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EFFICACY OF ENROFLOXACIN IN DRINKING WATER ON SOME POULTRY PATHOGENS WITH REFERENCE TO ITS TOXIC EFFECT

BY

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Ganadexil® (enrofloxacin 10 % oral solution) is a newly pharmaceutical preparation used in poultry industry for treatment of many diseases like colibacillosis, salmonelosis and infections caused by Mycoplasma. The activity of this drug was studied against E.coli, Salmonella gallinarum pullarum and Mycoplasma galliseptlcum where the minimum inhibitory concentration was recorded for each pathogen. Another study was performed on poultry with the aim of studying the effect of this drug on antibody titer against Newcastle disease virus. The possible side effect and toxopathological alteration due to enrofloxacine was recorded .Also the effect of enrofloxacine on the performance and tissue residues were investigated.

Introduction

Ganadexil® is a new antibacterial pharmaceutical preparation used for control of different diseases in veterinary medicine e.g. Colibacillosis, salmonellosis and Mycoplasma infection in poultry ; respiratory infection, bronchopneumonia and mycoplasmosis in calves. The active ingredient is enrofloxacin, which is one of the quinolones family. It is a newly synthetic fluoroquinolone used as a bactericide and mycoplasmeicide **(Bauditz, 1987)**. So the enrofloxacin is widely used in Egypt to decrease the mortality percentage and growth enhancer.

Enrofloxacin (enro.) was the first fluoroquinolone used in veterinary medicine **(Vancutsem et al., 1990)**. It is highly suspected that it modulate the activity of hepatic cytochrome p 450 isoenzymes **(Novotny and Shaw, 1991)**which would influence the biotransformation of other drugs **(Vancutsem and Babish, 1996)**.

Rzedzick, et al., (1991) stated that the fluemequine (one of the quinolone family)has been proved an immunostimulant to N.D and that enrofloxacin was less immunostimulant than fluemequine.

Wieliczko et al., (1991) stated that enrofloxacin is widely used in Egypt to decrease the percentages of mortality, growth enhancer, in addition to its major antibacterial activity. The drug can eliminates the disease carriers when used as preventive measures of disease.

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Helal, et al., (1995) recorded significant increase of antibody titers of ND in broiler chickens treated by 20, 40, 60, mg/kg b.w of enrofloxacine. However, they recorded significant increase of serum AST enzyme activity and creatinine concentration.

The present study aimed to explore the antibacterial activity of the drug against some poultry pathogen invitro and to study side effect and toxopathological effect of this drug invivo.

Material & Methods

Drug: Ganadexil® (Enrofloxacin) 10% oral solution produced by INDUSTRIAL VETERINARIA , S.A. – INVESA . BARCELONA (SPAIN).

Experiment one:-

Tested Microorganism:

Salmonella gallinarum pullarum, E.coli and Mycoplasma gallisepticum were kindly supplied from vaccine and serum production institute , Abbasia , Egypt.

Media used:

The media that used in this study were nutrient broth , nutrient agar,(Oxoid) ; Salmonella shigella agar (Difco) ; Eosin Methylene blue for E.coli (Oxoid) and Modified media for Mycoplasma gallisepticum according to **frey et al., (1988).**

Determination of Minimum inhibitory concentration (MIC).

Minimum inhibitory concentration for each microorganism was determined for the Ganadexil® on the previous tested microorganisms where different dilutions were made starting from 5% till reaching the minimum concentration that has the ability to inhibit the growth of the microorganism completely .This concentration is known as the minimum inhibitory concentration and was carried out after **Jones et al., 1985.**

Experiment Two :- Poultry, dosing and grouping:-

One hundred and twenty (one day old) broiler chickens were divided into four groups of thirty chickens. The first group was used as a control and received water without Ganadexil® and the other groups (second ; third and fourth) were given Ganadexil® in drinking water at doses of 10, 20, 40 mg/kg b.wt respectively three days per week for six weeks . All groups of chickens were vaccinated against N.D by lentogenic F.strain (Hitchner B1) at 7 day, lasota at 18 th and 30 th day. Body weight were recorded every week.

Biochemical analysis were carried on for (GOT , GPT) and alkaline phosphatase according to **Reitman and Frankel ,(1957)** and **Bessey et al., (1946)** respectively. Total protein, Albumin were analyzed according to **Doumas, et al., (1981),** and **Pinell and Northam, (1978)** respectively and serum globulin was determined according to **Coles, (1974).** Serum urea and creatinin were determined after **Patton and crouch ,(1977)** ; and **Henry, (1974)** respectively.

Specific antibody titers against ND determined after two and four weeks using haemagglutination inhibition (HI) test after **Beard and Wilkes, (1973)**

Tissue residues of enrofloxacine were determined after (1, 3, 6, 9, 12) days of the last dose where the birds were slaughtered and the drug residues were determined in liver ; kidney ; breast muscle ; thigh muscle and fat by microbiological method using staphylococcus arueous as a test organism according to **Migliol and Dorigo, (1989).**

For histopathological investigation specimens of some tissues were taken, fixed in 10 % formalin and proceed for histopathology according to **(Durry and Wallington, 1984).**

Results &Discussion

Enrofloxacin is a newly synthetic flurorquinolone having a bactericidal effect against Gram positive, Gram negative and mycoplasma as well as inhibiting chlamydia psittaci. Recently, enrofloxacine is widely used in animals, poultry and fish farms as a new antibiotic for treatment of some important diseases-**(Chiang, et al., (1990).**

In our study concerning the efficacy of the drug Ganadexil® (enrofloxacin) against Salmonella gallinarum pullorum, E.coli and Mycoplasma gallisepticum table (1) shows that the most sensitive organism to the effect of Ganadexil® was E.coli where its minimum inhibitory concentration (MIC) was 0.04 ug/ml and was followed by salmmonella gallinarum pullorum with MIC of 0.08 ug /ml and Mycoplasma gallisepticum showed the highest resistance with (MIC) of 0.15 ug/ml . These results indicates that the drug is highly effective with a relative degree on these organism. Our results partially agree with **Body et al., (1983)** who recorded the sensitivity of the a aforementioned species against norfloxacin (floroquinolones derivatives). Also **Barry et al., (1984)** recorded the sensitivity of these species against norfloxacin and ciprofloxacin and **Hannan et al., (1989)** who stated that mycoplasma gallisepticum was sensitive to some fluoroquinolones (Ciprofloxacine, pefloxacin, enoxacin and norfloxacin.

In relation to the effect of Ganadexil® on body weight of chicken at different period of experiment , table (2)indicated that a highly significant increase in body weight was recorded from the beginning of third week in group 2 (10 mg/kg b.wt.) and group 3 (20 mg /kg b.wt.) where mean body weight was 409.14 + 12.33 and 385.57 + 13.83 in the second and third group respectively versus 352.71 + 14.26 in control group. This may confirm that enrofloxacin act as growth enhancer as previously recorded by **Wieliczko et al.,(1991).**However mean body weight of group 4 (40 mg/k b.wt.) did not show significance variation throught the experiment . We suggest that the stoppage of body weight gain in this group may be due to the different metabolic and histopathological alterations that recorded in our study.

Concerning to biochemical alteration, table (3) showed that Ganadexil increased markedly the activity of GOT , GPT , Alkaline phosphatase which refer to alteration of hepatic function. Our histopathological investigations revealed some degenerative disorders due to enrofloxacin in high doses (Fig. 1).

Total protein and globulin showed a significant increase in serum . This give and indication for immunopotentiation effect due to this drug and here we agree with the result of **Rzedzick, et al., (1991)**. However, elevated level of serum urea and creatinin have been recorded. This may refer to the involvement of renal function due to this drug. This was also confirmed in our histopathology (Figs 2 & 3).

Table (4) shows the effect of Ganadexil® on specific antibody titer against N.D virus. The results of haemagglutination inhibition test revealed that Ganadexil® increased the level of antibody titer against ND virus after two weeks of administration when given at 10 , 20, 40 mg /kg b.wt. The immunostimulant effect of enrofloxacin have been reported in previous studies e.g **Helal et al., (1995) ; Rzedzick et al., (1991).** However , this immunostimulant effect did not persist where non significant reduction of antibody titer have been recorded in group3 after four weeks and significant reduction of antibody titer have been recorded in group 4 and groups 3 & 4 after four and six weeks respectively of Ganadexil® administrations. This may be due to the various metabolic and histopathological alteration which have been recorded in our study.

One of the major public health significance with utilization of antibiotic in poultry is the residues of these antibiotic in different tissues especially edible ones. Our results in table (5) indicated the presence of various tissue residues of Ganadexil® in liver, kidney, breast muscle , thigh muscle and fat after 1, 3, 6, 9, 12 days of slaughter in treated birds . Liver contained residues of the drug till the 6^{th} day of slaughter in groups 1 & 2 and till the 9^{th} day in group 3 . Similar results were recorded for kidneys. Breast and thigh muscle were free from residues after 1 day in groups 1, 2 but thigh muscle in group 3 contained 5.57 ug /g Ganadexil® residue till the third day of slaughter. Residues of Ganadexil in fat was not present in group one after 1day while it was prolonged in groups 2 &3 to the third and sixth day respectively. The presence of tissue residues of enrofloxacin was previously recorded by **Vancutsem, et al., (1990).**

Regarding to histopathological findings , our results indicated that Ganadexil® resulted in various histopathological alterations especially when given at high doses for longer periods. The worst adverse effect were observed in liver and kidneys (Figs. 1&3) and this results were in agreement with the results of **Fuchs et al., (1994)** and **Helal et al., (1995)** who found histopathological changes in liver, kidneys and lungs of broilers given intramuscular injection of enrofloxacine. We also reported histopathological changes in other tissues e.g heart (Fig. 4) ; spleen (Fig. 5), intestine (Fig. 6-) ; lung (Fig. 7) and testis (Fig. 8) . However, our results disagree with that reported by **Hue et al., (1993)** and **Rutgers, et al., (1994)** who mentioned that no adverse reaction were observed in rainbow trout or dog after enrofloxacin therapy and this differences is almost due to species variations. Finally we conclude that Ganadexil® (enrofloxacin) is a very effective drug against some microorganisms responsible for a great losses in poultry production and that it is immunostimulant and considered as a good growth promoter .Also, Ganadexil® should be used in the indicated concentrations because higher doses resulted in several disease disorder e.g (liver, kidney) dysfunction in addition to histopathological changes in various tissue. Lastly, we should keep in mind the public health significance arised from overdoses of enrofloxacin because it leaves tissue residues at higher concentrations therefore sufficient time should be elapsed for withdrawal of the drug from the tissue.

Table (1): Minimum inhibitory concentration of Ganadexil® (enrofloxacin)on Salm.gallinarum pullorum, E.coli and Mycoplasma gallisepticum.

Type of microorganism / *Concentration of the drug*	*Salm.gallinarum pullarum*	*E. coli*	*Mycoplasma gallisepticum*
5.00%	----	----	----
2.50%	----	----	----
1.25%	----	----	----
0.60%	----	----	----
0.30%	----	----	----
0.15%	----	----	----
0.08%	----	----	+
0.04%	+	----	+
0.02%	+	+	+
0.01%	+	+	+

Table (2) Effect of Ganadexil® on body weight of chicken at different periods of experiment in relation to control group (Mean ± SE)

Groups & treatment	**Body weight per gram**					
	1st week	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
Group 1 Control	33.93 ±2.02	142.43 ±5.89	352.71 ±14.26	585.0 ± 18.88	1001.07 ±30.98	1548.33 ±44.79
Group 2 10 mg/kg b. wt	36.64 + 1.91	141.64 ±5.15	409.14** ± 12.33	657.86** ± 19.53	1111.25* ±45.08	1724.44* ±87.81
Group 3 20 mg/kg b.wt.	35.64 + 2.30	138.36 ±4.28	385.57** ± 13.83	631.79** ±21.67	1147.92** ±38.18	1699.44* ±73.49
Group 4 40 mg/kg b.wt.	35.40 ± 2.50	136.57 ±4.34	350.79 ± 14.74	584.64 ± 20.51	1011.67 ±38.47	1497 ±43.89

-Table (3):Effect of Ganadexil® on some biochemical parameters in serum of chicken Compared to control group (Mean + S.E).

Parameter / Groups	G.O.T U/ml	G.P.T U/ml	Alkaline phosphatase U/100 ml	Total protein (U/L)	Albumin (U/L)	Globulin (U/L)	Urea gm%	Creatinine gm%
Group 1 (Control)	14.67±2.85	130.67±2.69	241.00±21.93	2.68±0.12	2.56±0.11	0.123±0.01	3.68±0.07	9.27±1.56
Group 2 (10mg/kg b.wt.)	20.33±1.86	167.67±6.36**	243.33±6.01	3.39±0.08**	2.76±0.07	0.635±0.16**	6.94±0.71**	16.57±0.68*
Group 3 (20mg/kg b.wt.)	23.33±1.20*	165.00±2.87**	344.00±58.10	3.506±0.08**	2.75±0.07	0.753±0.01**	5.86±0.33**	15.46±1.19*
Group 4 (40mg/kg b.wt.)	25.67±1.86*	158.00±1.15**	381.67±39.41*	3.648±0.09**	2.95±0.03*	0.694±0.13**	4.98±0.05	17.36±1.58**

Table (4):Effect of Ganadexil® on the haemagglutination inhibition (HI) specific antibody titer against Newcastle disease virus in serum of treated chiken versus to control group (Log base –2 titer).

Period after vaccination / Groups & treatment	Two weeks	Four weeks	Six weeks
Control	0.8.3±0.20	2.107±0.26	0.903±0.32
Group 2 (10 mg /kg b wt.)	1.505±0.23**	2.107±0.27	0.803±0.27
Group 3 (20 mg / kg b.wt.)	1.204±0.26*	1.906±0.25	0.502±0.23*
Group 4 (40 mg / kg b.wt.)	1.60±0.24**	1.304±0.23*	0.503±0.23*

Table (5): Ganadexil® residues (ug/g) in liver , kidney , breast muscle , thigh muscle and fat of treated chickenl.

Timeof slaugter / Tissues	Time of slaughter after the last dose														
	Group 1					Group 2					Group 3				
	one day	Three days	Six days	Nine days	Twelve days	one day	Three days	Six days	Nine days	Twelve days	one day	Three days	Six days	Nine days	Twelve days
Liver	15.2	6.40	1.87	---	---	19.20	12.30	5.80	---	---	24.37	17.4	12.03	6.17	---
Kidney	13.0	5.03	2.30	---	---	16.67	7.37	3.23	---	---	20.90	17.07	10.97	4.30	---
Breast muscle	1.53	---	---	---	---	3.00	---	---	---	---	7.27	---	---	---	---
Thigh muscle	1.63	---	---	---	---	3.93	---	---	---	---	7.83	5.57	---	---	---
Fat	4.20	---	---	---	---	5.60	2.47	---	---	---	7.70	5.90	2.30	---	---

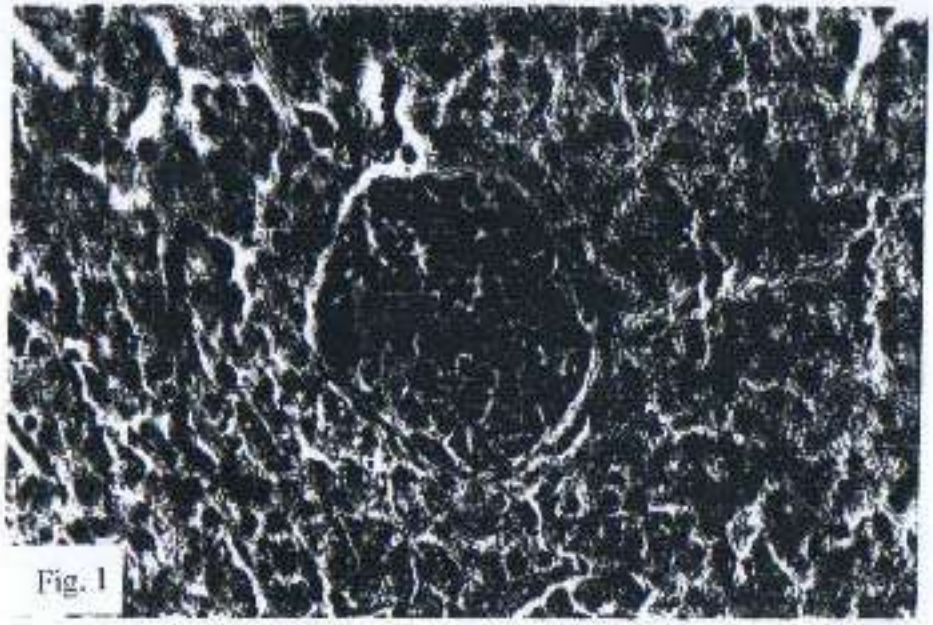
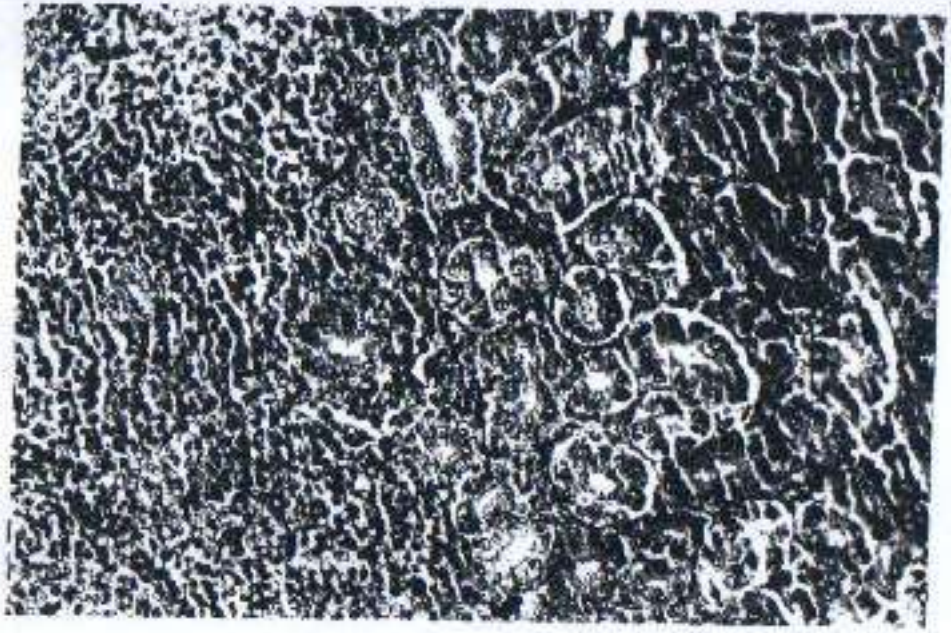


Fig. 1



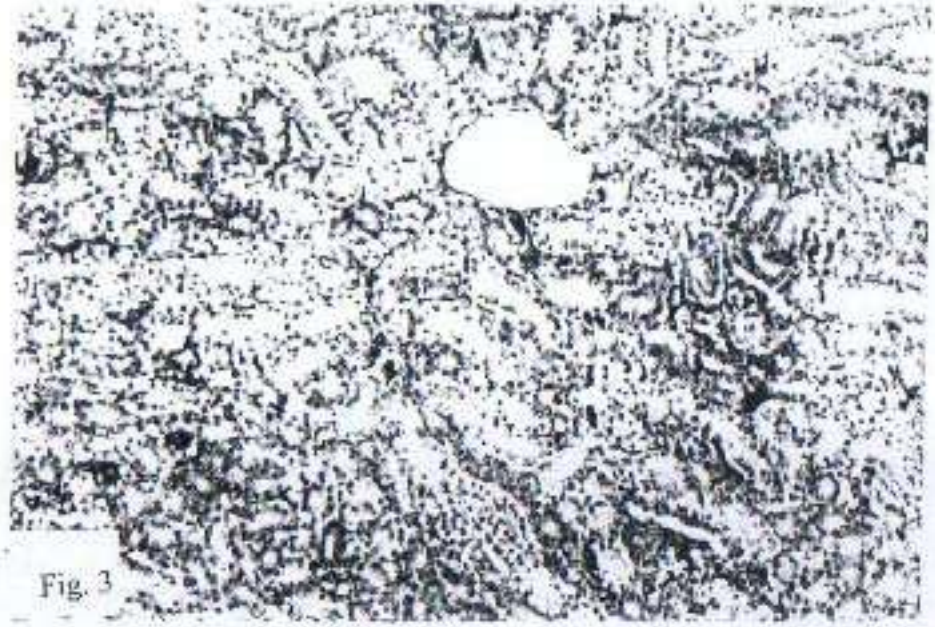


Fig. 3

Fig. 4

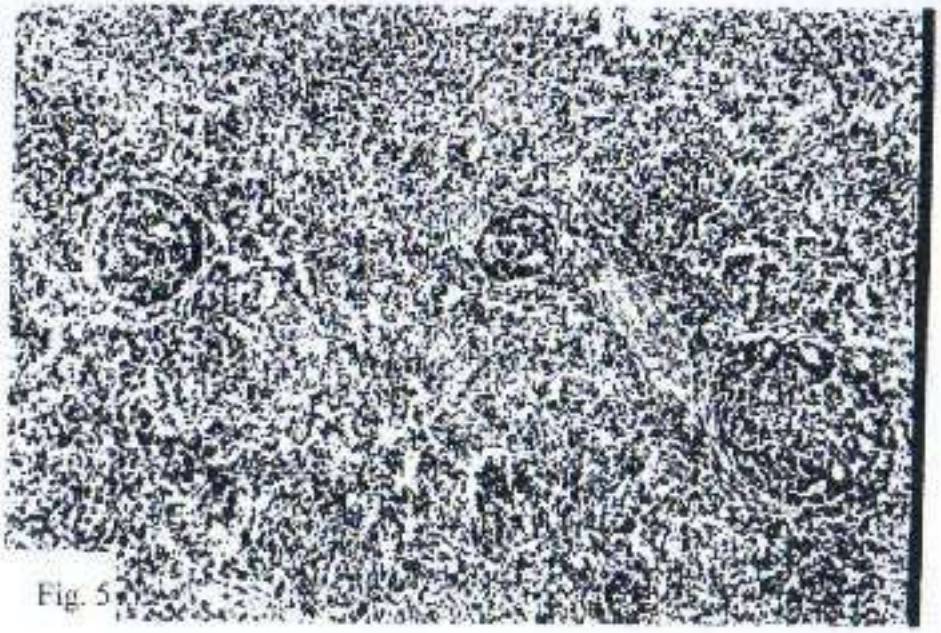


Fig. 5

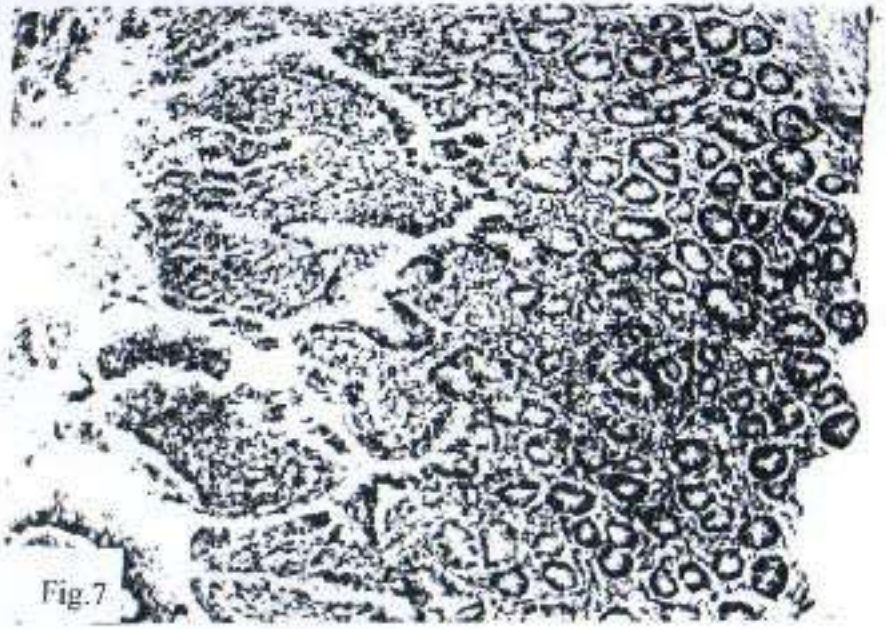


Fig.7

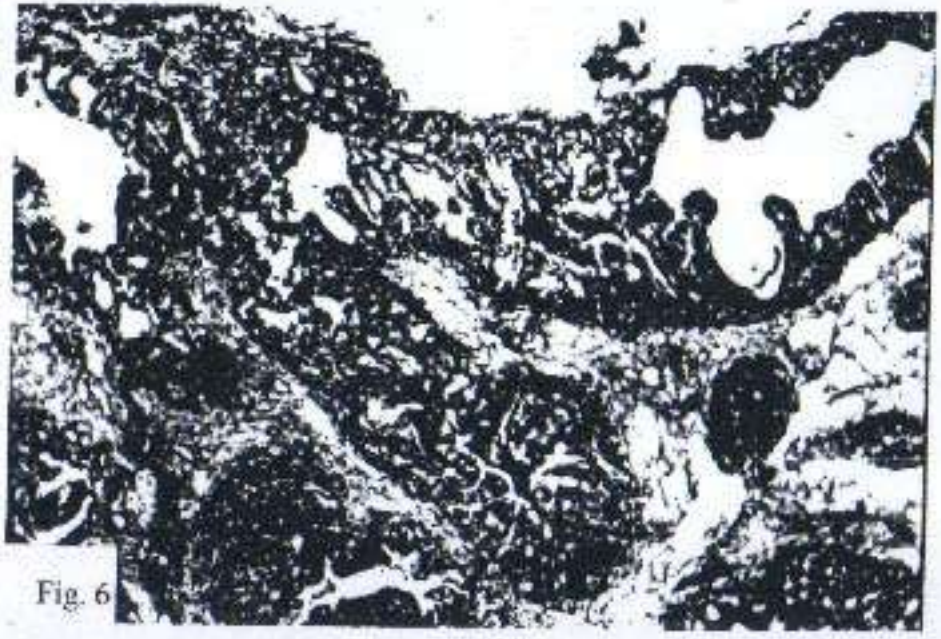
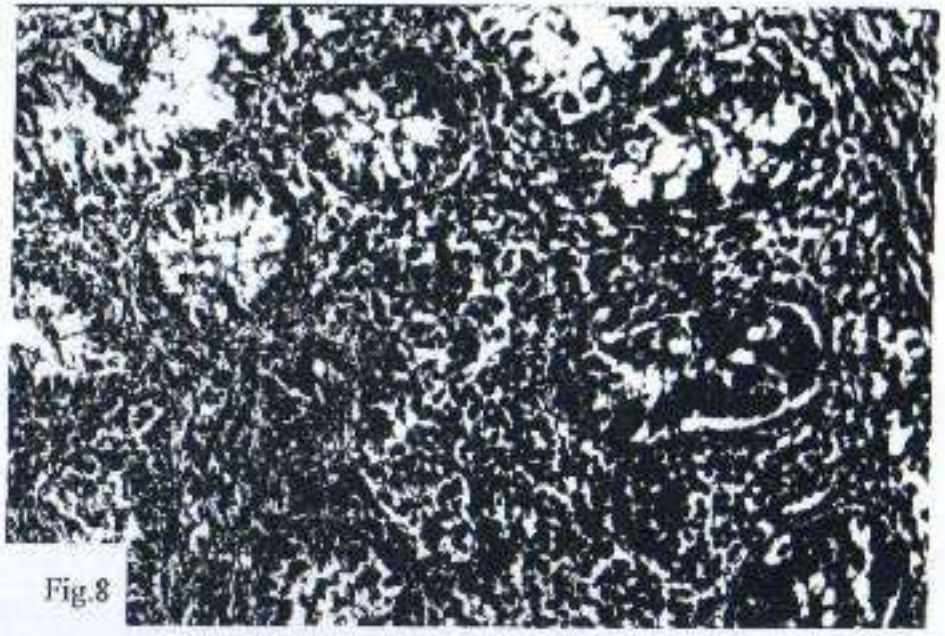


Fig. 6



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الملخص العربي

مدي كفـــاءة المضـــاد البكتيري جانادیکسیل علي بعض مسببات الأمراض في الدجاج مع الإشارة إلي تأثيره السمي

تم دراسة كفـــاءة المضـــاد البكتيري جانادیکسیل خارجيا علي ميكروبات الأشريشيـــا كـــولاي و سالمونيلا الدجاج و الميكوبلازما حيث تم تقدير أقل تركيز يمنع النمو لكل من الميكروبات الســـابقه. تلي ذلـــك دراسة علي الدجاج استهدفت تأثير هذا العقار علي إنتاج الأجسام المضادة لفيروس مرض النيوكاسل بالأضافة إلي التأثير السمي و الباثولوجي الناتج عن استخدام هذا العقار في الدجاج. أيضا تم دراسة تأثير هذا العقار علي معدل النمو في الطيور و متبقياته في بعض الأنسجه . أثبتت الدراسه وجود كفـــاءة عاليه لهذا العقار ضد ميكروبـــات الأشريشيـــا كولاي وسالمونيلا الدجاج و الميكوبلازما بالإضافة أنه يزود انتاج الأجســـام المضـــادة و لكنـــه في الجرعات العالية أدي إلي حدوث إصابات مختلفة في الكبد و الكلى و تغيرات باثولوجيه في الأنسجه المختلفة مـــع وجود متبقيات في هذه الأنسجه لذلك ننصح باستخدام هذا العقار في حدود الجرعات الموصي بهـــا.