, the actual WDT of FF would be longer than the WDT original form of FF and may be reach to eleven days.

2. MATERIALS AND METHODS

2.1. Drug:

Florfenicol was obtained as oral solution (10%) from Pharma Swede Egypt under trade name Floricol®. each one milliliter contains 100 mg florfenicol base.

Birds 160 clinically healthy CObb chicks' unsexed one day old were obtained from private commercial hatchery. Classified into four groups each of which 40 chicken. Each group was kept in a separate pen with a layer of saw dust on the floor and given commercial chick basal diets. All groups are vaccinated against Newcastle disease virus Hitchner B1 at 7th and Lasota vaccine at 16th, 26th and 36th day of age and Gumboro vaccine against Gumboro diseases virus at 12thand 22th day of age and Classified into four groups as follows:

G 1: given florfenicol 120 mg/kg b.wt orally in drinking water once /aday-4days /week. G 2: given florfenicol 60 mg/kg b.wt (double therapeutic dose) orally in drinking water once /aday-4days /week. G 3: given florfenicol 30mg/Kg b.wt (therapeutic dose) orally in drinking water once/day- 4 days/week. G 4: kept as control group and allowed to drink clean water.

2.2. Sampling:

Organ weight samples: Slaughtering 10 birds of each group at 20thand 10 birds at 40th day of age to obtain organ weight as relative organ weight (gm of organ/ 100 gm body weight) was estimated (18). Blood allowed to stand for one hour at room temperature and centrifuged at 3000 r.p.m for fifteen minutes for separation of serum.

- serum samples were stored at -20C^{o}-samples for detection of Florfenicol residues obtained by slaughtering of 4 birds after 2th, 4th, 6th, 8th day from last dose to obtain liver, kidney, lung, heart, spleen, brain ,thigh and breast muscle.

Detection of florfenicol residue: Carried by Reference laboratory for Veterinary Quality Control on Poultry Production (R.L.Q.P) and Animal Health Research Institute (A H R I). According to (16).

2.3. Histopathological investigation:

According to previous methods [10], Samples from liver, kidneys, spleen, thymus, heart, brain & bursa of fabrecious. Were preserved in 10% formalin.

2.4. Statistical analysis:

The data were calculated as mean ± standard error. All statistical analysis was carried out according to (34).

3. RESULTS

Effect of treated Chicken with Florfenicol on relative organ weight and % to body weight at 20th day and at 40th day showed in Table (1). Highly significant and significant reduction in weight of liver, heart, lung, brain and proventriculus, with significant increase in weight of kidney and gizzard in G1(120 mg/kg b.wt) and G2 (60mg/kg b.wt) respectively with non-significant effect on chicken organs of G3 (60mg/kg b.wt) compared to G4 (control). - Effect of Florfenicol on Total Protein TP, Albumin, Globulin, in blood serum of treated broiler chicken at 20th day. Table (2) Show reduction of T.P, alb, globulin and A/G ratio in G1(120 mg /kg b.wt) and G2(60 mg/kg b.wt) with non-significant reduction in G3(30mg/ kg b.wt) compared to control. -Effect of Florfenicol on Creatinine, ALT and AST in blood serum of treated broiler chicken at 40th day. Table (3) Highly significant increase of Creatinine, AST and ALT in broiler chicken

of G1(120mg/kg b.wt) and G2 (60mg/kg b.wt) with non significant effect on G3 (30mg/kg b.wt) comparable to G4(control). - Concentration of Florfenicol Residues (µg/g) in treated chicken organs using HPLC-UVD: Table (4) show high concentration of Florfenicol in kidney, liver, spleen, lung, heart, thigh and breast muscle 2-dayes and 4-dayes after last dose and showing moderate concentration of Florfenicol 6-dayes after last dose and showing low concentration of Florfenicol in kidney and liver only detectable 8-dayes after last dose.

1. DISCUSSION

Concerning to the effect of Florfenicol on organ weight of treating chickens demonstrated in table (3,4,5) where we can notice a decrease in weights of liver, bursa, thymus, brain and proventriculus, spleen and heart with increase in weights gizzard , kidney, of all treated groups. Highly significant in G1where broiler chicken received (120 mg/Kg body weight) and significant in G2 where broiler chicken of received (60 mg/Kg body weight) at 20 and 40th of age, with non-significance in G3 where broiler chicken of received (30 mg/Kg body weight) at 20 and 40th of age compared to G4 (control). The significant decrease in weight of liver in G1 and G2 compared by G3 and G4 may be due to toxic effect of Florfenicol on liver, which confirmed by histopathological changes in our results photo (2 and 3) as there where areas of necrosis with evidence of calcification.

These results agreed with (32) in swine, dogs and rats. While opposite results were recorded by (30) in broiler chicken. Concerning to effect of florfenicol on serum biochemical parameters table (2) showed liver function enzymes as serum transferases (ALT and AST) so there were highly significant increase in serum ALT and AST in G1 where broiler chicken received (120 mg/Kg body weight) and significant increase in G2 where broiler chicken received (60 mg/Kg body weight) with non significant in G3 where broiler chicken received (30 mg/Kg body weight) at 20th and 40th day of age compared to G4 (control). These results agreed with (24) also with (14) the increase in A.S.T may be attributed to toxic effect upon heart muscle, liver cells and kidney and consequently liberating their intracellular enzyme into the blood stream (15). An increased level of Creatinine in the circulation is generally due to disorders that cause a reduction of glomerular filtration rate (GFR) (prerenal), sever kidney disease that adversely affects the number and/or microanatomy of the glomeruli (renal) and obstructive disorder that impair its elimination in urine (26).

Concerning to residues of florfenicol in treated broiler chicken determined by (HPLC–UVD) in table (17) recorded the concentration of Florfenicol (µg/g) after 2,4,6 and 8 days from the last dose of administration in kidney, liver, Spleen, heart, lung, thigh muscle and breast muscle where we noticed that the highest concentration of florfenicol presented after 2 days and decreased till become zero after 8 days from last dose except in liver, kidney and spleen of G1 where broiler chicken received (120 mg/Kg body weight) and G2 where broiler chicken received (60 mg/Kg body weight) and in liver and kidney of G3 where broiler chicken received (30 mg/Kg body weight).
Our results agreed with (17) Also agreed with (35) But our results disagreed with (9) The longer withdrawal period in our results may be due to long period of treatment, also the higher doses in G1 where broiler chicken received (120 mg/Kg body weight) and G2 where broiler chicken received (60 mg/Kg body weight) and manner of dosing by oral administration where the bioavailability of florfenicol after oral administration was high with approximately 55.3%of being absorbed

Table (1) Effect of Florfenicol Treated Chicken on relative Organ Wt.& % to B. Wt. at 40th Day

Organ	Para meter	G1	G2	G3	G4
Liver	Wt(gm)	11.66**±1.12	12.73*±0.07	13.16±0.48	13.9±0.79
	%	3.93	4.24	4.34	4.3
Kidneys	Wt(gm)	4.82**	4.0*	3.80	3.30
	%	2.062	1.8	1.5	1.026
Heart	Wt(gm)	1.02±0.03	1.83±0.09	2.27±0.09	2.37±0.07
	%	0.244	0.639	0.747	0.736
Lung	Wt(gm)	1.22	1.90	2.2	2.6
	%	0.150	0.663	0.725	0.808
Brain	Wt(gm)	1.33±0.03	1.43±0.03	1.53±0.03	1.60±0.06
	%	0.47	0.48	0.51	0.50
Gizzard	Wt(gm)	22.2	20.7	11.2	10.4
	%	2.53	2.93	3.03	3.23
Proven triculus	Wt(gm)	1.5	1.9	2.3	2.5
	%	0.22	0.66	0.76	0.78

Table (2) Effect of Florfenicol on Total Protein TP, Albumin and Globulin in blood serum of treated broiler chicken at 20th day

	TP(g/dl)	AL(g/dl)	GB(g/dl)	A/G
G 1	2.89±0.17	1.22±0.16	0.96±0.08	1.27
G 2	3.26±0.18	2.18±0.09	1.08±0.12	2.02
G 3	4.27±0.25	3.16±0.17	1.67±0.08	1.89
G 4	4.43±0.26	2.36±0.17	2.02±0.11	1.17

Table (3) Effect of Florfenicol on Creatinine, ALT and AST in blood serum of treated broiler chicken at 40th day.

	CR(mg/dl)	ALT(u/l)	AST(u/l)
G 1	4.96 ** ±0.03	15.72** ±1.30	91.4**±1.69
G 2	1.75 *±0.03	9.17 *±0.17	49.26*±3.53
G 3	0.37±0.04	3.51±0.55	45.52±3.97
G 4	0.34±0.04	3.28±0.31	35.41±5.34

Table (4) Concentration of Florfenicol Residues (µg/g) in treated chicken organs using HPLC-UVD

	2-dayes after last dose			4-dayes after last dose			6-dayes after last dose			8-dayes after last dose		
Organ	G 1	G 2	G 3	G 1	G 2	G 3	G 1	G 2	G 3	G 1	G 2	G 3
Liver	360.8 ±0.03	260.6 ±0.018	244.4 ±0.11	130.4 ±0.08	129.7 ±0.8	118.4 ±0.03	75.4 ±0.14	67.3 ±0.18	37.1 ±0.14	11.3 ±0.02	1.9 ±0.11	0.28 ±0.12
Kidney	637.3 ±0.04	330.9 ±0.02	245.37 ±0.17	385.7 0.02	157.3 ±0.17	129.7 ±0.12	95.3 ±0.15	79.9 ±0.13	57.4 ±0.21	23.5 ±0.18	13.4 ±0.07	0.93 ±0.11
spleen	220.7 0.01	190.3 ±0.85	117.8 ±0.15	103.2 ±0.15	87.8 ±0.13	32.7 ±0.18	37.3 ±0.9	46.1 ±0.28	31.7 ±0.02	0.89 ±0.22	0.22 ±0.1	-
Lung	209.3 ±0.01	200.1 ±0.17	130.1 ±0.19	75.9 ±0.23	39.3 ±0.15	30.1 ±0.18	22.1 ±0.21	11.3 ±0.08	7.3 ±0.11	-	-	-
Heart	180.51 ±0.04	87.8 ±0.24	39.7 ±0.21	63.7 ±0.11	29.3 ±0.27	27.2 ±0.21	32.3 ±0.02	12.4 ±0.18	15.8 ±0.18	-	-	-
Thigh muscle	164.8 ±0.01	109.7 ±0.11	40.3 ±0.24	87.8 ±0.11	46.9 ±0.23	11.89 ±0.13	15.8 ±0.22	15.8 ±0.02	11.2 ±0.07	-	-	-
Breast muscle	158.4 ±0.01	59.7 ±0.02	25.7 ±0.2	110.1 ±0.15	97.8 ±0.16	63.3 ±0.9	27.3 ±0.22	15.9 ±0.02	0.98 ±0.11	-	-	-

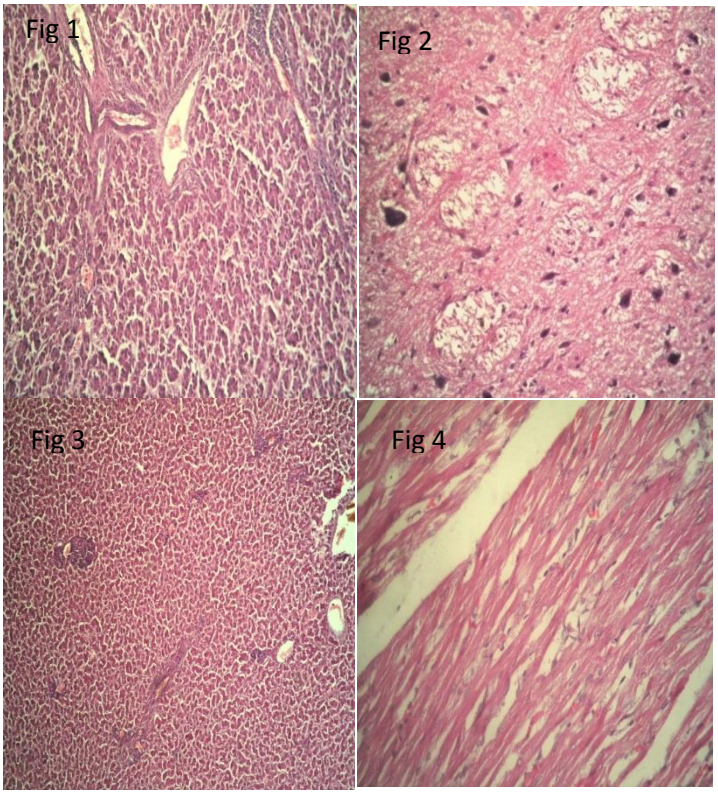

Fig (1) of Brain of broiler chicken of G1 (120 mg/kg b.wt) showing multi areas of malecia. X 400. Fig (2) of Liver of broiler chicken of G1 (120 mg/kg b.wt) showing distortion of hepatic cords, thickening of portal area associated with lymphocytic cellular infiltration. X 200. Fig (3) of liver of broiler chicken of G2 (60 mg/kg b.wt) showing small foci of perivascular inflammatory cells infiltration with congestion of portal vessels, sinusoid, central vessels and some degeneration of hepatocytes. X200. Fig (4) of heart broiler chicken of G2 (60 mg/kg b.wt) showing severe edema within the muscle bundles

Furthermore, the elimination half life was long (1), The prolonged presence of residues of florfenicol and florfenicol-amine in edible tissues can play an important role in human food safety, because the compounds could give rise to a possible health risk. A withdrawal time of 6 days was necessary to ensure that the residues of florfenicol were less than the maximal residue limits or tolerance established by the European Union (16).

Concerning to microscopic examination of Liver of broiler chicken of G1 (120 mg/kg b.wt) showing distortion of hepatic cords, thickening of portal area associated with lymphocytic cellular infiltration, also showing multiple scattered foci of inflammatory cells and areas of necrosis with evidence of calcification. While Liver of broiler chicken of G2 (60 mg/kg b.wt) showing small foci of perivascular inflammatory cells infiltration with congestion of portal vessels, sinusoid, central vessels and some degeneration of hepatocytes. But in liver of broiler chicken in G3 (30 mg/kg b.wt) show mild degree of inflammation if form of minute foci of infl-amematory cells aggregation. This microscopic picture reflected the elevation of ALT and AST.

Microscopic examination of lung of broiler chicken of G1 (120 mg/kg b.wt) showing large nodule of inflammatory cells, while lung of broiler chicken of G2 (60 mg/kg b.wt) showing small aggregation of inflammatory cells. But in lung of broiler chicken of G3 (30 mg/kg b.wt) showing absence of inflammatory nodules.

Heart of broiler chicken of G2 (60 mg/kg b.wt) showing severe edema within the muscle bundles. While heart of broiler chicken of G3 (30 mg/kg b.wt) showing moderate degree of edema within the myocyte bundles.

Spleen of broiler chicken of G3 (30 mg/kg b.wt) showing well demarcation/ proliferation of white and red pulps. Our results agreed with (36), (16), (10), (35), (32), (30) and (26).

Conclusion

Florfenicol was absorbed rapidly; distributed and eliminated slowly it may be a suitable for treatment of common bacterial infections in broiler chicken. Moreover, our study provides data for its prudent use in suggesting a rational dosing with the withdrawal time to guarantee its safety for consumers. florfenicol in its trade mark Floricol® should be withdrawn at least 8dayes before marketing to ensure that the drug is completely eliminated from chicken tissue. Using HPLC method is a highly rapid and sensitive method in determining Florfenicol residues in chicken organs and tissues to detect the health hazard by consumption of chicken treated with florfenicol and the withdrawal time not admitted, as meat of chicken considered a cheap source of protein than others and more popular in Egypt.

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دراسة التأثير السمي لعقار الفلورفنيكول على دجاج التسمين وتقدير متبقياته فى الانسجة

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[1]مديرية الطب البيطري بالقليوبية، [2]قسم الطب الشرعي و السموم كلية الطب البيطري جامعة بنها

الملخص العربي

الفلورفنيكول هو أحد المضادات الحيوية الشائعة الاستخدام في قطاع الإنتاج الداجني. في هذه الدراسة تم إعطاء دواء الفلوريكول لدجاج عمر يوم واحد في ماء الشرب ولمدة ستة أسابيع وذلك لدراسة أثاره السمية على الأعضاء المختلفة، وظائف الكبد والكلى وحساب المتبقيات له في الأنسجة المختلفة. و في هذه الدراسة استخدم 160 كتكوت تسمين ابيض و قسمت الطيور إلى أربعة مجموعات الأولى تناولت الفلورفنيكول بنسبة 120 مجم/كجم من وزن الجسم كأربع أضعاف الجرعة العلاجية و المجموعة الثانية (60 مجم/ كجم من وزن الجسم) كضعف الجرعة العلاجية و المجموعة الثالثة (30 مجم/ كجم من وزن الجسم) كجرعة علاجية أما المجموعة الرابعة فتركت كمجموعة ضابطة تشرب مياه عادية و أدى استخدام الفلورفينيكول إلى نقص مستوى البروتين الكلى ،الألبومين والجلوبيولين مع زيادة في تركيز الكرياتينين و AST, ALT و وذلك في الجرعات العالية 60و120 مجم/ كجم أما عن المتبقيات من الفلورفينيكول في أنسجة الدجاج فكان أعلى تركيز للدواء في الكبد، الكلى ، الطحال، القلب والرئتين وكان أقل تركيز في عضلات الفخذ والصدر وقد استمر فترة السحب الدوائي من الجسم لتمتد إلى ثمانية أيام ليصل إلى الصفر.

(مجلة بنها للعلوم الطبية البيطرية: عدد 24 (1)، يونيو 2013: 209–217)