

Study on The effectiveness of florfenicol in the treatment of coli-septicaemia infection in Muscovy duckling.

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ABSTRACT

The activity of florfenicol In-vitro against freshly clinical isolate of E. Coli (O.78) was tested. Antibiogram study revealed that florfenicol, enrofloxacin and gentamycin were the most effective antibacterials against E.coli isolated from naturally infected ducklings, the minimal inhibitory concentrations MICs of florfenicol against tested E. coli was 0.125 ug/ml.

In-vivo florfenicol was highly effective against naturally infected ten – day old Muscovy duckling with colisepticaemia when given at a dose level of 30 mg / kg. b. wt (therapeutic dose) and 60 mg /kg. b.wt. (double therapeutic dose) for three successive days in drinking water compared to infected non treated ducklings. The drug at a dose level of 15 mg / kg. b. wt(half therapeutic dose) reduce mortality from 35% (infected, non treated group) to 5% whereas the therapeutic and double therapeutic doses produced no mortalities . Also infected treated duckling showed significant increase in body gain, feed consumption and feed conversion rate. The study revealed that the drug at 30 mg / kg.b. wt had no adverse effect on erythrocytic count, haemoglobin and packed cell volum and some liver and kidney functions tests of infected ducklings. The forementioned haematological and biochemical parameters were disturbed in infected ducklings treated with 60 mg/kg. b. wt. and returned to its normal condition one week post medication. Histopathological changes in infected and treated ducklings were demonstrated.

INTRODUCTION

Colisepticaemia is one of the most serious bacterial diseases of duckling result in severe economic losses. The disease is caused by E. coli infection. Infection of young birds with E. coli evokes a high mortality start in 2 – 3 days post-exposure (1). Chloramphenicol is an old commonly used drug to control disease out breaks nevertheless, resistance to this antimicrobial agent has been recorded (2,3). In addition, this antimicrobial has toxic effect (4). Therefore continuous research had led to development of new generation with a high efficacy and low toxicity as thiamphenicol or florfenicol, these drugs are similar in structure with substitution of fluorine atom in the site of hydroxyl group of chloramphenicol in case of florfenicol. They act by inhibition of bacterial protein synthesis, binding to 50s ribosomal subunits of susceptible bacteria (5,6). Bacterial resistance to both chloramphenicol and thiamphenicol is due to the presence of chloramphenicol acetyltransferase (CAT). Several bacterial strains that are highly resistance to both chloramphenicol and thiamphenicol are highly sensitive to

florfenicol. Also the structural modifications in the design of florfenicol have given it distinct advantages relating to safety and efficacy over thiamphenicol and chloramphenicol (5, 7, 8).

Florfenicol, like thiamphenicol has sulfomethyl group instead of the nitro group of the chloramphenicol aromatic ring that has been associated with chloramphenicol induced non dose related irreversible aplastic anemia in humans (9). Hence, since August 1994 its use in food producing animals was banned in the European union (10). Florfenicol has been introduced into the veterinary medicine to replace chloramphenicol (11).

The aim of this study was planned to evaluate the therapeutic efficacy of florfenicol for the treatment of natural infection of E. coli in Muscovy duckling besides In-vitro activity of the drugs on the isolated E. coli compared with other commonly used antimicrobials.

MATERIAL AND METHODS

Drugs

Florfenicol (Florecol)^R is an oral 10% solution From Pharmaswede – Egypt 10th of Ramadan city.

Bacterial strain

E. coli Strain (O.78) was isolated and identified biochemically and Serologically according to (12) from duck farms infected with colisepticaemia in Diarb Nigm city sharkia Governorate.

Commercial sensitivity discs

These discs were florfenicol (FFC 30ug), Enrofloxacin (En, 5ug), Gentamycin (Gn, 10 ug), Tetracycline (T, 30ug) Doxycycline (Dc, 30 ug) combination of sulfamethoxazol and trimethoprim (SXT, 25ug) and chloramphenicol (C, 30 ug).

Media

MacConkey agar, MacConkey broth, Mueller Hinton agar and nutrient broth (oxoid LTD, England) were used for determination of in vitro activity of florfenicol.

In vitro study

The isolated strain of *E. coli* was tested for its sensitivity to different antimicrobial agents using disc – diffusion method (13). The (MICs) of florfenicol and other antimicrobials were determined using the serial dilution tube technique (14).

In vivo study

One hundred, ten day old Muscovy ducklings from farms in sharkia Governorate weighing about 150-200 gm were employed for this study. Twenty of them were apparently clinically healthy free from *E. coli* and the rest were naturally infected with coli-septicaemia . Water and commercial feed (five stars) were provided ad libitum. At the age of ten days all ducklings were grouped into five equal groups each of twenty.

The first group was left as a control (non infected non treated). The other four group were naturally infected with *E. coli* , the second group was left as a positive control (infected non treated), the third group was orally treated with half therapeutic dose of florfenicol (15 mg / kg. b. wt) for 3 successive days, the fourth group was orally treated with the therapeutic

doses(30 mg / kg. b. wt) of the drug for 3 successive days while fifth group was treated with double therapeutic dose (60 mg / kg. b. wt) for 3 successive days.

Birds were examined daily for clinical signs of disease and mortality . post-mortem lesions in dead duckling were recorded and trials for reisolation of *E. coli* from liver and heart of infected groups were conducted using MacConkey broth and agar.

Total feed consumption and body gain were recorded before and post – treatment.

At the end of treatment and one week post treatment, five ducklings from each group were sacrificed, two blood samples were collected, the first sample was collected on sodium EDTA for haematological studies. Erythrocytic count was performed using an improved Neubauer haemocytometer and Nutt and Herrick solution as a special diluent for chickens blood (15). Haemoglobin estimation was performed by the test-kit (16) The packed cell volume (PCV) was estimated by the microhaematocrite capillary method (17). Mean corpuscular volume was calculated from the above haematological parameters. The second blood samples were collected and the serum was separated for evaluation of some biochemical parameters: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) (18) total proteins and albumin (19). Serum creatinine (20) were assayed and serum uric acid were estimated (21).

Samples from liver, kidneys, and heart were taken from sacrificed birds and fixed in 10% formalin solution and prepared for the histopathological examination (22).

The obtained data were tabulated and statistically analysed (23).

RESULTS

Bacteriological examination of the diseased duckling revealed the presence of *E. coli* . The mean zones of inhibition, and MICs for florfenicol and other antimicrobial

agents against isolated *E. coli* are shown in Tables 1 and 2.

The clinical signs of diseased ducklings were general signs of an illness, diarrhoea, lameness and respiratory signs followed by death.

The gross lesions were pericarditis, perihepatitis and air sacculitis.

The previous symptoms and gross lesions were completely disappeared under the influence of the therapeutic and double therapeutic doses of the drug. Florfenicol with half therapeutic dose reduced mortality from 35% to 5% compared to infected non treated ducklings Table 3.

The infected duckling showed a significant decrease in body gain besides the feed conversion rate (F.C.R.) when compared with the control group. The infected and treated duckling showed significant increase in body weight gain, higher than that infected untreated ducklings especially on 7th day post medication Table 3. The infected duckling with *E. coli* showed a significant decrease in RBC count, Hb concentration and PCV compared with the control group as seen in Table 4. The results of haematological studies in infected and treated duckling with double therapeutic dose on the 1st day post medication showed a significant decrease in RBCs count, Hb concentration and PCV compared with control, thereafter on the 7th day post medication these parameters were improved toward the normal level Table 4.

Alteration of some serum biochemical values in infected duckling and infected treated with different doses of florfenicol was detected Table 5. Treatment of infected duckling with florfenicol improved these parameters toward its normal levels after seven day post medication.

Histo pathological examination

Heart; heart of infected non treated duckling showed pericarditis with thickened oedematous membrane containing congested blood capillaries, inflammatory fluid and lymphocytic cells Fig 1. In the treated duckling the inflammatory changes in the pericardium disappeared Fig 2.

Liver: the hepatic cells of infected duckling suffered from degenerative changes and the hepatic tissue showed numerous leukocytic infiltration in the portal areas, the hepatic sinusoides and blood vessels appeared congested and engorged with blood and the wall of blood vessels was thickened Fig 3. In case of group treated with half therapeutic dose the degenerative changes of hepatic cells were slightly ameliorated and the blood vessels were dilated and slightly engorged with blood Fig 4.

Kidneys: Kidneys of infected ducklings showed degenerative changes of the tubular epithelium with congestion of blood vessels and inter – renal capillaries Fig 5. Duckling treated with therapeutic dose showed slight degeneration in the tubular epithelium beside slight congestion and dilatation of blood vessels Fig 6.

Table 1. *In-vitro* susceptibility of obtained *E. coli* (O78) isolated from infected duckling to florfenicol and some commonly used antimicrobial drugs. .

Antimicrobial agent	Disc potency UG	Standard degree of sensitivity	Mean zone of inhibition (m.m)
Florfenicol	FFC 30	< 18	23
Enrofloxacin	EN 5	< 22	23
Gentamycin	GM 10	< 15	19
Tetracycline	T 30	< 14	17
Doxycycline	DO 30	< 18	16
Chloramphenicol	C 30	< 18	12
Sulfa and trimethoprim	SXT 25	< 16	13

Table 2. MICs of florfenicol and other commonly used antimicrobial drugs against isolated *E.coli* (ug/ ml)

Antimicrobial agent	M.I.C ug /mL
Florfenicol	1.0
Enrofloxacin	0.06
Gentamycin	0.5
Tetracycline	16.0
Doxycycline	8.0
Chloramphenicol	16.0
Sulfa and trimethoprim	250

Table 3. Mortality rate percent, mean body weight gain , feed consumption and feed conversion rate (FCR) on the 1st and 7th day post oral medication with florfenicol for 3 successive days .

Group	Mortality rate %	1 st -day post medication			7 th -day post medication		
		Body weight gain(gm)	Feed consumption gm/bird	FCR	Body weight gain(gm)	Feed consumption gm/bird	FCR
1-control non infected, non treated	-	155 ^a	350	2.25	350 ^a	820	2.34
2-infected non treated	35%	80 ^c	260	3.25	160 ^c	450	2.81
3- Infected and treated with 15mg/kg.b.wt	5%	120b ^c	310	2.58	330 ^{bc}	730	2.60
4- Infected and treated with 30mg/kg.b.wt	-	147 ^a	345	2.35	330 ^{ab}	800	2.42
5-Infected and treated with 60mg/kg.b.wt	-	140 ^{ab}	340	2.41	320 ^{ab}	780	2.43

The mean values with different letters are significantly different with each other (P < 0.05).

Table 4. Effect of oral administration of florfenicol for 3 successive days on some haematological parameters in naturally infected duckling with *E.coli* (n = 5).

Group	1 st day post medication			7 th day postmedication		
	RBCs 10 ⁶ /UL	H.b. gm/dl	PCV gm/dl	RBCs 10 ⁶ /UL	H.b. gm/dl	PCV gm/dl
1-control non infected, non treated	3.05 ± 0.31	11 ± 0.33	37.5 ± 1.09	3.1 ± 0.26	11 ± 0.37	39.5 ± 2.96
2-infected non treated	2.46 ± 0.12*	8.96 ± 0.91*	32.1 ± 1.29*	2.1 ± 0.19**	9.1 ± 0.51**	33.9 ± 1.12
3- Infected and treated with 15mg/kg.b.wt	2.87 ± 0.36	9.98 ± 0.32*	34.9 ± 0.31*	2.87 ± 0.16	10.1 ± 0.66	35.7 ± 1.72
4- Infected and treated with 30mg/kg.b.wt	2.95 ± 0.19	10.1 ± 0.15	35.1 ± 0.22	3.03 ± 0.21	11.0 ± 0.71	39.6 ± 2.12
5-Infected and treated with 60mg/kg.b.wt	2.65 ± 0.21*	9.30 ± 0.42*	33.7 ± 1.21*	3.01 ± 0.15	10.8 ± 0.86	39.7 ± 2.36

* Significant at (P < 0.05).

** Significant at (P < 0.01).

*** Significant at (P < 0.01).

Table 5. Effect of oral administration of florfenicol for three successive days on some serum biochemical values in naturally infected duckling with *E. coli* (n = 5)

Group	1 st day post medication						7 th day post medication					
	AST IU/l	ALT IU/l	Total proteins gm/dl	Albumin gm/dl	Uric acid mg/dl	Creatinine mg/dl	AST IU/l	ALT IU/l	Total proteins gm/dl	Albumin gm/dl	Uric acid mg/dl	Creatinine mg/dl
1-control non inf. non treated	36 ± 3.36	0 ± 0.43	3.8 ± 0.04	1.62 ± 0.17	3.7 ± 0.43	0.5 ± 0.08	37.2 ± 0.9	2.5 ± 0.35	3.7 ± 0.03	1.51 ± 0.09	3.1 ± 0.07	0.5 ± 0.3
2- Inf. non treated	56 ± 2.20**	11.2 ± 0.5***	4.3 ± 0.16*	1.12 ± 0.12*	6.8 ± 0.59**	2.3 ± 0.36**	65 ± 1.2***	16.21 ± 0.57***	3.69 ± 0.19*	0.99 ± 0.03*	7.2 ± 0.5**	2.6 ± 0.6***
3- inf. and treated with 10mg/kg. b. wt	4 ± 1.60*	± 0.14**	± 0.6*	± 0.06*	± 0.19*	± 0.31*	± 0.16	± 0.58*	± 0.19	± 0.03	± 0.21*	± 0.1*
4- inf. and treated with 30mg/kg. b. wt	35 ± 1.7	8.90 ± 0.42	3.32 ± 0.16	1.56 ± 0.1	3.5 ± 0.16	1.1 ± 0.04*	35.2 ± 0.13	8.74 ± 0.41	3.5 ± 0.2	1.9 ± 0.9	3.2 ± 0.1	0.81 ± 0.9
5- inf. and treated with 60mg/kg. b. wt	44.1 ± 0.46*	12.3 ± 0.12**	4.26 ± 0.09**	1.25 ± 0.06*	4.6 ± 0.47**	1.9 ± 0.37**	36.4 ± 0.9	8.71 ± 38	3.4 ± 0.17	1.8 ± 0.7	3.3 ± 0.12	0.86 ± 0.6

* Significant at (P < 0.05)

** Significant at (P < 0.01).

*** Significant at (P < 0.001).

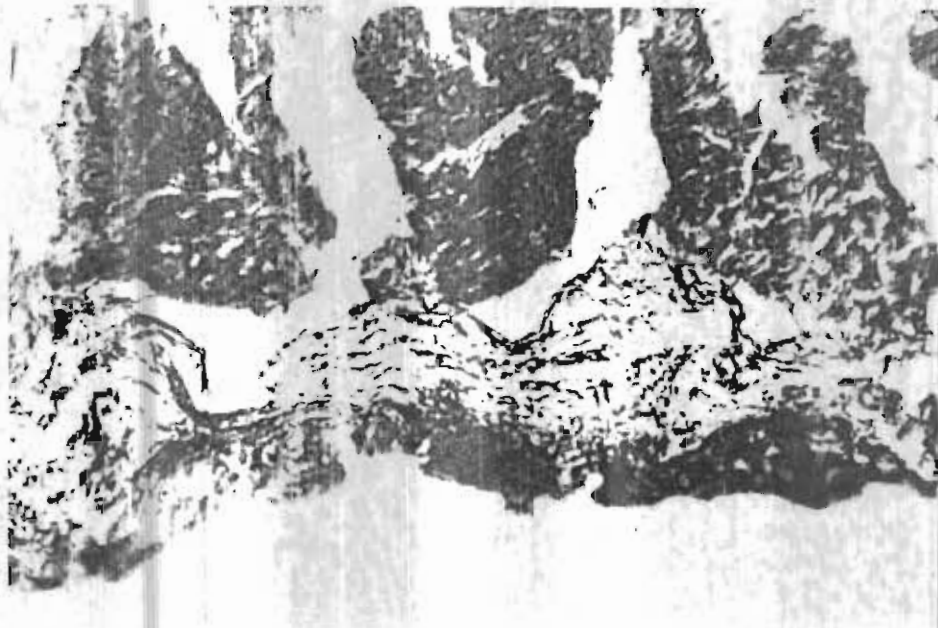


Fig 1 . Heart section of infected non treated duckling showing fibrinous pericarditis. (H and E, X.300).

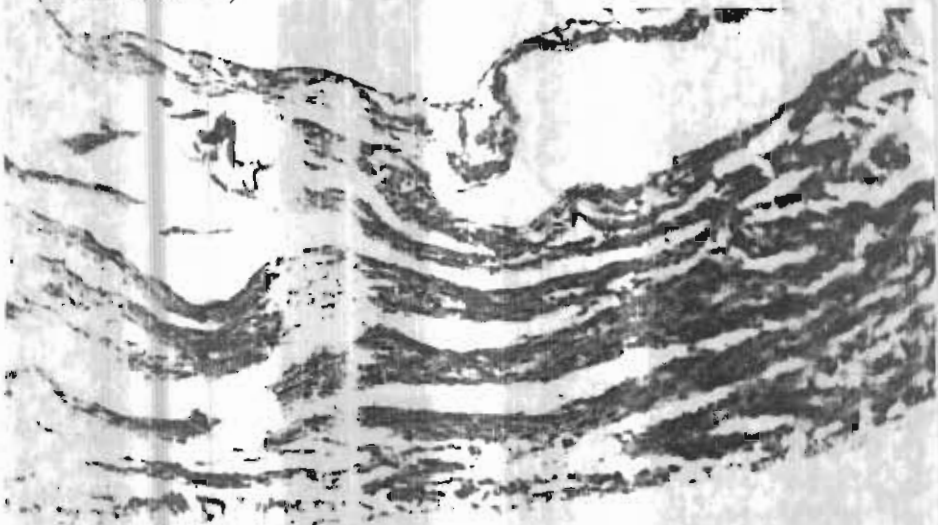


Fig 2 . Heart section of infected duckling treated with therapeutic dose of florfenicol showing disappearance of inflammatory changes of the pericardium (H and E, X 300).

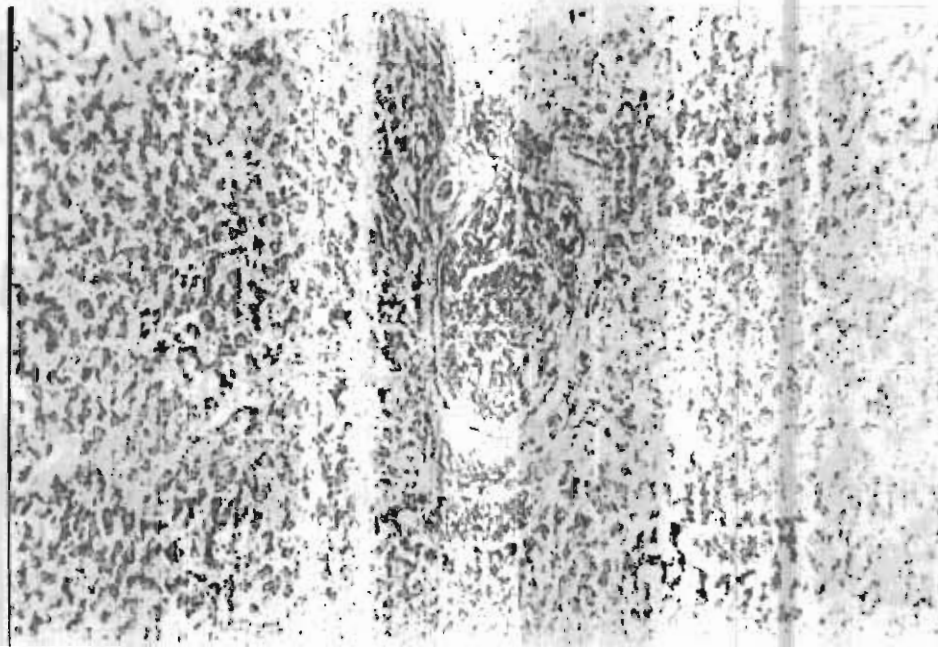


Fig 3'. Liver section of infected non treated duckling showing hepatic cell degeneration and congestion of blood vessels (H and E ,X 300).

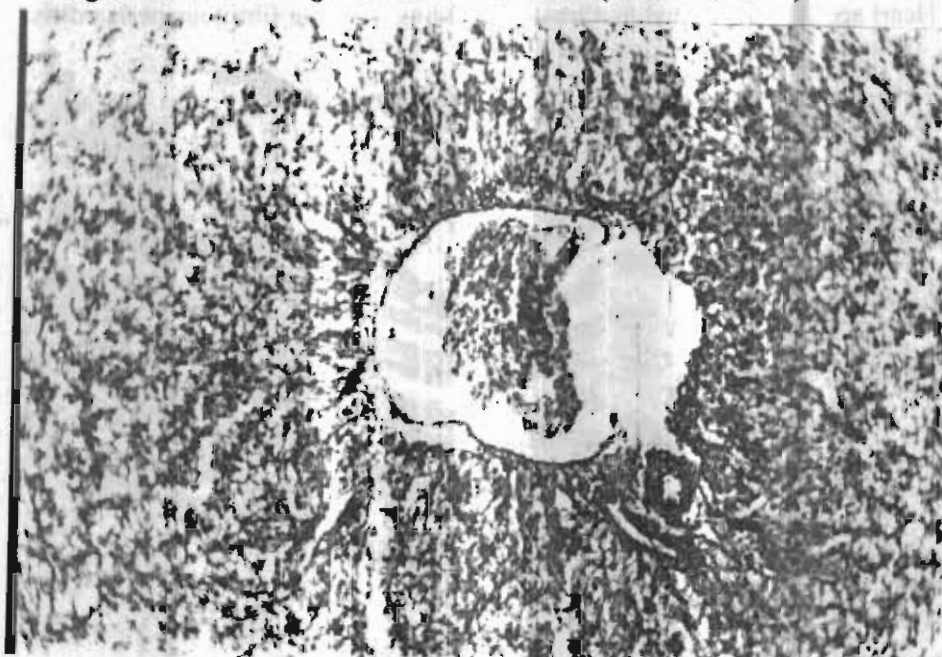


Fig 14. Liver section of infected duckling treated with half therapeutic dose of florfenicol showing slight hepatic cell degeneration (H and E, X 300).

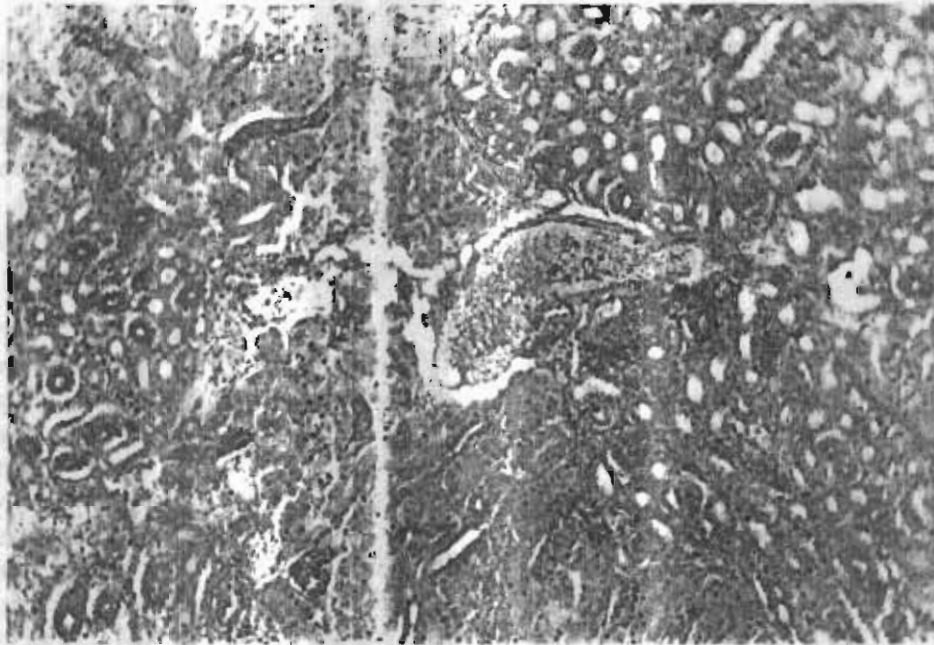


Fig 5, Kidney section of infected, non treated duckling showing tubular degeneration with congestion of blood vessels (H and E, X 300).

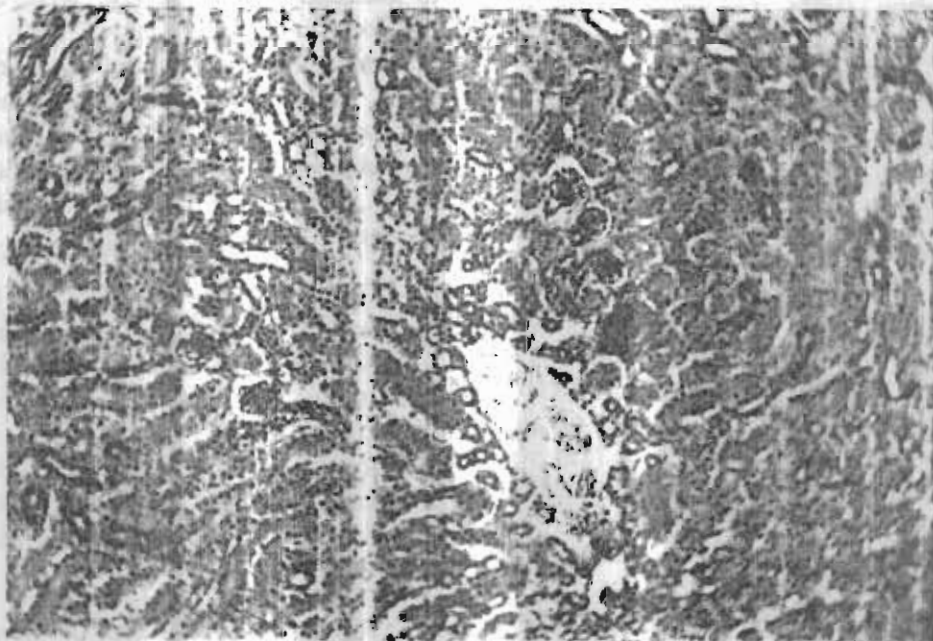


Fig 6, Kidney section of infected, duckling treated with therapeutic dose of florfenicol showing slight tubular degeneration with slight congestion of blood vessels (H and E, X 300).

DISCUSSION

Florfenicol, a structural analogue of thiamphenicol, is of great value in the treatment of infectious diseases (5). Recently it has been reported that florfenicol showed greater activity than chloramphenicol and thiamphenicol, especially against *Pasteurella*, *Salmonella*, *E.coli*, and *Staphylococcus aureus* (24,8).

In the present investigation, using the disc diffusion test, comparison of antimicrobial activity of florfenicol (30ug) with other widely used antibiotics revealed more potent activity of the drug on *E. coli* than other tested drugs (Inhibition zone 33 mm), the same results were previously reported in chickens (25) and in muscovy duckling (26). In addition, the MIC of the drug was determined as a value (1.00 ug/ml) below the average of concentration of the drug (3.2 ug/ml) in serum of Muscovy ducks after oral dosing of 30mg/kg. b. wt (27).

The obtained results showed that florfenicol at a dose level of 15mg/kg.b.wt reduced mortality rate from 35% (infected non treated) to 5% whereas the drug at 30 and 60mg/kg. b.wt completely prevented mortality in duckling infected with *E.coli*. This may be attributed to pharmacodynamic characters of florfenicol. Florfenicol has low protein binding and reaches higher level in tissues than serum and so it reaches clinically effective concentrations at sites of infection (27,28). Our results are in agreement with (29) who recorded the least mortality rate in ducklings experimentally infected with *E.coli* and treated with florfenicol.

In the present study administration of florfenicol with different doses resulted in a significant increase in the body weight, feed consumption and feed conversion rate on 7th days post treatment and the body weight returned to the normal level. This may be attributed to the antimicrobial effect of the

drug which consequently improves the metabolic activity of the bird (30). Our results are in agreement with (29) who recorded that no difference in live weight gain was found among the duckling experimentally infected with *E.coli* and treated with florfenicol and the healthy non infected ducklings.

Although the drug at a dose level of 60 mg/kg. b. wt succeeded in preventing mortality due to *E.coli* infection, however a significant decrease of RBCs count, Hb concentration and PCV, were recorded similar results were reported (4) which showing occurrence of normocytic normochromic anaemia in buffalo after administration of florfenicol. Our results revealed that therapeutic dose of florfenicol displayed non significant changes in measured haematological parameters previously cited report (31). The results of haematological study in the infected group revealed macrocytic anaemia as that RBCs counts and Hb concentration and PCV. were decreased significantly. This may be due to haemorrhagic effect of *E.coli* or its endotoxin which cause intravascular destruction of erythrocytic cells in the body tissues as seen histopathologically in the liver and kidney tissues. These Nearly similar results were reported (1,15).

The significant increase in the liver enzymes (AST and ALT) in the present study in the infected ducklings could be due to the degenerative changes, necrosis and haemorrhage induced by bacteria or its toxins with liberation of these enzymes into serum. The increase activity of AST and ALT have been associated with hepatocellular damage in ducks (32).

The antibacterial effect of the drug was evident by improvement of the biochemical parameters of the infected and treated ducklings especially at 7th day post medication with therapeutic dose where these enzymes were decreased significantly compared with infected non treated one, this may be due to

decreased liver cell damage which was demonstrated histopathologically. These recorded results were supported by previous results (7). On the contrary, the administration of double therapeutic dose of florfenicol showed a significant increase in serum AST, ALT on 1st day post medication, this change was reversible returned to normal level after one week post treatments, the same result was reported in rats injected with florfenicol (100 mg/kg) and showed decreased weight gain and severe damage to kidney and liver (33).

Nevertheless hypoalbumenia, a significant increase of serum total proteins in infected group in our study was detected. Similar findings were mentioned (32), this may be due to destructive effect of bacteria and its toxin on the liver cells which is the main sources of albumin and protein synthesis in the body (34). Our results on 7th day post medication with florfenicol revealed improvement the level of total proteins, and albumin this may be due to decrease liver cell destruction.

The biochemical findings of the kidney function tests of the infected non treated duckling in this study denoted very highly significant increase in the level of serum uric acid and creatinine as compared with healthy control group. These may be attributed to the renal damage which could be due to bacteria and its toxin, these parameters showed significant decrease post treatment with therapeutic and double therapeutic doses of florfenicol. These results indicated that florfenicol could protect the kidney from nephrotoxicity induced by coli-septicaemia when used as treatment after infection. Using florfenicol at therapeutic and double therapeutic dosage for the treatment of bovine respiratory infection had an encouraged results (35).

The histopathological findings could be attributed to septicemic effect of *E. coli* upon

the blood vessels, serous membranes and the parenchymatous organs (1,15).

Measurement of the forementioned liver and kidney functions one week post medication with therapeutic and double therapeutic doses of florfenicol revealed an improvement of altered parameters towards the normal levels. These results denote that the liver and kidney tissues was not severely damaged these findings coincide with several studies that referred to safety of the drug (7,36). In conclusion these results proved the efficacy of florfenicol in the control of a natural colisepticaemia infection in Muscovy ducklings.

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الملخص العربي

دراسة على فاعلية الفلور فينيكول في علاج الإصابات بالميكروب القولوني العصوي بالبطن المسكوفي

أسامة السعيد عبدالله أحمد محمود أحمد حمودة صبري محمد محمد العكشة

الفلور فينيكول هو مضاد بكتيري حديث مشابه في تركيبه للكلورمفينيكول ولكنه ذو كفاءة وفاعلية أعلى من الكلورمفينيكول وأكثر أماناً عند استعماله.

وفي هذه الدراسة تم عزل ميكروب القولوني العصوي من أحد مزارع البطن المسكوفي بدير نجم - شرقية وأجرى له اختبار حساسية باستخدام أقراص الحساسية وسنبيب التخفيف واتضح من اختبار التأثير الفلور فينيكول القوي على الميكروب المعزول مقارنة بالعقاقير الأخرى شائعة الاستخدام.

بالإضافة لما سبق فقد تم تقييم فاعلية الفلور فينيكول في علاج كتاكيت البطن المسكوفي المصابة طبيعياً بميكروب القولون العصوي حيث تم أخذ البطن (عدد 100) من مزرعتين إحداهم سليمة (20 بطة) والأخرى مصابة (80 بطة) وقسم البطن السليم والمصاب على خمسة مجموعات كلاً منها يحتوي على 20 بطة على النحو التالي :-

المجموعة الأولى: مجموعة ضابطة سليمة وغير معالجة.

المجموعة الثانية: مصابة وغير معالجة.

المجموعة الثالثة: مصابة ومعالجة بالفلور فينيكول بمعدل 10 ملجم / كجم وزن حي نصف الجرعة العلاجية".

المجموعة الرابعة : مصابة ومعالجة بالفلورفينيكول بمعدل ٣٠ ملجم /كجم وزن حي "الجرعة العلاجية".

المجموعة الخامسة : مصابة ومعالجة بالفلورفينيكول بمعدل ٦٠ ملجم /كجم وزن حي " ضعف الجرعة العلاجية".

استمر العلاج لمدة ثلاثة أيام متتالية عن طريق مياه الشرب واستنادا إلى الأعراض الأكلينيكية ونسبة النفوق والصفة التشريحية ومعدل استهلاك العلف ومعدل التحويل الغذائي بالإضافة إلى الوزن المكتسب في كل المجاميع يمكن القول بكفاءة العقار بالجرعة العلاجية الموصى بها في التغلب على الإصابة بالميكروب القولوني في كتاكيت البط المسكوفي

علاوة على ذلك تم دراسة تأثير الجرعات المختلفة من الفلورفينيكول على صورة الدم ووظائف الكبد والكلى بالبط المسكوفي المصاب وأظهرت الدراسة عدم وجود تأثيرات ضارة للفلورفينيكول على صورة الدم ووظائف الكبد والكلى في البط المصاب والمعالج بالجرعة العلاجية بينما حدثت بعض التغيرات المؤقتة بالجرعة الضعف علاجية ولكنها رجعت لمعدلاتها الطبيعية بعد أسبوع من نهاية العلاج.

وبدراسة التغيرات الهستوباثولوجية في البط المصاب والمعالج وجد أن العقار له تأثير واضح في علاج الأعراض المرضية للمرض.

خلاصة القول أن هذه النتائج أثبتت كفاءة الفلورفينيكول في جرعاته العلاجية (٣٠ ملجم / ك وزن حي) في التغلب على العدوى الطبيعية بميكروب القولون العصوى الذى يصيب البط ويسبب خسائر فادحة.