



IMMUNO TOXICITY OF FLORFENICOL IN BROILER CHICKEN

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ABSTRACT

Florfenicol, (structural analogue of thiamphenicol) is of great value in veterinary treatment of infectious diseases. The present study was designed to investigate the immune-suppressive action of Florfenicol either on humeral or cellular immunity. 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 decrease of body weight, decreased no of RBCs and WBCs counts, Hb and PCV, decrease of phagocytic activity, decreased (DLC) (Lymphocytes, Basophil, Oesinophils, heterophil and monocytes and about humeral immunity investigated that Florfenicol administration decreased serum albumin and α , β and γ globulins, also decreased ND antibody titers the decrease in these parameters is highly significant in G1 and significant in G2 and non significant in G3 compared to the control, from our results we concluded that Florfenicol is an immune-suppressive drug in dose dependent manner.

KEY WORDS: florfenicol, Immunotoxicity, 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 the poultry industry. In addition, antimicrobial drugs are used as feed additives to enhance growth and feeding efficiency of food animals. (17).Florfenicol is a monofluorinated analogue of thiamphenicol, has antibacterial activity against a broad spectrum of bacterial strains, including enteric bacteria that are resistant to chloramphenicol and thiamphenicol. Its activity is the same as that of thiamphenicol and chloramphenicol, inhibiting bacterial protein synthesis at the

ribosome (8). But differs in that it does not cause a dose-related reversible bone marrow suppression or irreversible aplastic anemia in people. Although it acts at the same site as chloramphenicol and thiamphenicol, the pharmacological composition of florfenicol makes it more resistant to deactivation by bacteria. (21). Florfenicol can induce immunosuppression in mice by inhibition of IgG1&IgG2 antibody production in serum, proliferation of spleen cells (3) and significantly inhibited in vitro phagocytosis activity of bovine blood neutrophils (21).

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 12th and 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 30 mg/Kg b.wt (therapeutic dose) orally in drinking water once/day- 4 days/ week. (1) & (10). G (4) : kept as control group and allowed to drink clean water.

2.2. Sampling:

Body weight: Individual b.wt determined weekly and estimated means b. wt.

Organ weight samples: Slaughtering 10 birds of each group at 20th and 10 birds at 40th day of age to obtain organ weight as relative organ weight (gm of organ/ 100 gm body weight) was estimated (15).

Blood samples: collected and divided into two parts: The first part: The blood sample was taken quickly (in heparinized tubes for phagocytic activity test)–(EDTA containing tubes for counting red blood cells (RBCS), total leukocytic count(WBCS), packed cell volume (PCV) and hemoglobin (Hb) and (sodium citrate containing tubes for differential leukocytic count).

The second part: blood was allowed to stand for one hour at room temperature and then centrifuged at 3000 rpm for 15 minutes for separation of serum.

Serum samples: serum samples was collected and stored at -20C° for humeral immunity tests (Electrophoresis of serum proteins) and (Haemagglutination inhibition HI specific antibody titer against ND) and also for serum biochemical tests (AST, ALT, Creatinine, total protein, Alb, Glob).

2.3. Histopathological investigation:

According to (9) Samples from spleen, thymus, & bursa of fabricius were preserved in 10% formalin.

2.4. Statistical analysis :

The data were calculated as mean \pm standard error. All statistical analysis was carried out according to (23).

3. RESULTS

Effect of treated chicken with Florfenicol on body weight showed in Table (1) highly significant decrease in body weight in G1 and G2 compared to the control. G3 showed that Florfenicol maintained body weight resemble that of the control if used in therapeutic dose. Effect of Florfenicol on relative Organ Weight and % to body weight at 40th Day of Bursa, Spleen, Thymus and number of follicles are showed in Table (2). The results showing highly significant and significant reduction in weight of Bursa, Spleen and Thymus in G1(120mg/kg b.wt) and G2 (60 mg/ kg b.wt) with non-significant effect on G3(30mg/kg b.wt) comparable to G4 (control), reduced No of follicles of bursa in all treated groups at 40th day. Table (3) Showing highly significant and significant reduction in count, Hb and PCV % in G1 (120 mg/ kg b.wt and G2 (60 mg/ kg b.wt) respectively with non-significant to G4

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Table (1) Effect of treated chicken with Florfenicol on body weight (mean \pm S.E.).

Period	1 st day	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week
Groups							
G1 (120 mg / kg)	50.77 ± 1.37	87.55 ± 4.33	280.09 ± 9.25	500.90 ± 14.41	933.70 ± 18.17	1200.14 ± 35.39	1543.83** ± 19.40
G2 (60 mg/kg)	50.57 ± 1.46	102.55 ± 3.270	390.66 ± 11.48	550.12 ± 14.05	950.50 ± 18.80	1305.5 ± 33.18	1609.17* ± 7.23
G3 (30 mg/kg)	50.15 ± 1.30	110.75 ± 3.36	400.25 ± 4.41	685.90 ± 15.95	1100.70 ± 17.28	1450.67 ± 19.94	1820.17 ± 7.23
G4	51.40 ± 1.24	102.50 ± 4.06	350.25 ± 6.80	650.80 ± 5.87	1066.70 ± 11.17	1411.14 ± 35.39	1849.66 ± 12.22
Control							

Table (2) Effect of Florfenicol on relative Organ Weight and % to B. Wt of Bursa, Spleen, Thymus and number of follicles at 40th day.

Organ		G1	G2	G3	G4
Bursa	Wt	0.12**	0.43*	0.67	0.87
	(gm)	± 0.03	± 0.33	± 0.03	± 0.09
	%	0.63	0.146	0.219	0.269
No of Folli		19.66	17.00	17.00	15.33
		± 0.58	± 0.058	± 0.58	± 0.67
Spleen	Wt	0.10**	0.13*	0.17	0.20
	(gm)	± 0.60	± 0.033	± 0.033	± 0.06
	%	0.04	0.05	0.055	0.06
Thym	Wt	0.50**	0.73*	0.77	1.07
	(gm)	± 0.29	± 0.09	± 0.15	± 0.66
	%	0.17	0.25	0.25	0.33

Table (3) Effect of Florfenicol on some Blood parameters (RBCs and WBCs count) of broiler chicken at 40th day.

	RBCs $\times 10^6$ /mm ³	WBCs $\times 10^3$ /mm ³	Hb (g/100mlbl.)	PCV (%)
G 1	2.12** ± 0.24	17.94** ± 0.89	11.67** ± 0.43	11.00** ± 1.25
G 2	2.63* ± 0.14	19.0* ± 2.42	12.87* ± 0.89	12.67* ± 0.54
G 3	3.65 ± 0.14	22.67 ± 8.24	15.93 ± 0.97	27.33 ± 1.79
G 4	4.00 ± 0.13	24.0 ± 1.91	16.07 ± 0.14	31.33 ± 1.91

Table (4) Effect of Florfenicol on Phagocytic Activity (m)

Groups	Phagocytosis %	
	At 20 th day	At 40 th day
G1	71.4**	68.4**
G2	69.2**	74.7**
G3	75.9*	77.3*
G4	84.4	85.3

reduction in G3 (30mg/ kg b.wt) compared (control). Table (4) Showing reduction of phagocytosis % in all treated groups highly significant in G1 (120 mg /kg b.wt) and G2 (60 mg/kg b.wt) and significant in G3(30 mg/kg b.wt) compared to G4 (control).

Table (5) Showing significant and highly significant reduction of lymphocytes, basophiles and eosinophils with non-significant increase in heterophiles and monocytes in G1 (120 mg/kg b.wt) and G2 (60mg/kg b.wt) with non-significant effect on G3 (30 mg/kg b.wt) compared to G4 (control). Table (6) Showing decrease in HI titer in G 1(120 mg/kg b.wt) and G 2 (60 mg/kg b.wt) after 2 weeks from ND vaccination compared to G 3 (30 mg/kg b.wt) and G 4(control). Effect of Florfenicol on serum proteins albumin and (α , β and γ globulin) in tested chicken Sera by Electrophoresis (KD) Showing highly significant reduction in albumin, α , β and γ globulin in G1 (120 mg/kg b.wt) and significant reduction in G2 (60 mg/kg b.wt) compared to G3(30mg/kg b.wt) and G4(control) (Table 7).

1. DISCUSSION

Florfenicol is a broad-spectrum, primarily bacteriostatic, antibiotic with a range of activity similar to that of chloramphenicol, including many gram-negative and gram-positive organisms

however, florfenicol does not carry the risk of inducing human aplastic anemia that is associated with chloramphenicol. Florfenicol has been demonstrated to be active in vitro and in vivo. It also has activity against some chloramphenicol resistant strains of bacteria possibly because it is less affected by the major enzyme produced in plasmid-mediated bacterial resistance against chloramphenicol and thiamphenicol (24). Florfenicol becomes increasingly utilized in poultry industry in the last few years. For the following purposes: (1) to treat diseases (2) as growth promoters, and (3) to improve feeds' nutritional efficiency (16). But their side effects cannot be excluded and so the uncontrollable use of florfenicol may lead to hazard effects on medicated broiler chicken. Concerning to table (1) showed growth retardation and significant decreased body weight in broiler chicken of G1 which received (120 mg/Kg body weight) and G2 broiler chicken which received (60 mg/Kg body weight) these results similar to results noticed by (27), (24) & (11) Sever weight loss may be due to decreased feed consumption or improper assimilation of feed due to its effect on liver which confirmed chemically on the study of liver function and in our histological study The decrease in body weight in our study may be due to tissue degeneration as recorded in our histological study.. but florfenicol maintained body weight resemble that of the control in broiler chicken of G3 received therapeutic dose (30 mg/Kg body weight) if compared to broiler chicken of G4 (control). Our results agreed with the results of (27) & (11). These results may be due to the anabolic effect of florfenicol and due to bacteriostatic action (8). And this explain its use as a routine work in the farm for its prophylactic effect, this difference may be due to the anatomical and physiological variations between the different species, or due to the manner of dosing where the bioavailability of florfenicol after I/M and oral administration was high with approximately 96.6% and 55.3% of being

Table (5) Effect of Florfenicol on different Blood Cell Count of broiler chicken at 40th day.

	Lymph	Basoph	Oesinoph	Heteroph	Mono
G1	14.24 ** ±0.11	0.23 ** ±0.24	0.93 ** ±0.07	71.42 ±0.31	16.91 ±0.70
G 2	16.25** ±0.80	0.45 * ±0.12	1.25 * ±0.24	69.76 ±0.05	14.11 ±0.43
G 3	20.85 ±0.20	1.75 ±0.03	1.91 ±30	62.80 ±0.30	11.30 ±0.25
G 4	21.65 ±0.77	2.85 ±0.30	2.15 ±0.55	63.71 ±0.42	9.80 ±1.57

Table (6) Effect of Florfenicol on HI specific antibody titer against Newcastle Disease ND in serum of treated chicken versus to control group

Groups	At 14 th day	At 21 th day	At 40 th day
G1	0.8 ±0.22	1.304 ±0.23	0.304 ±0.24
G2	0.8 ±0.22	1.906 ±0.25	0.502 ±0.23
G3	0.9 ±0.23	2.107 ±0.27	0.803 ±0.27
G4	0.8 ±0.20	2.107 ±0.26	0.903 ±0.32

gdgdgdg

Table No (7) Effect of Florfenicol on serum proteins albumin and (α, β and γ globulin) in tested chicken Sera by Electrophoresis (KD).

Serum proteins(KD)	G1	G2	G3	G4
Albumin	32.63 ±0.39	34.6±1.8	46.75±2.6	52.16±2.2
α globulin	62.87±0.21	79.14±1.6	72.52±2.24	62.68±2.24
β globulin	84.93±0.69	97.48±1.1	106.6±2.2	103.99±2.6
γ globulin	120.1±0.46	173.22±1.5	195.72±2.8	154.97±2.8

Histopathological Effect of Florfenicol on Bursa of Fabrecious:

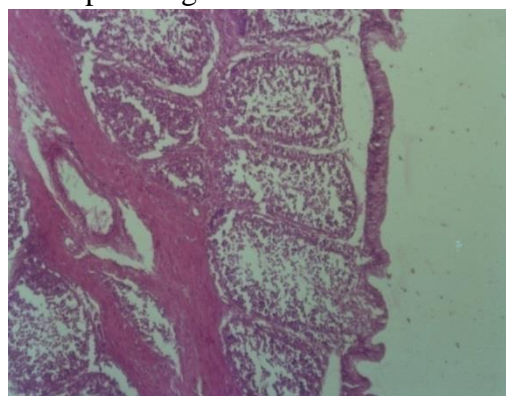


Fig No (1) Bursa of Fabrecious of G1 administered administered 120 mg/ Kg B. Wt Show depletion of lymphoid follicles and desquamation of mucosal epithelium

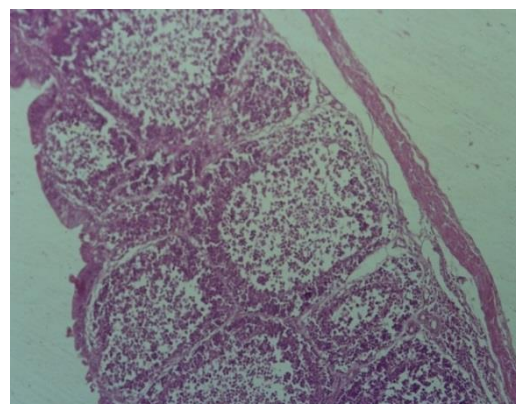


Fig No (2) Bursa of Fabrecious of G 2 administered 60 mg/ Kg B. Wt Show focal desquamation of some lining epithelium and slight lymphoid.

absorbed respectively. Furthermore, the elimination half-life after I/M was longer than that after oral administration indicating slow release and absorption from injection site (1). Concerning to effect of Florfenicol on weight of bursa, thymus and spleen in table (5) showed significant reduction in weight of organs of immunity in G1 where broiler chicken received (120 mg/Kg body weight) and G2 where broiler chicken of received (60 mg/Kg body weight) this indicate that Florfenicol is immune--suppressant organ. Our results agree with (5)& (18), as we do our best, there are insufficient previous published data concerning the effect of Florfenicol on organ weight, Also agree with (3) showed that florfenicol damaged the immune organs irreversibly in short time, The result showed that florfenicol has no harm to spleen of chickens, but thymus, cortex of bursa, tonsil of Fabricius to some extent were harmed severely. Regarding to the effect of florfenicol on blood parameters of chicken, data of table (6 and 7) showed that there was a highly significant decrease in RBCs count, WBCs count, Hb and PCV% in G1 where broiler chicken received (120 mg/Kg body weight) slight significant in G2 where broiler chicken received (60 mg/Kg body weight) and 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 (12), (5) Also agree with (24), (24)& (16). Similar results were recorded by (13) that more ever toxic changes in the. From results concerning the effect of florfenicol on some blood parameter we can suggest that the observed results similar to that obtained by chloramphenicol where florfenicol is a fluorinated derivative of chloramphenicol and thiamphenicol which has a fluorine atom instead of hydroxyl group located at C-3. (22). So that, because of the well-known risk of a plastic anaemia of chloramphenicol, its use in human and veterinary medicine is limited by its toxicity. Theoretically possible

that florfenicol could cause some dose-dependent, reversible bone marrow suppression, but it has not been clinically reported. This phenomenon is not considered a side/adverse effect with normal clinical use, but an awareness of this possibility may be useful if long-term therapy with this medication is considered (23). Our results disagreed with (19). Concerning the effect of florfenicol on phagocytic activity % in table (12) showed decreased phagocytic activity in all treated groups highly significant in G1 where broiler chicken received (120 mg/Kg body weight) and significant reduction in G2 where broiler chicken received (60 mg/Kg. b. wt) and G3 where broiler chicken received (30 mg/Kg. b. wt) compared to G4 (control). These results agreed with (7), (25) and (19). On contrary our results are disagreed with (22) who showed no effects were observed for florfenicol on phagocytosis Transmission. Electron micro-scopic examination showed that at the high concentration of florfenicol 99% of the treated neutrophils were abnormal. Results indicated that florfenicol don't altered neutrophils function but they did alter neutrophils morphology. Table (13) showed different white blood cells (Lymphocytes, Basophil, Oesinophils, heterophil and monocytes). There were significant reduction in all treated groups of lymphocytes, but highly significant in G1 and G2 .significant reduction in basophile and non-significant decrease in oesinophile with highly significant increase in monocytes and heterophile. These results agreed with (5). Decreased lymphocytes (lymphopenia) in G1 and G2 may be due to toxic effect of florfenicol on lymphoid tissues as lymphoid depletion in bursa of fabricius and thymus was recorded in our histological results fig(1) and fig(2), also oesinopenia and basopenia caused by florfenicol due to it acts as stress factor. Nearly similar results were reported by (18). Also, agree with Chrzastec *et al.*, (2011). Also with (17) Similar results were recorded by (2 & 3). Neutrophils number increase due

to their sensitivity to chemotactic or leukotactic effect of florfenicol. These results disagree with (22 & 19). Regarding to the effect of Florfenicol on humeral immunity firstly by titration of ND antibodies, we discovered reduction of AB titer in all treated groups after 2 weeks highly significant in G1 where broiler chicken received (120 mg/Kg body weight) and G2 where broiler chicken received (60 mg/Kg body weight) with non significant effect in G3 where broiler chicken received (120 mg/Kg body weight) compared to G4(control). Our results agree with (7) Also in pig agree with (15) against classical swine fever virus with (16), (17) and (3). These results may be attributed to the immune-suppressor effect of florfenicol. However, not agreed with (28). Secondly effect on globulins specially γ globulin which formed extra-hepatically in lymph nodes and other cells of reticulo-endothelial system of spleen and bone marrow. Florfenicol decreased γ globulin highly significantly in G1 where broiler chicken received (120 mg/Kg body weight) and G2 where broiler chicken received (60 mg/Kg body weight) with non significant effect on G3 where broiler chicken received (30 mg/Kg body weight) compared to G4(control), these results may be due to its toxic effect on organs of immunity (bursa, spleen and thymus) which clearly observed from decrease relative weight and from histopathology. Our results agree with (18, 27 and 3).

Conclusion:

Our results indicate that Floricol® Florfenicol oral solution each one milliliter contains 100 mg florfenicol base showed a protective effect on chicken's body weight if used in therapeutic dose (30 mg/Kg B.wt) but in G2 (60 mg/Kg B.wt) and G1 (120 mg/Kg B.wt) lead to lowering body weight. Also, leads to lowering the relative chicken weight of (liver, heart, lung, brain and proventriculus) and increase the relative chicken weight of kidney and gizzard in G1 and G2, on blood

parameters Floricol® lowering RBCs, WBCs count, Hb and PCV in G1 and G2. Concerning to effect of Floricol® on immunity the drug is immunosuppressive on G2 (60 mg/Kg B.wt) and G1 (120 mg/Kg B.wt) broiler chicken showed reduction in phagocytic activity in all treated groups but significantly in G1 and G2, also broiler chicken showed reduction of different leukocytic count in all treated groups but significantly in G1 and G2, Floricol® also reduced humeral immunity showed lowering of Haemagglutination inhibition antibody titer against Newcastle disease virus vaccine (NDVV) significantly in G1 and G2 and non significantly in G3 compared to control.

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دراسة بعض التأثيرات السمية المناعية لعقار الفلورفنيكول في دجاج التسمين

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الملخص العربي

الفلورفنيكول هو أحد المضادات الحيوية الشائعة الاستخدام في مجال الإنتاج الحيواني لعلاج الأمراض المختلفة. وهذه الدراسة تمت لبيان الآثار السمية للفلورفنيكول على الحالة المناعية لدجاج تسمين تناولت عقار الفلورفنيكول في ماء الشرب لمدة ستة أسابيع. وفي هذه الدراسة استخدم 160 كتكوت تسمين ابيض و قسمت الطيور إلى اربعة 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 فتרכت كمجموعة ضابطة تشرب مياه عادية فقد أدى استخدام الفلورفنيكول إلى نقص في عدد كرات الدم الحمراء و البيضاء، الهيموجلوبين مما نتج عنه أنيميا نقص الهيموجلوبين لدجاج التسمين وذلك في الجرعات العالية 60 و 120 مجم/كجم. إما عن الاختبارات المناعية فقد أدى استخدام الفلورفنيكول إلى نقص في نشاط الخلايا الأكلة بجسم الطائر وذلك في كل المجموعات المعالجة بالفلورفنيكول ويزداد النقص في الجرعات 60 و 120 مجم/كجم وذلك مقارنة بالمجموعة الثالثة والمجموعة الضابطة. وأدى أيضا استخدام الفلورفنيكول إلى نقص في عدد الخلايا الليمفاوية والخلايا السرية الاصبغ بالاصباغ القاعدية والخلايا الوردية وذلك مع زيادة الخلايا الأحادية والخلايا المتعددة الأشكال. كما لوحظ نقص في معدل الثغرة الدموي HI لتحسين النيوكاسل و ذلك في كل المجموعات المعالجة بالفلورفنيكول ويزداد النقص في الجرعات 60 و 120 مجم/كجم وذلك مقارنة بالمجموعة الثالثة والمجموعة الضابطة كما لوحظ أيضا نقص في كمية γ globulin وذلك باستخدام التحليل الطيفي للسيرم كما أدى إلى تآكل الخلايا الليمفاوية في الطحال وكيس فابريشيس مما سبق نستنتج إن الفلورفنيكول هو مثبط مناعي في الجرعات العالية على دجاج التسمين.

(مجلة بنها للعلوم الطبية البيطرية: عدد 24 (1)، يونيو 2013: 230-239)