EFFICACY OF PEFLOXACIN FOR THE TREATMENT OF BROILER CHICKENS EXPERIMENTALLY INFECTED WITH *ESCHERICHIA COLI* O78:K80

By

K. MADIAN[*], WAFAA. A. ABD EL-GHANY[*] AND GEHAN M. KAMEL[**]

[*]Dept. of Poultry Dis., [**] Dept. of Pharmacology, Faculty Veterinary Medicine, Cairo University

SUMMARY

This study was conducted to assess the efficacy of pefloxacin in controlling the adverse effects of experimentally induced colibacillosis in broiler chickens. Two- hundreds and thirty, one-day old broiler chicks were used and randomly allocated into four experimental groups; group (1) uninfected-untreated, group (2) infected-untreated, group (3) infected-treated with pefloxacin in the drinking water and group (4) infected-treated with pefloxacin by intramuscular injection (I/M) injection. The drug was given at dose level of 10 mg/kg b.wt/day for 3 consecutive days. Experimental colibacillosis was induced at 21-day of age by I/M inoculation of *Escherichia coli (E.coli)* serotype O78: K80. Clinical signs, mortalities, mean lesion scores, performance including body weight and feed conversion efficiency, frequency of *E.coli* re-isolation and histopathological alterations were recorded and evaluated. Also, clinical chemistry parameters including serum levels of total bilirubin and liver enzymes (liver function parameters) as well as uric acid, blood urea nitrogen and creatinin (kidney function parameters) were estimated. The humoral immune response to sheep red blood cells (SRBC) and serum protein electrophoresis were measured. The pharmacokinetic of pefloxacin were investigated. The Results showed that infected-pefloxacin treated chickens had less pronounced clinical signs, significant (P<0.05) lower mortalities, lower average gross pathology scores, higher body weight and better feed conversion than infected-untreated birds. Serum levels of total bilirubin and liver enzymes as well as uric acid, blood urea nitrogen and creatinin were significantly (P<0.05) decreased toward normal values in chickens of infected-pefloxacin treated groups when compared with those in infected-untreated group. The haemagglutinating antibody titers to SRBC and serum levels of gammaglobulins (albumin/globulin ratios) were significantly (P<0.05) increased in the treated groups than untreated ones. The results of pefloxacin pharmacokinetics

cleared that higher drug serum concentration was recorded following I/M injection than after oral dosing. The absorption half life time ($t_{1/2ab}$) was 0.53±0.08 and 0.21±0.026 h following oral and I/M administration, respectively. The maximum serum concentration (Cmax) recorded after oral dosing (2.71±0.14 µg.ml^{-1}) and (1.68±1.15 µg.ml^{-1}) following I/M injection. The tissues residues results revealed that pefloxacin was not detected on the 9th day following cessation of water medication; however, the drug was still detected in the liver, kidney and skin of I/M injected birds. The results of pefloxacin pharmacokinetic and serum drug concentrations cleared that pefloxacin have good absorption with high bioavailability, long half-life time and excellent tissue and body fluid penetration. In the present study, although there were no convincing differences in clearing the clinical or pathological features of *E.coli* between the two methods of pefloxacin administration, but I/M route was more efficacious than drinking water method because of its ability to completely eliminate *E.coli* from its site of action and so it may could minimize the risk of disease reoccurring. In conclusion, pefloxacin administration (regardless to the method) at 10 mg/kg b.wt /day for 3 consecutive days is efficacious for the treatment of colibacillosis in broiler chickens evident by improvement of all investigated parameters and good

Pharmacokinetic profile.

INTRODUCTION

Escherichia coli (*E.coli*) is a member of Gram negative bacteria. Avian pathogenic *E.coli* (APEC) frequently infects broiler chickens inducing severe diseased conditions with great economic losses (**Chansiripornchai and Sasipreeyajan, 2002**). All ages of poultry are susceptible to APEC infection, however, the birds are mostly infected at 4-5 weeks old (**Chansiripornchai et al., 1995**). The main clinical forms of *E.coli* infection in chickens vary from acute colisepticaemia with sudden death to subacute fibrinopurulent serositis at 2-8 weeks old (**Leitnes and Heller, 1992**). Administration of antibiotics is the most common and fast way for treating of APEC infection in broiler chickens, but the major problem associated with the treatment is the development of

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drug resistant strains to the most commonly used drugs (**Vandemaele et al., 2002**). Hence, it's necessary to search for new therapeutic agents to control this infection and to define the most effective route of administration. Much has been reported about the resistance of *E.coli* to fluoroquinolones (**Toyfour et al., 2001; Ruiz et al., 2002 and Xiapei et al., 2002**).

The fluoroquinolones are a relatively new class of synthetic antimicrobials for which the clinical use, toxicity as well as pharmacological characters in animals have been reviewed (**Vancutsem et al., 1990**). They have bactericidal activity against Gram-positive and Gram-negative aerobic bacteria (**Stamm et al., 1985 and Debbia et al., 1987**). Pefloxacin is a novel and the latest generation of fluoroquinolones with a chemical structure [1-ethyl-6-fluro-1-4-oxo-7(4-methyl-1-piprazinyl) quinolone-3 carboxylic aid], this chemical structure rendered it more active than other fluoroquinolones against Gram-negative and some Gram-positive bacteria and also it improved the pharmacokinetic profile of the drug through enhanced lipid solubility (**Berg, 1988 and Neer, 1988**).

The drug is partially metabolized (demethylated and oxidized) in the liver to norfloxacin (a primary metabolite) which itself is a potent antimicrobial agent used in human and veterinary practice (**Stein, 1987; Robson, 1992; Spoo and Riviere, 1995 and Sarközy et al., 2004**). Pefloxacin inhibits Topoisomerase II of the bacterial DNA gyrase enzyme and DNA replication (**Badet et al., 1982; Kayser, 1985 and Smith, 1986**) so, it exhibited broad spectrum rapid bactericidal activity at relatively low concentration and posses a pronounced post-antibiotic effect (**Jenkins and Friedlander, 1988; Vancutsem et al.,**

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1990 and Brown, 1996). Favorable kinetic properties of pefloxacin like good absorption, high bioavailability, long elimination half life time, low protein binding, excellent tissues and body fluid penetration and large volume of distribution have been documented by **Barre et al., (1984); Dow et al., (1986); Andriole (1988); Gonzalez and Henwood, (1989) and Andon (1992).** These characters of pefloxacin make it an ideal and suitable antibacterial drug in poultry medicine. Pefloxacin posses considerable *in-vitro* potency against bacterial infectious diseases in poultry such as coliform infections, salmonellosis, infectious coryza, avian mycoplasmosis, complication due to chronic respiratory disease, fowl cholera, avian tuberculosis, clostridial infections and avian chlamydiosis **(Gonzalez and Henwood, 1989 and Mohamed and Dardeer, 2001).**

Meanwhile, literatures about using of pefloxacin either in chemoprophylaxis or treatment of poultry pathogens are relatively sparse. Pefloxacin pharmacokinetic and its tissue residues in different avian species were available only in normal non diseased birds **(Moutafchieva, 1997; Moutafchieva et al., 1997; Ershov et al., 2001; Isea et al., 2003; Jehan, 2004; Mohan et al., 2004; Pant et al., 2005; Babu et al., 2006 and Moutafchieva and Yarkove, 2006)**. However, no previous studies on pefloxacin in diseased conditions of poultry are recorded.

Therefore, the purpose of the present study was to determine the efficacy of pefloxacin in the treatment of *E.coli* infection in the broiler chickens when administered either in the drinking water or via intramuscular (I/M) injection and the pharmacokinetic profile of pefloxacin.

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MATERIALS AND METHODS

I. Experimental chicks:

A total of two hundreds and thirty clinically healthy one-day old Hubbard broiler chicks of both sexes obtained from commercial hatchery were used in the present study. At arrival and before experiment, the chicks were tested to be free from *E.coli* by bacteriological culture of liver, heart, blood, spleen and yolk sac of ten randomly selected chicks and they prove negative isolation for *E.coli*. Chicks were individually identified with leg band rings and kept under complete observation in separate thoroughly cleaned and disinfected pens. Feed and water were provided adlibitum for the entire experimental period. A commercial unmediated broiler ration that formulated to meet NRC recommendation **(NRC 1994)** was used. All chicks were vaccinated against Newcastle disease (ND) using Hitchner B1 and Lasota vaccines and against infectious bursal disease (IBD) using 228E vaccine at 5, 12 and 19 days of age; respectively via eye-drop instillation according to a standard vaccination program implementation on local broiler farms.

II. Bacteria (The inoculum's bacteria):

A virulent *E.coli* strain, serotype O78: K80 was used in the present study as it was kindly supplied from Animal Health Research Institute; Dokki, Egypt. The strain originally had been isolated from a field case of colisepticaemia (generalized *E.coli* infection) and had been fully identified, classified and serotyped according to **Edwards and Ewing (1972) and Quinn et al., (1994)**. The *E.coli* inoculum was a logarithmic phase culture produced by overnight incubation of *E.coli* in nutrient broth **(Sekizaki et al., 1989).** The number of

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bacteria per milliliter was determined by plating ten-fold serial dilution of the nutrient broth suspension on plate count agar (PCA). Titers were expressed as colony forming unit (CFU) per ml (CFU/ml) **(Fernandez et al., 2002).** Strain of *E.coli* was firstly demonstrated to be pathogenic in preliminary infectivity trial (pilot experiment) according to **(Lublin et al., 1993).**

III. Antibiogram:

The *in-vitro* antibiotic sensitivity test on *E.coli* serotype O78: K80 used in this study was performed using pefloxacin and most common antibiotics including (chroramphenicol, gentamycin, ciprofloxacin, enrofloxacin, neomycin and norfloxacin) to determine the susceptibility pattern of *E.coli* serotype to antimicrobial drugs by the standard disc diffusion technique **(Smith, 1970 and Prasad et al., 1997)** using Oxiod multi-discs (Oxoid, Hants, UK).

IV. Pefloxacin:

Pefloxacin standard pure powdered drug (100%) (1-ethyl-6-Fluro-1.4 dihydro-7-4 methyl -1- pipeazinyl- 4-oxo 3- quinolon-carboxlic acid) was kindly obtained from Pharmasweed Pharmaceutical Company, Egypt. The pefloxacin pure powder was dissolved in sterilized de-ionized distilled water to prepare pefloxacin 1% solution (according to pharmaceutical company's recommendation) which is used in drinking water or through intramuscular (I/M) injection at the dose level of 10 mg/kg b.wt **(Sachan et al., 2003).** The minimum inhibitory concentration (MIC) of pefloxacin against the used *E.coli* strain was determined as 0.06 µg/ml according to the method of **Raemdonk et al., (1993).**

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V. Experimental design:

Two hundreds and thirty, one-day old broiler chicks were randomly allocated into four groups, each consists of 50 chicks. Each group was randomly assigned into two replicates of 50 chicks per each. The experimental groups were divided into; uninfected-untreated (group1), infected-untreated (group 2), infected and treated with pefloxacin in drinking water (group 3) and infected and treated with pefloxacin via intramuscular (I/M) injection (group 4). The chicks in group 2, 3 and 4 were experimentally infected with single dose of *E.coli* at 21 days of age. For studying pefloxacin pharmacokinetics, 2 groups (5 and 6) of ten chicks per each were used. The chicks in groups (5 and 6) were experimentally infected with *E.coli* at the same age and treated with single dose of pefloxacin orally and via intramuscular (I/M) injection in groups (5 and 6); respectively at 2 days post inoculation (starting of clinical signs appearance). The experiment started from day-old and terminated at 42 days of age.

VI. Experimental Infection (Inoculation of bacteria):

At 21 days of age, experimental colibacillosis was induced as described by **Fernandez et al., (2002).** A dose of 0.3 ml of inoculum (broth culture) containing 12 X 10^9 CFU *E.coli*/ ml (3.6 X10^8 CFU *E.coli*/ chicks) was intramuscularly injected in the pectoral muscle of the chicks in the infected groups.

VII. Pefloxacin-Treatment regimen:

Medication with pefloxacin was initiated when clinical signs were started to be appear (2 days post inoculation). Immediately prior to treatment, all birds in

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each treated group were weighed in order to accurately calculate the required daily amount of pefloxacin based on the therapeutic dose of 10 mg/kg body weight **(Raemdonck et al., 1993 and Sachan et al., 2003).**

Drinking water regimen (continuous dosing):

Three days prior to treatment, the daily water consumption of birds was monitored in order to determine the amount of water consumed daily (24 hours period). The entire daily drug dose was administered continuously (continuous dosing regimen) during 24 hours period in a volume of water which was consumed in the same period. Identical dosing regimen was repeated during two subsequent days for a total of 3 consecutive days of according to **(Tanner et al., 1992)**. Fresh drug solution was mixed with drinking water daily and replaced at the same time each day.

Intramuscular injection (I/M) regimen

Birds of groups (4 and 6) were injected daily for 3 consecutive days via I/M injection with the required daily amount of pefloxacin prepared solution **(Raemdonck et al., 1993)**

VIII. Clinical follow up:

1- Clinical signs and mortalities:

All chicks were clinically inspected or observed each day for any health-related problems. The clinical signs were monitored and recorded daily for fifteen days following experimental infection. In every group, all mortalities were recorded daily and the dead birds were necropsied.

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2- Post-mortem examination (lesion scoring):

Dead birds were necropsied immediately after detection of their death and macroscopical lesion scores were reregistered. Also, on weekly basis, at 7, 14, 21, 28 days post experimental infection five birds of surviving chickens from each group were authonized or sacrificed and necropsied. A detailed post-mortem examination was performed on the air-sacs, pericardium, perihepatic capsule, trachea and lung, in addition to conventional post-mortem examination and lesion scores were recorded **(Nakamura et al., 1992).** Post-mortem lesions indicative of affected organs were scored severity on a scale of 4 points scoring system ranging from 0 to 3 as follow; 0= no lesions, 1= mild, 2= moderate and 3= severe **(Raemodnock et al., 1993 and Fernandez et al., 2002).** The following criteria were used to score the severity of lesions according to **(Fernandez et al., 2002):**

Heart: 0= no lesions, 1= little fibrin in pericardial sac, 2= definite fibrin in pericardium and 3 = extensive fibrin and adhesion of the pericardium.

Air-sacs: 0= no lesions, 1= mildly cloudy air-sacs, 2= definitely cloudy air-sacs (multifocal white or yellow materials), 3= definitely cloudy air-sacs with large amount of caseous exudates.

Liver: 0= no lesions, 1= little fibrin, 2= definite fibrin on liver surface 3= extensive fibrin. The severity index of post-mortem (macroscopic) lesions was described previously by **Nakamura et al., (1987) and (1990)**. The severity index was calculated by adding the lesion scores of the organ or tissue examined and dividing by the sum of the total number of chickens subjected to post-mortem examination.

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3- Re-isolation of *E.coli* O78:

To re-isolate the challenge organism, five randomly selected birds from each group were sacrificed at the end of 1ˢᵗ, 2ⁿᵈ and 3ʳᵈ week following initiation of therapy. In these birds, swabs from liver, spleen, trachea, air-sacs and pericardium were inoculated into MacConkey broth and then platted on MacConkey agar for 24h and at 37ºC (**Fernandez et al., 2002**). The organism was identified on the basis of cultural characters according the procedure of **Cruickshank et al., (1975).** Recovered *E.coli* isolate were lasted for susceptibility to pefloxacin.

4- Profits (Performance):

On a weekly basis along the experimental period from placement through day to 42, the chicks in each group were individually weighed and average body weight per group was obtained. Feed consumption for each group was recorded weekly for calculation of feed conversion rate (feed efficiency). Also European Production Efficiency Factor (EPEF) per group was calculated to evaluate the performance.

5- Histopathology:

When chickens reached 6 weeks of age, five randomly selected birds were sacrificed and organs including air-sacs, heart, liver, lung, spleen, bursa of fabricus and thymus were excised for apprizing the influence of infection and treatment on the histopathological alterations. Tissue samples were fixed in 10% neutral buffered formalin, fixed tissues were dehydrated in methanol, cleared in xylene, trimmed, embedded in paraffin sections at 4um and stained with hematoxyline and eosin (**Bancroft et al., 1996**). All tissues were

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examined microscopically and the histopathological alterations were graded in blind vision microscopic lesions.

IX. Laboratory Follow up:

1. Blood Sampling:
On 42 day of age, blood samples were collected by wing vein puncture from randomly selected ten birds (two replicates of five birds each) from each group. The sera were separated by centrifugation at 3000 rpm for 5 minutes and were stored at -20°C till using for evaluation the effect of the infection and pefloxacin-treatment on the clinical chemistry parameters (liver and kidney functions) and on serum protein fraction profile.

2. Estimation of clinical chemistry parameters
Serum levels of clinical chemistry parameters including [Total bilirubin, aspertate aminotransferase (AST) and alanine aminotransferase (ALT)] (to investigate liver function), also the kidney function tests including uric acid levels, blood urea nitrogen and creatinine were determined according to **Santurio et al., (1999) and Sachan et al., (2002)** employing commercially available diagnostic kits from Bio-merieux-France according to manufacturer's instructions with the help of BM Hitachi 704 clinical analyzer.

3. Humoral immune response to sheep red blood cells (SRBC):
At 28 days of age, all the birds in experimental group (1, 2, 3 and 4) were inoculated intramuscularly with 0.5 ml/bird of 5% suspension of washed SRBC in phosphate buffer saline (PBS). Blood samples were collected from ten birds/ group at 3, 6, 9 and 12 days post SRBS sensitization. The total haemagglutinating antibody titers produced in response to SRBC were determined by micro-agglutinating technique (**Van der Zijpp and Leenstra**

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1980), and expressed as the Log_2 of the reciprocal of the highest dilution of serum giving visible agglutination.

4- Profile of serum proteins*:*

Electrophoresis of serum protein fractions [albumin, alpha globulin (αG), Beta-globulin (β-G) and gamma-globulin (γ-G)] was made on cellulose-acetate membrane according to method performed by **(Epstein and Karcher, 1994)** using one pooled serum sample of randomly collected ten ones per group to asses the effect of experimental infection and pefloxacin on immunoglobulins and albumin: globulin ratio.

X. Pharmacological assay*:*

1. Blood samples:

A. For pharmacokinetics studies:

Blood samples were collected via vein puncture from each bird in groups (5) and (6) at 10, 20, 30, and 45 minutes, 1, 2, 3, 4, 5, 6, 8 and 10 hours post drug administration.

B. For determination of the daily serum pefloxacin concentrations:

Blood samples were collected from certain ten marked chickens in the 3rd and 4th group at 24 hours after each pefloxacin administration.

2. Tissue samples:

Three birds from group 3 and 4 were sacrificed at the 1st, 3rd, 5th, 7th and 9th day after cessation of drug administration. Tissue samples of different organs including liver, kidneys, heart, gizzard, breast muscle and skin were excised and collected from each bird for estimation of pefloxacin concentration in tissues. One gram of each tissue samples was homogenized with 3 ml of

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phosphate buffer saline (pH 7.2) and centrifuged at 3000 rpm for 15 minutes. The supernatant fluids were collected and stored at -20°C until analyzed.

3. Pefloxacin analytical procedure:

Pefloxacin concentration in serum and tissues were determined by microbiological assay procedure **(Arret et al., 1971)** using non pathogenic *E.coli* (ATCC 25922) that measure the antibacterial activity of the parent drug and its metabolites according to the method of **Moutafchieva and Djouvinov (1997) and Moutafchivea and Yarkove (2006)**. Pefloxacin standard curves were constructed using antibacterial free sera collected from infected-untreated group and phosphate buffer solution (pH 7.2). The lower detectable limit of pefloxacin assay by this method was 0.012 μg/ml serum. *In-vitro* serum protein binding percent of pefloxacin was determined both in serum of birds in the uninfected-untreated group and in the infected-untreated one according to the method of **Craig and Suh (1980)** using the concentration of 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078.

4. Determination of pefloxacin pharmacokinetic:

A computerized curve-stripping program (R Strip, Micromath Scientific Software, Salt Lake City, UT) was used to analyze pefloxacin serum concentration-time curves for each individual bird in the pharmacokinetic study. Serum concentration-time curves were obtained for each bird and fitted to the following equation:

$$C = Ae^{-K_{ab}t} + Be^{-K_{el}t}$$

Where C is the serum concentration, A and B are the intercepts of the absorption and elimination phases with the concentration axis, respectively. K_{ab} is the absorption rate constant expressed in units of reciprocal time (h^{-1}),

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K_{el} is the elimination rate constant expressed in units of reciprocal time (h^{-1}) and e is the base of natural logarithm. The peak of drug concentration (Cmax) and time to peak concentration (Tmax) were calculated according to the statistical moment theory of **Yamaoka et al., (1978).** The absorption half-life time ($t_{1/2ab}$) and the elimination half-life one ($t_{1/2el}$) are calculated as $Ln2/K_{ab}$ and $Ln2/K_{el}$, respectively. The area under the concentration-time curves (AUC) were calculated by trapezoidal rule **(Gibaldi and Perrier, 1982).** The mean residence time (MRT) for the tested drug was calculated as AUMC/AUC, where AUMC is the area under the first moment curve.

XI. Statistical analysis:

The collected data were computed using analysis of variance (ANOVA) where difference in means was obtained; also the least significant difference (LSD) test was measured to distinct different treatments **(Snedecor and Corchran, 1980)**. Results of the pharmacokinetic parameters, daily serum concentration and tissue residues were statistically analyzed using student *t*-test **(Snedecor and Corchran, 1980)**.

RESULTS AND DISCUSSION

Escherichia coil (*E.coli*) is responsible for heavy economic losses to poultry industry, by its association with various disease conditions, either as primary pathogen or as a secondary pathogen. Treatment protocols for *E.coli* infections include the use of antibiotics with broad spectrum activity **(Brans and Gross, 1997).** Many antibiotics are active against *E.coli*, but this bacterium has often developed resistance to different antimicrobial agents **(Blanco et al., 1996)**. Hence, it is necessary to search for new therapeutic agents to control such

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infection and to define the most effective dose and route of administration. Pefloxacin is a newer member of the fluoroquinolone antimicrobials and is a potent broad spectrum antimicrobial agent with a pharmacokinetic profile characterized by high bioavailability after administration, good penetration into tissues and body fluid and long half-life time (**Moutafchieva and Yarkove, 2006).** These properties necessitate using of pefloxacin in the poultry field against the most important common bacterial pathogens.

1. Antibiogram:

Results of *in-vitro* antibiotic sensitivity pattern revealed that *E.coli* challenge organism used in this study was highly sensitive to pefloxacin than other tested antibiotics (chroramphenicol, gentamycin, ciprofloxacin, enrofloxacin, neomycin and norfloxacin). This obtained result agrees with that of **Prasad et al., (1997)** who study the antibiogram pattern of *E.coli* isolated from many pathological lesions and reported that *E.coli* showed highest sensitivity to pefloxacin when compared with other antibiotics. The minimum inhibitory concentration (MIC) of pefloxacin that determined in the present study was 0.06 µg/ ml and it was agree with the finding of **Sharma et al., (1994).** The recent introduction and the limited using of pefloxacin are attractive explanations for the higher sensitivity of *E.coli* strains to pefloxacin (**Prasad et al., 1997)**. Also our result concerning the antibiotic sensitivity pattern is consistent with that reported by **Hui and Das (2000) and Tai and Fang (2000)** who noticed high sensitivity of *E.coli* strains isolated from chickens and ducks to pefloxacin *in-vitro*. Moreover, **Rolinski et al., (2002)** mentioned that different *Salmonella* species showed high sensitivity to pefloxacin *in-vitro* sensitivity test.

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2. Clinical signs:

No clinical signs were observed in the uninfected-untreated group. Reduced birds activity, dyspnea, snicking, mucopurulent nasal discharges, decrease appetite and depression were present among the infected-untreated birds and were estimated between 40-60% within 24 hours following experimental *E.coli* infection. Within 24 hours following initiation of treatment pefloxacin reduced the clinical signs which were present in infected birds prior to treatment and the signs continued to improve in the next days post-medication. Infected Birds treated with pefloxacin through I/M route improved clinically during and after the 3-days treatment period and clinical signs disappeared within 5 to 7 days following treatment. The response to treatment with pefloxacin in drinking water was less pronounced as signs continued to improve post-treatment but more slowly and few affected birds showed mild signs of respiratory distress at the end of the first week following initiation of medication. In contrast, the incidence of clinical disease in surviving infected-untreated birds was estimated as 20-50%, seven days following infection. Data presented in table (1) show and summarize the effect of different pefloxacin treatment regimen on mortality and mean lesions score over three weeks observation period following initiation of treatment.

3. Mortalities:

Following medication, the total cumulative mortality percentages attributed to generalized *E.coli* infection (septicaemia and serositis) was significantly ($P<0.05$) lower (2%) and (4%) in birds administrated pefloxacin through I/M injection and those and received pefloxacin in the drinking water; respectively when compared with uninfected-untreated group (34%) that had a quite high

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cumulative mortality during the experiment. Mortalities during 2 days post-infection were reduced by the 3rd day of pefloxacin treatment and completely stopped by the 7th day of medication. These results indicated that pefloxacin reduced the mortality rate when administered through I/M injection more than through drinking water.

There were no mortalities recorded among chickens of uninfected-untreated group. Our findings are partially constant with those reported by **Adayel and Abdalla (2007)** who found that treatment of *Salmonella enteritidis*-infected chicks with pefloxacin at dose level of 5 mg and 10 mg/kg b.wt for 3 successive days partially reduced clinical signs and decreased mortality rate from (38%) to (6%) and (2%); respectively, while using of pefloxacin at dose level 5 mg/kg b.wt but for 5 successive days ameliorate clinical symptoms and reduce mortality rate to 2%.

4. Gross lesions and lesion scores:

Colibocillosis was confirmed in both dead and sacrificed birds on the basis of post-mortem inspection for typical lesions of tracheitis, air-saculitis, pericarditis and perihepatits, also the subsequent recovery of *E.coli* isolate. Macroscopic lesions observed in dead and sacrificed birds of infected-untreated group post infection were represented tracheitis with purulent exudates in the tracheal lumen and bronchi, pericardits and perhepatitis with deposits of fibrin on the pericardium and the perihepatic capsule as well as presence of fibrinous air-saculitis on both the thoracic and abdominal air-sacs. The mean lesion score of infected-untreated birds was significantly (P<0.05) higher than those of infected-treated birds in groups (3) and (4). Infected chickens that received pefloxacin via I/M injection had significantly (P<0.05)

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lower average gross pathology scores than did chickens that received pefloxacin in drinking water (table 1). No survived birds in both infected-treated groups (groups 3 and 4) were scored 3 or 4 compared to infected-untreated group. Seven days following initiation of pefloxacin treatment lesions virtually disappear.

Table (1): Mortality rates (No. of deaths/total No. of chickens percentage in parentheses) and mean macroscopic lesion score for broilers in pefloxacin treated and untreated groups.

Experimental groups	Mortality	Number of Examined chickens		Mean macroscopic lesion scores			
		Sacrificed	Dead	Tracheitis	Air-saculitis	Pericarditis	Perihepatitis
Uninfected-untreated	0/50 (0.0%)	15	0	0	0	0	0
Infected-untreated	17/50 (34%)	15	17	2.60 ±0.14[a]	2.37 ±0.17[a]	2.29 ±0.20[a]	2.03 ±0.23[a]
Pefloxacin in DW	2/50 (4.0%)	15	2	0.34 ±0.18	0.28 ±0.15[b]	0.46 ±0.17[b]	0.34 ±0.50[b]
Pefloxacin by I/M injection	1/50 (2.0%)	15	1	0.26 ±0.15[b]	0.17 ±0.80[c]	0.30 ±0.14[c]	0.25 ±0.12[c]
L.S.D				1.87	1.63	1.36	1.20

Values within a column represent means ± SEM. L.S.D: least significant difference.
Values in a column not sharing a common letter are significantly different.
Lesions were graded as: absent, mild, moderate or sever for scoring, the following scoring point system was used (lesion absent = 0, mild = 1, moderate = 2 and sever = 3). Mean lesion (severity index) was calculated by adding the lesion scores of tissue examined and dividing by the sum of the total number of examined bird **(Nakamura et al., 1987)**
DW= Drinking water   I/M= Intramuscular

5. Re-isolation of *E.coli* O78: K 80 from organs:

All chickens in uninfected-untreated group were negative for isolation of

the challenge bacteria (Table 2). Seven days following initiation of

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treatment with pefloxacin, *E.coli* could no longer be recovered (re-isolated) from liver, spleen, trachea, air-sacs and pericardium of the sacrificed birds in the group treated pefloxacin by injection, while *E.coli* could be recovered from tissues in some birds in birds treated in the drinking water with re-isolation rate of 6.6% to 13.4%. Infected-untreated group had a higher frequency of *E.coli* re-isolation that ranged from 33.3 to 66.6% from different organs than those from treated birds. These results demonstrated that pefloxacin reduced the number of birds from which bacteria were isolated.

The rate of re-isolation of the challenge bacteria of more than 50% was recorded by other authors (**Timms et al., 1989, Freed et al., 1993, Kempf et al., 1995**) who studied the effect of the other antibiotics on colibacillosis. From almost all of chickens in the untreated-infected group, *E.coli* was re-isolated, whereas no re-isolation of the organism from birds given the pefloxacin by injection. A low percentage of the birds were infected with *E.coli* in group treat with pefloxacin in drinking water. The failure to re-isolate *E.coli* from the infected-treated chickens indicates the advantage in using pefloxacin for treatment of this organism.

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Table (2): Re-isolation of *E.coli* O78: K80 (No. of positive samples /No. of broiler examined, percentage in parentheses) from the organs in pefloxacin treated and untreated groups.

Experimental Groups	Number of Examined chickens	Number and percentage of positive chickens with *E.coli* isolation from				
		Liver	**Spleen**	**Trachea**	**Air-sacs**	**Pericardium**
Uninfected-untreated	15	0/15 (0.00)[a]	0/10 (0.00)[a]	0/15 (0.00)[a]	0/15 (0.00)[a]	0/15 (0.0)[a]
Infected-untreated	15	8/15 (53.3)[b]	10/15 (33.3)[b]	10/15 (66.6)[b]	7/15 (46.6)[b]	8/15 (53.3)[b]
Pefloxacin in DW	15	2/15 (13.4)[a]	0/15 (6.60)[b]	1/15 (6.60)[a]	2/15 (13.4)0[ab]	1/15 (6.60)[a]
Pefloxacin by I/M injection	15	0/15 (0.00)[a]	0/15 (0.00)[a]	0/15 (0.00)[a]	0/15 (0.00)[a]	0/15 (0.00)[a]

DW= Drinking water   I/M= Intramuscular

6. Profits (Performance):

Average body weight, cumulative feed conversion and European Production Efficiency Factor (EPEF) data are presented in table (3). From the table, it could be observe that during the 1st, 2nd, 3rd week post *E.coli* infection, the weekly average body weight of broiler chickens in infected-untreated group was significantly ($P<0.05$) lower than that of the birds in uninfected-untreated group. On the other hand, during the same period, treatment of infected broiler chickens with pefloxacin either by I/M injection or in the drinking water resulted in significantly ($P<0.05$) greater average body weight when compared to body weight of surviving infected-untreated ones, although this average in the treated birds was still less than that of the un-infected control birds.

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Concerning the cumulative feed conversion, chickens infected with *E.coli* but not treated with pefloxacin had feed conversion of (2.32) when compared with that of uninfected-untreated group (1.84). Infected treated birds with pefloxacin in groups (3) and (4) had better cumulative feed conversion than those infected and kept without medication. On the basis of the higher values, the better performance, the calculated EPEF revealed good performance in *E.coli*-infected broiler chickens when treated with pefloxacin either through I/M injection or in drinking water. These data indicated that medication with pefloxacin can improve the overall performance of broiler chickens when it is faced with an *E.coli* infection or challenge.

Table (3): Average body weight, cumulative feed conversion and EPEF of pefloxacin treated and untreated groups.

Treatment group	Average body weight/gm						CFC	EPEF[*]
	Age/week							
	Before challenge			After challenge				
	1	2	3	4	5	6		
Uninfected-untreated	135.71± 4.51[a]	292.1 ±7.83[a]	623.4 ±18.9[a]	856.6 ±23.4[a]	1200.3 ±46.6[a]	1779 ±33.41[a]	1.84	207.14
Infected-untreated	133.64± 6.86[a]	296.0 ±9.99[a]	440.0 ±17.6[b]	619.33 ±24.84[b]	729.50 ±26.61[b]	1338.0 ±25.42[b]	2.32	139.34
Pefloxacin in DW	130.91± 5.20[a]	285.0 ±8.13[a]	554.75 ±10.95[c]	726.33 ±21.86[c]	952.0 ±32.33[c]	1531.0 ±46.63[d]	2.07	177.01
Pefloxacin by I/M injection	139.22± 3.85[a]	290.0 ±7.5[a]	582.5 ±15.32[c]	747.50 ±19.18[c]	1048.0 ±54.98[c]	1651.0 ±47.82[c]	1.95	200.57
LSD	16.45	25.2	30.1	63.48	115.80	119.75		

DW= Drinking water   I/M= Intramuscular   CFC= Cumulative feed conversion
EPEF[*] = European Production Efficiency Factor. The higher the value, the better the performance.
LSD= Least significant difference as determined by Fisher's protected LSD procedures.
Means within the column with no superscripts are significantly different (p<0.05).
Values of body weight represent the means ± SEM of 20 broiler chickens per treatment.

The decrease in body weight post *E.coli* infection may be attributed to the deleterious effect of the microorganism which invade the host and retard its metabolic activity **(Abdullah, 1993).** Meanwhile the improvement of body gain of chickens in infected-treated birds might be attributed to the bactericidal effect of drug on the infection and consequently improvement of general health condition **(Brown, 1996).** Our obtained data concerning the bird's performance were supported by the results of **Adayel and Abdalla (2007)** who mentioned that oral administration of pefloxacin at dose levels of 5 and 10 mg/kg b.wt for 3 successive days to *Salmonella enteritidis* infected chicks improved the performance of the infected chicks. The low feed conversion among infected-treated chickens in groups (3) and (4) was attributed to the rapid resolution of illness, allowing these chickens to return to their genetic performance potentially and more quickly than those of infected-untreated counterparts.

The clinical signs, mortality pattern, lesion scores and reduced broiler performance recorded in the present work are typical of colibacillosis and similar to those reported by a number of authors **(Piercy and West, 1976; Cheville and Arp, 1978; Smith et al., 1985; Nakamura et al., 1992; Sasipreeyajan and Pakpinyo 1992; Raemdonk et al., 1993; Mognet et al., 1997; Pourbakhshs et al., 1997; Fernandez et al., 2002 and Glisson et al., 2004)** and by using of these parameters as indices of *E.coli*, we evaluated the efficacy of pefloxacin treatment. The magnitude of suffering and losses among chickens infected with *E.coli* are obvious and immense and can be attributed to high virulence of *E.coli* strain O78: K80 that used in experimental infection in this study. **Sekizaki et al., (1989) and Frenandez et al., (2002)** mentioned

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that *E.coli* strain O78: K80 is highly virulent for poultry and induces high mortality in a short time. Also cytotoxins of *E.coli* seem to be important factors in the pathogenesis of the disease as they are the most potent bacterial toxins **(Marks and Robert, 1993).** These toxins in active host cell ribosoms disrupt protein synthesis and cause cell death **(Obrien et al., 1992).**

Using of pefloxacin at dose level of 10 mg/kg b.wt/day for 3 successive days either in the drinking water (continuous dosing) or by I/M injection for the treatment of *E.coli*-infected broiler chickens resulted in rapid control of experimental colibacillosis as evidenced by rapid resolution of clinical signs, significant reduction in mortalities, lesion score, rate of *E.coli* re-isolation and improvement of performance (greater body weight and more efficient feed conversion). All the aforementioned criteria indicated the efficacy of pefloxacin and provided reasons for the efficacy in controlling *E.coli* infection in broilers.

The obtained results concerning the efficacy of pefloxacin can be explained as a result of the following: 1- The potent bactericidal activity of pefloxacin as it inhibits and interferes with the activity of DNA gyrase and Topoisomerase II enzymes which are needed for the transcription and replication of bacterial DNA **(Badet et al., 1982 Smith, 1986; Dudley, 1991; Glisson, 1994 and Chansiripornachai et al., 1995)**. As a result of inhibition of transcription and replication of bacterial DNA, it exhibited broad spectrum, rapid and high excellent bactericidal activity **(Vanctsem et al., 1990 and Brown, 1996).** 2- Favorable pharmacokinetic profile of pefloxacin that documented in the present study and previously by **Isea et al., (2003) and Moutafchieva and**

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Yarkove (2006) including rapid absorption, high bioavailability, excellent tissue and body penetration, long elimination half-life time, low protein binding and large volume of distribution concluded that administration of pefloxacin at 10 mg/Kg in chickens might ensure serum and tissue concentrations therapeutically effective for most pefloxacin-sensitive pathogens (Gram-negative aerobes and Gram–positive aerobes and anaerobes). 3- High *in-vitro* sensitivity of *E.coli* to pefloxacin that observed in this work and by **Prasad et al., (1997)**. Very little reports were published about the efficacy of pefloxacin for treatment of colibacillosis in broiler chickens. On the other hand, numerous reports have indicated the effectiveness of pefloxacin in the treatment of *Salmonellae* infections **(Wille et al., 1988; Nagah et al., 2004 and Adayel and Abdalla, 2007)**. They recorded that drinking water administration of pefloxacin at dose level of 5 and 10 mg/ kg b.wt for 5 successive days was highly effective in controlling experimental with *Salmonellae* spp infections in broiler chickens as evident by reduction of clinical signs, mortality rate and re-isolation of the organism as well as improving the body gain.

Our results are supported by the work of many authors who found that all new quinolone derivatives including enrofloxacin, danofloxacin and sarafloxacin were very active against *E.coli* infection in broiler chickens. **Glisson et al., (2004)** reported that chickens infected with *E.coli* and medicated with enrofloxacin had significantly ($P<0.05$) less mortality, lower average gross pathology scores, lower re-isolation rate of the bacteria, higher average live weight and better feed conversion ratio than infected not treated chickens. In a three field studies and in a disease model study, treatment of *E.coli*-infected

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broiler chickens with danofloxacin at 5mg/kg b.wt/day for 3 consecutive days resulted in rapid resolution of clinical signs, significant reduction in moralities and mean lesion scores and improving performance of broilers (**Raemdonck et al., 1993**)**.** In the same manner, obtained data here were supported and in agreement with the findings of **McCabe and Rippel (1993); Joong Kim (1995); Chansiripornchai and Sasipreeyajan (2002) and Zhenling et al., (2002)** who found a significant increase in the average daily gain and feed conversion ratio and reduction in mortalities of broilers treated with sarafloxacin (third generation of quinolones) than those not received treatment after experimental infection with *E.coli* serotype O78.

7. Histopathology:

Septicaemic, serosal respiratory lesions and lymphocytic depletion of bursa of fabricius and thymus were the main microscopical lesions demonstrated in broiler chickens infected with *E.coli* and kept without treatment (group 2) (Fig. 1F, 2F, 3F, 4F, 5F, 6F and 7F). The septicaemic lesions consists of focal coagulative necrosis in diffuse heavy manner all over the hepatic tissues with sever dilatation of portal, central vein and sinusoid of liver as well as hyperemia of red pulps with focal necrosis in diffuse manner all over the splenic tissues associated with thickening of the splenic capsule and depletion of lymphoid cells in the white pulp. The serosal lesions consists of fibrinopuulant inflammation (fibrin exudates) in the serosal system including pericardium, epicardium, air-sac with infiltration of fibrin threads, dead heterophilis and other mononuclear leucocytes inflammatory cells in associated with dilatation of blood vessels and capillaries. The respiratory microscopical lesions consist of pneumonia and air-saculitis and showing

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dilatation in the interlobular blood vessels of the lung and edema in the interstial stroma. The epithelium of air-sacs showed hyperplasia and cellular infiltration associated with congestion and edema in addition to cellular infiltration in sub-epithelial tissues. Marked lymphocytic depletion was noticed in the bursa and thymus of chickens affected with fibrinopurulent serositis. There were no significant histopathological lesions in any of the chickens in uninfected-untreated group (Fig. 1E, 2E, 3E, 4E, 5E, 6E and 7E)

Table (4) showed the degree of severity of histopatholgical changes recorded in different organs collected from the different experimental groups. The severity and incidence of septicaemic, serosal, respiratory lesions and lymphocytic depletion of lymphoid organs were less in chickens of *E.coli*-infected-pefloxacin treated groups (groups 3 and 4) (Fig. 1 G,H; 2 G,H; 3 G,H; 4 G,H; 5 G,H; 6 G,H; and 7 G,H) when compared with chickens infected with *E.coli* and kept without treatment (group 2). The results revealed that infected broiler chickens that received pefloxacin via I/M injection had very mild histoptholgical lesions (nearly normal structure) in different organs (Fig. 1G, 2G, 3G, 4G. 5G, 6G and 7G) than those treated with pefloxacin in drinking water. These observations indicated that pefloxacin can alleviate the severe histopathogical lesions caused by *E.coli*. The results of the present study agree with the finding of **Nakamura et al., (1986, 1987 and 1990) and Kutkat et al., (2002)** who reported that both bursa and thymus showed marked lymphocytic depletion with privascular edema in the splenic pulp in broiler chickens experimentally infected with *E.coli.* Also our findings were similar to those observed by **Nakamura et al., (1985 and 1992); Kutkat et al., (2002) and Sahar and Elshazly (2002)** who found that infection of chicken with

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E.coli serogroup (O78) induced mononuclear leucocytes inflammatory cells infiltration with dilatation of the portal veins, per hepatitis and vascular degeneration of the hepatocytes. They found a picture of severe acute pericarditis and heterophilis infiltration in the myocardium as well as absence of cilia, hyperplasia and cellular infiltration associated with congestion in the epithelium of air-sacs.

Table (4): Histopathological changes in the organs collected from in pefloxacin treated and untreated groups.

Organ	Lesion	Treatment regimen			
		Uninfected-untreated	Infected-untreated	Pefloxacin in DW	Pefloxacin I/M injection
Liver	Focal necrosis	-	++++	++	-
	Hyperemia	-	++++	+++	++
	Perihepatitis	-	++++	+++	++
Heart	Pericarditis	-	++++	++	+
Lung	Hyperemia	-	+++	++	+
	Oedema	-	+++	++	-
Air-sacs	Airsacculitis	-	++++	++	-
Spleen	Necrosis	-	++++	-	-
	Depletion	-	++++	-	-
	Hyperemia	-	++++	+	+
Bursa of fabricus	Follicles	-	-	-	-
Thymus glands	Depletion	-	++	+	-
	Hyperemia	-	++	+	+

++++= Very severe   +++= Severe   ++= Moderate   += Mild   -= Nil   DW= Drinking water   I/M= Intramuscular

8. Biochemical analysis:

The effect of pefloxacin treatment on clinical chemistry parameters is given in table (5). The serum analysis of untreated, *E.coli*-infected broiler chickens denoted a significant ($P<0.05$) increase in the activity of liver enzymes including, aspertate aminotransferase (AST) and alanine aminotransferase

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(ALT) and serum level of total bilirubin as well as significant (P<0.05) increase the kidney parameters considering serum levels of creatinin, uric acid and blood urea nitrogen, all of these findings indicating dysfunction of both liver and kidney. On the other hand, abovementioned parameters in infected-treated broiler chickens of groups (3) and (4) decreased significantly (P<0.05) toward the values that recorded in uninfected-untreated birds indicating the good therapeutic effect of pefloxacin against *E.coli* and its safety effect on liver and kidney function when clinically used at the recommended dose and duration. The significant improving in biochemical parameters in pefloxacin treated groups are consistent with the observations of **Aday1 and Abdalla (2007)** who detected significant elevation in serum levels of ALT, AST, creatinin and uric acid in chicks infected with *Salmonella enteritidis* in comparison with those that treated with pefloxacin at dose level of 10 mg/kg b.wt for 3 successive days in drinking water.

The significant (P<0.05) increase in the serum levels of liver enzymes determined in chickens of infected-untreated group may be attributed to the severe histopatholgoical changes (liver damage) caused by *E.coli* infection that observed in the present study. Also, **Campell and Coles (1986)** concluded that the increased activity of liver enzymes has been associated with hepatocellular damage in birds infected with *E.coli* as well as increase in creatinin, blood urea nitrogen and uric acid (kidney function parameters) may be attributed to septicemia caused by *E.coli* and also due to effect of its toxin on kidney **(Pai et al., 1984 and Tizipori et al., 1987). Omaima (1987) and Mona and Osfor, (2002)** observed significant increase in serum levels of liver enzymes, creatinin, uric acid and blood urea nitrogen in chickens infected with *E.coli*

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and referred that increase to liver and kidney damage associated with bacterial infection. Moreover, **Sachan et al., (2003)** reported on the absence of possible hepatotoxicity and nephrotoxicity potential of pefloxacin in broilers when administered daily at 10mg/kg b.wt for 5 successive days in drinking water. Other investigations **(Sachan et al., 2000 and 2002 and El- Boushy et al., 2006)** recorded similar results as they concluded that pefloxacin had no adverse effect on the liver enzymes at the clinically used dose and duration.

Table (5): Effect of pefloxacin administration on serum chemistry parameters in pefloxacin treated and untreated groups.

Treatment group	Serum biochemical values					
	Hepatic function parameters			Renal function parameters		
	Total bilirubin	AST*	ALT*	Uric acid	Blood urea nitrogen	Creatinin
Uninfected-untreated	0.24 ±0.026^a	193.5 ±7.94^a	69.6 ±5.90^a	8.78 ±0.23^a	0.63 ±0.039^a	0.39 ±0.020^a
Infected-untreated	0.36 ±0.025^b	245.3 ±5.09^b	87.1 ±3.36^b	12.20 ±0.11^b	0.78 ±0.031^b	0.53 ±0.031^b
Pefloxacin in DW	0.31 ±0.021^b	224.7 ±10.42^{bc}	80. ±3.60^{bc}	10.44 ±0.60^c	0.71 ±0.028^{bc}	0.48 ±0.025^b
Pefloxacin by I/M injection	0.29 ±0.023^a	219.6 ±8.22^c	74.2 ±2.05^{ac}	9.32 ±0.31^a	0.65 ±0.028^{ac}	0.44 ±0.034^a
LSD	0.073	24.42	11.93	1.091	0.096	0.085

Values represent the mean ± SEM of 10 broiler chickens each per treatment (n=10).
Means in a column with no common superscripts differ significantly (P<0.05).
LSD= Least significant difference as determined by Fisher's protected LSD procedure.
AST**= Aspertate aminotransferase.   ***ALT= Alanine aminotransferase.   DW= Drinking water
  I/M= Intramuscular

9. Immune Response:

  Data presented in table (6) show the total haemagglutinating antibody titers against SRBC in different experimental groups. These data clearly

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demonstrated that broiler chickens inoculated with *E.coli* at 21 day of age followed by SRBC injection at 28 day of age had significant (P<0.05) and marked decrease in antibody titers against SRBC at 3, 6, 9, 12 days post-SRBC inoculation when compared with the chickens in uninfected-untreated group (1), while antibody titers to SRBC were found to be increased significantly (P<0.05) in broiler chickens infected with *E.coli* and treated with pefloxacin either via I/M injection or in drinking water.

Table (6): Total haemagglutinating antibody titers (Log_2) of pefloxacin treated and untreated groups.

Treatment group	Agglutination to sheep red blood cells (SRBC)			
	Days after sensitization to SRBC			
	3	**6**	**9**	**12**
Uninfected-untreated	5.1±0.31[a]	5.9±0.23[a]	5.5±0.34[a]	4.8±0.41[a]
Infected-untreated	3.2±0.20[b]	4.2±0.22[b]	3.4±0.26[b]	2.7±0.26[b]
Pefloxacin in DW	4.3±0.21[cd]	5.7±0.26[a]	4.5±0.27[c]	3.8±0.35[cd]
Pefloxacin by I/M injection	4.6±0.22[ad]	5.9±0.37[a]	5.2±0.44[ac]	4.4±0.30[ad]
LSD	0.69	0.79	0.96	0.97

Values are Log_2 of reciprocal of the highest serum dilution to cause agglutination.
LSD= Least significant difference as determined by Fisher's protected LSD procedures.
   Values are geometric means ± SEM (n=10).
  Means in the column accompanied by different superscripts differ significantly (P<0.05).
        DW= Drinking water   I/M= Intramuscular

Table (7) and figure (8) presented serum immunoglobulin concentration and albumin/ globulin ratio in different groups analyzed by serum protein electrophoresis. As shown in the table, immunoglobulin concentration was lower in sera from infected-untreated birds (group 1) than in uninfected-untreated birds (group 2). Conversely, the serum electrophoresis of chickens in infected-pefloxacin treated birds in group (3) and group (4) showed high serum gamma globulin in comparison with infected-untreated one.

Table (7): Electrophoretic-separated serum protein fractions in pefloxacin treated and untreated groups.

Treated group	Electrophoretic serum protein fractions										Albumin/ Globulin ratio (A/G)
	Albumin		Globulins								
			Alpha(α)				Beta(β)		Gamma(γ)		
			α₁		α₂						
	%	g/d L	%	g/d L	%	g/d L	%	g/d L	%	g/d L	
Uninfected-untreated	31.0	1.02	0.5	0.02	24.9	0.8	2.6	0.06	41.0	1.3	0.45
Infected-untreated	50.8	1.4	14.0	0.4	8.1	0.2	14.8	0.4	12.3	0.3	1.03
Pefloxacin in DW	44.8	1.3	13.4	0.4	9.4	0.3	19.9	0.6	12.6	0.4	0.81
Pefloxacin by I/M injection	33.7	1.4	5.8	0.2	18.7	0.6	18.9	0.6	12.9	0.4	0.78

Values represent serum protein fractions of one pooled serum sample of three replicates of ten broiler chickens each per treatment (n=10).
DW= Drinking water   I/M= Intramuscular

Our results implied the possible immunosuppressive effect of *E.coli* as evident by significant reduction in haemagglutinating (HA) antibody response to SRBC and in serum gammaglobulins of birds in infected-untreated group, nevertheless this effect diminished by pefloxacin treatment either through I/M injection or in the drinking water. These results are consistent with those of **Nakamura et al., (1994); Hassanein et al., (2001) and Abdel-Fattah et al., (2003)** who reported that broiler chickens experimentally infected with *E.coli* pre or concurrent to vaccination with Newcastle disease (ND) vaccine had lower antibody titers as compared with non-infected vaccinated birds and they pointed-out on the possible immunosuppressive role of *E.coli* and its adverse effect on the immune response to ND vaccination.

The efficacy of immune system in chickens is dependent on the bursa of fabricus for initiating humorally-related antibodies **(Glick, 1970)** and on

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thymus for antibody cellular-related antibodies **(Cooper et al., 1965).** Modification of cellular integrity of these tissues by infection and chemical or physical agents results in immunosuppression **(Glick, 1967).** Since the potential of lymphoid tissues to produce antibodies is dependent on the bursa and thymus, the marked bursal and thymic lymphocytic depletion (inhibition of specific immunological tissues) induced by experimental infection with *E.coli* that observed in this study and previously reported works **(Nakomura et al., 1986, 1987, 1990 and 1992; Mona and Hassanean, 1998; Hassan and Hassanein, 1999; Hassanein et al., 2001 and Kutkat et al., 2002)** is regarded as an attractive explanation for immunosuppressive ability of *E.coli* and would be expected to resulted in impaired (HA) response to SRBC and reduction in serum globulins levels of infected-untreated birds.

10. Pharmacological assay:

The pharmacokinetics of pefloxacin have been studied enough in mammals, however this has gained a minimal investigation in the infected broiler chickens. Regarding the pharmacokinetic studies, our results showed that pefloxacin serum concentration after oral and I/M administration in a single dose (10 mg/kg b.wt) in *E.coli* experimentally infected broiler chickens were best to be described by a one compartment open model. Similar findings were reported in healthy broiler chickens by **Pant et al., (2005)** and in goats following I/M injection **(Malik et al., 2002)**.

The calculated pharmacokinetic parameters following a single oral and I/M administration in *E.coli* experimentally infected broiler chickens was given in table (8) and figure (9). Comparing the disposition variables of pefloxacin after a single oral and I/M administration in experimentally infected broiler

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chickens, the drug was slowly absorbed following oral dosing than after I/M injection. This was indicated from the prolonged absorption half life time ($t_{1/2ab}$) after oral dosing (0.53±0.08 h) compared with that (0.21±0.026 h) after I/M injection. The absorption half life time recorded following oral dosing in this study was significantly shorter than 1.19±0.22 h that recorded after oral administration in healthy chickens **(Pant et al., 2005)**. This indicates more rapid absorption of pefloxacin in the infected birds which may attribute to losing of certain amount of the administrated dose with the diarrhea in these diseased birds and/or enhancement of the drug absorption as a result of its higher serum proteins binding affinity in the infected chickens than healthy ones. The tested drug reached a maximum serum concentration (Cmax) of 2.71±0.14 µg.ml^{-1} following oral administration which is significantly lower than 11.68±1.15 µg.ml^{-1} after I/M injection. However, the time taken to reach this significant high concentration (Tmax) after I/M route (1.17±0.103 h) was significantly shorter than the calculated one after oral dosing (1.62±0.11 h). This could be explained by a rapid absorption of pefloxacin from I/M injection site than oral administration and/or losing of great amount of the orally administrated dose with the diarrhea affecting these diseased birds.

It's really known that pefloxacin is metabolized to norfloxacin **(Moutafchieva, 1997 and Srivastava et al., 2000)** and the bioassay method used in this study can't differentiate between the active metabolites and the parent drug. So the recorded Cmax values in this study either following oral dosing (2.71±0.14 µg.ml^{-1}) or I/M injection (11.68±1.15 µg.ml^{-1}) are represent the total concentration for pefloxacin and norfloxacin together. Otherwise, the

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Cmax values recorded following oral administration in normal broiler chickens by **Pant et al., (2005)** (3.78±0.23 µg.ml^{-1}) and **Suresh Babu et al., (2006)** (2.69±0.1.9 µg.ml^{-1}) represent the pefloxacin concentration itself according to their assay method by HPLC.

The mean residence time (MRT) for pefloxacin after I/M injection (11.36±1.24 h) was significantly longer than the recorded one (5.18±0.42 h) after oral dosing indicating more continued absorption of pefloxacin after I/M injection than oral administration. Comparing with the recorded values in healthy chickens, a prolonged MRT (14.32±1.94 h) was recorded after oral dosing in healthy chickens the study of **Pant et al., (2005)** that revealed more continued absorption of the tested drug in non infected birds than in *E.coli* infected ones.

Walker (2000) and Toutain et al., (2002) suggested that the potency of the antibacterial efficacy of fluoroquinolones is determined by two main factors which are Cmax/MIC ≥ 10 and AUC/MIC ratio ≥ 100. These are the critical break points determine the efficacy of fluoroquinolones **(Forrest et al., 1993 and Meinen et al., 1995)**. According the MIC value of pefloxacin for *E.coli* (0.06 µg.ml^{-1}) **(Raemdonk et al., 1993 and Pant et al., 2005)** and the Cmax and AUC values obtained in this trial, the Cmax/MIC and AUC/MIC ratios obtained following either oral or I/M administration were higher than 10 and 100, respectively. So, from a clinical point of view the oral and I/M administration of pefloxacin in a single dose of 10 mg/kg b.wt would sufficient enough to achieve a potent antibacterial efficacy against *E.coli* infection in chickens.

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Table (8): Mean ± S. E. of pefloxacin pharmacokinetic parameters after oral and I/M administration in a single dose (10 mg/ kg b.wt) in *E.coli* experimentally infected broiler chickens (n=10).

Parameter	Unit	Oral	I/M
A	$\mu g.ml^{-1}$	3.99±0.62	9.46±1.35**
K_{ab}	h^{-1}	1.51±0.21	3.06±0.41**
$t_{1/2ab}$	h	0.53±0.08	0.21±0.026**
B	$\mu g.ml^{-1}$	4.26±0.73	13.62±1.08***
K_{el}	h^{-1}	0.163±0.01	0.091±0.02**
$t_{1/2el}$	h	4.03±0.52	7.41±0.85**
Tmax	h	1.62±0.11	1.17±0.103**
Cmax	$\mu g.ml^{-1}$	2.71±0.14	11.68±1.15***
AUC	$\mu g.ml^{-1}h^{-1}$	30.08±3.75	155.29±10.68***
AUMC	$\mu g.ml^{-1}h^{-2}$	164.37±13.82	1645.7±38.91***
MRT	h	5.18±0.42	11.36±1.24***

** Significant at $p \geq 0.01$ Significant at $p \geq 0.001$ I/M= Intramuscular

Consecutive administration of pefloxacin (10 mg/kg b.wt) either in the drinking water or by I/M injection once daily for 3 successive days in *E.coli* infected broiler chickens revealed a daily increase in the drug serum concentration (table 9). Concerning the drinking water medication, the drug concentration increased gradually from 0.068±0.01 $\mu g.ml^{-1}$ at 24 h after the first administration to 1.042±0.064 $\mu g.ml^{-1}$ at 24 h from last one. However, after I/M injection the drug concentration elevated from 0.207±0.014 $\mu g.ml^{-1}$ to 5.37±0.28 $\mu g.ml^{-1}$ at 24 h from the first injection till the last one, respectively. These concentrations exceeded the MIC value (0.06 $\mu g.ml^{-1}$) for the tested infected organism, which demonstrates that consecutive administration of pefloxacin either in drinking water or by injection at 24 h intervals could be sufficient for the treatment of *E.coli* infection in broiler chickens.

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Table (9): Mean ± S. E. of daily pefloxacin serum concentration following drinking water and I/M injection (10 mg/ kg b.wt) once daily for 3 successive days in *E.coli* experimentally infected broiler chickens (n=10)

Treatment regimen	Daily drug serum concentration ($\mu g.ml^{-1}$)		
	1st	2nd	3rd
Pefloxacin in DW	0.068±0.01	0.22±0.016	1.042±0.064
Pefloxacin by I/M injection	0.207±0.014***	2.15±0.13***	5.37±0.28***

*** Significant at p≥ 0.001   D W= Drinking water   I/M= Intramuscular

Tissue concentrations of the tested drug determined following the consecutive administration of pefloxacin were tabulated in table (10). Tissue residue study revealed wide distribution of pefloxacin in different tissues of *E.coli* infected birds. Similar results were reported by **Pant et al., (2005) and Mohan et al., (2006)** in non infected and Japanese quail, respectively. Higher drug tissue concentrations were determined after I/M injection than drinking water medication. The highest concentrations were determined in liver and kidney, either in both medications. The drug was still detected in all of the tested tissue samples till the 5th day after stopping of I/M injection however the drug was stop detected in all tested samples at 3 days post cessation of oral dosing except kidney the drug can be measured till the fifth day. **Mohan et al., (2006)** found long persistence of pefloxacin in tissues of Japanese quail especially in muscles even ten days after drug withdrawal. These differences might be attributed to species differences and/or the higher dosage (12 mg/ kg b.wt) and longer time of administration (12 day) in his study than this present one.

The higher value of the *in-vitro* serum proteins binding percentage of pefloxacin for *E.coli* infected chickens (11.6%) than for non infected birds (10.4%) indicates its higher protein binding affinity in *E.coli* infected birds than healthy ones.

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Table (10): Mean± S.E. of pefloxacin tissue residues (µg/g) after administration in drinking water and I/M injection (10mg/kg.bwt) once daily for 3 consecutive days in *E.coli* experimentally infected broiler chickens(n=3).

Organ	Days after l[ast] dose	DW	I/M
Liver	1	0.483±0.07	1.26±0.15**
	3	0.025±0.02	0.37±0.05**
	5	ND	0.02±0.004
	7	ND	ND
	9	ND	ND
Kidney	1	0.57±0.08	1.42±0.12**
	3	0.083±0.006	0.55±0.03***
	5	0.012±0.001	0.026±0.003
	7	ND	ND
	9	ND	ND
Breast Muscles	1	0.18±0.02	0.64±0.04***
	3	0.012±0.001	0.03 ±0.005**
	5	ND	0.012±0.002
	7	ND	ND
	9	ND	ND
Gizzard	1	0.13±0.02	0.42±0.03***
	3	0.02±0.001	0.12±0.016**
	5	ND	0.02±.0.001
	7	ND	ND
	9	ND	ND
Heart	1	0.13±0.014	0.46±0.024***
	3	0.012±0.002	0.08±0.006***
	5	ND	0.012±0.002
	7	ND	ND
	9	ND	ND
Skin	1	0.12±0.02	0.33±0.02***
	3	0.03±0.01	0.08±0.005**
	5	ND	0.012±0.002
	7	ND	ND
	9	ND	ND

** Significant at $p \geq 0.0$ *** Significant at $p \geq 0.001$ ND: Non detected
DW= Drinking water   I/M= Intramuscular

The results showed that there were no convincing differences in the clinical or pathological features of *E.coli* infection by comparing the two methods of pefloxacin administration indicating that administration of the drug in the

drinking water is as effective as I/M injection. Although, there were no persuasive differences between both methods, the present study suggested that I/M injection be preferred and more efficacious than drinking water administration (continuous dosing) in the treatment of avian colibacillosis because of the drug ability to completely eliminate *E.coli* from its site of action and therefore it may be minimize the risk of the disease reoccurring.

In conclusion; in the present study, pefloxacin therapy at 10 mg/kg b.wt/day has been intensively evaluated in experimental infection in broiler chickens as shown to be highly effective, useful and good choice in the treatment of *E.coli* infection regardless the method of administration.
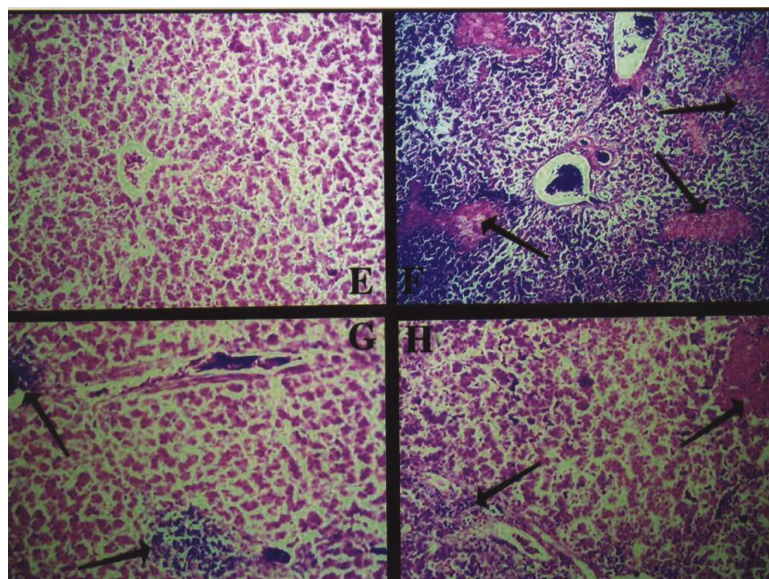
Fig. (1): E) Liver of the uninfected-untreated group showing normal histological structure without any histopathological alterations (H&E X40). F) Liver of *E.coli*-infected untreated birds showing focal necrosis with severe dilatation of portal vein, central vein and sinusoids (H&E X40). H) Liver of *E.coli*-infected and pefloxacin treated chickens in the drinking water showing few focal necrosis with mononuclear leucocytes inflammatory cells infiltration in the portal area and dilated central vein and siusoids (H&E X40). G) Liver of *E.coli*-infected and pefloxacin treated birds by I/M injection showing few focal mononuclear leucocytes aggregation with dilatation of portal vein (H&E X40).

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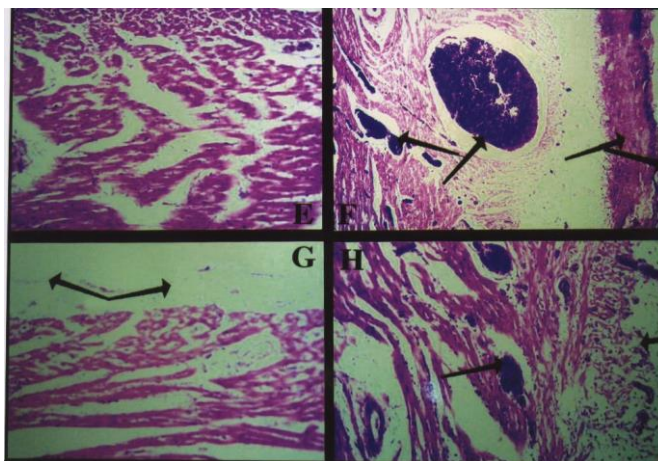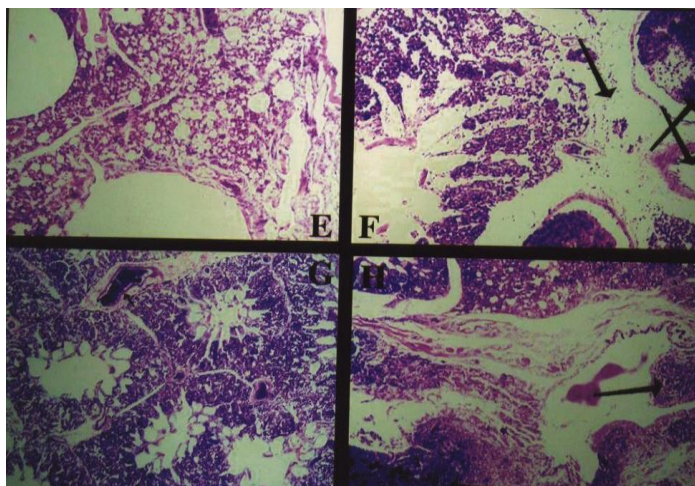
Fig. (2): E) Heart of birds in uninfected-untreated group showing normal histological structure without any histopathological alterations (H&E X40). F) Heart of *E.coli*-infected untreated birds showing fibrin exudates, dead heterophils and dilated blood vessels in the thick pericardium while the underling myocardium had dilated blood vessels and capillaries (H&E X16). H) Heart of *E.coli*-infected and pefloxacin treated chickens in the drinking water showing fibrin exudates in the pericardium with dilated blood capillaries, while the myocardium had hyperemic blood vessels and capillaries (H&E X40). G) Heart of *E.coli*-infected and pefloxacin treated birds by I/M injection showing oedema in the pericardial layer (H&E X40).

Fig. (3): E) Lung of chickens in the uninfected-untreated group showing normal histological structure without any histopathological alterations (H&E X40). F) Lung of *E.coli*-infected untreated group showing dilatation and oedema in the interlobular stroma (H&E X40). H) Lung of *E.coli*-infected and pefloxacin treated group in the drinking water showing mononuclear leucocytes inflammatory cells infiltration and aggregation in the peribronchiolar tissue (H&E X16). G) Lung of *E.coli*-infected and pefloxacin treated group by I/M injection showing mild dilatation of stromal blood vessels (H&E X16).
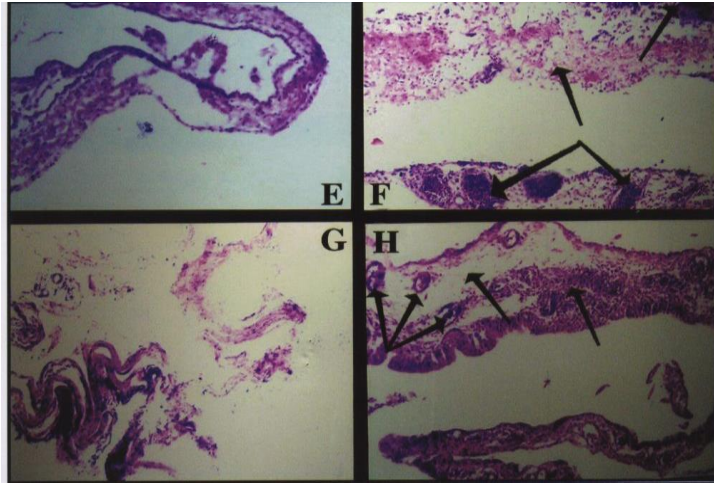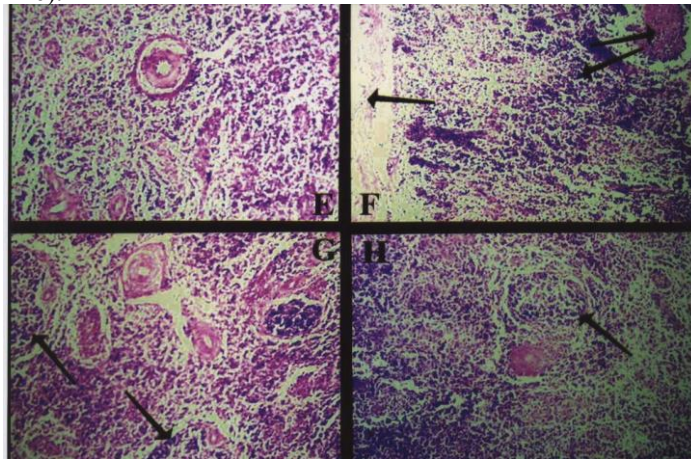
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Fig. (4): E) Air-sacs of birds in uninfected-untreated group showing normal histological structure without any histopathological alterations (H&E X40). F) Air-sacs of *E.coli*-infected untreated group showing fibrin exudates, dead heterophils and other inflammatory cells with dilated blood capillaries replacing the inner and outer layers as well as the intermediate one (H&E X16). H) Air-sacs of *E.coli*-infected and pefloxacin treated group in the drinking water showing hyperemic blood capillaries with inflammatory cells and fibrin exudates in the intermediate layer (H&E X40). G) Air-sacs of *E.coli*-infected and pefloxacin treated chickens by I/M injection showing no alterations (H&E X16).

Fig. (5): E) Spleen of chickens in uninfected-untreated group showing normal histological structure without any histopathological alterations (H&E X40). F) Spleen of *E.coli*-infected untreated chickens showing hyperemic red pulps and sinusoids with focal necrosis replaced the white pulps and thickening in the splenic capsule (H&E X40). H) Spleen of *E.coli*-infected and pefloxacin treated group in the drinking water showing few circumscribed round aggregation of lymphoid cells (H&E X40). G) Spleen of *E.coli*-infected and pefloxacin treated chickens by I/M injection showing focal circumscribed round aggregation of lymphoid cells (H&E X40).
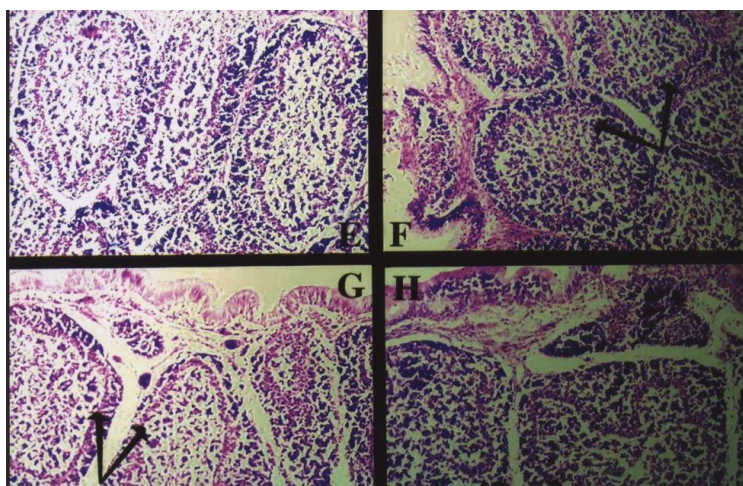
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Fig. (6): E) Bursa of fabricus (BF) of chickens in uninfected-untreated group showing normal histological structure without any histopathological alterations (H&E X40). F) BF of *E.coli*-infected untreated chickens showing active well developed follicles (H&E X40). H) BF of *E.coli*-infected and pefloxacin treated group in the drinking water showing mature well developed lymphoid follicles (H&E X40). G) BF of *E.coli*-infected and pefloxacin treated chickens by I/M injection showing well developed active follicles (H&E X40).
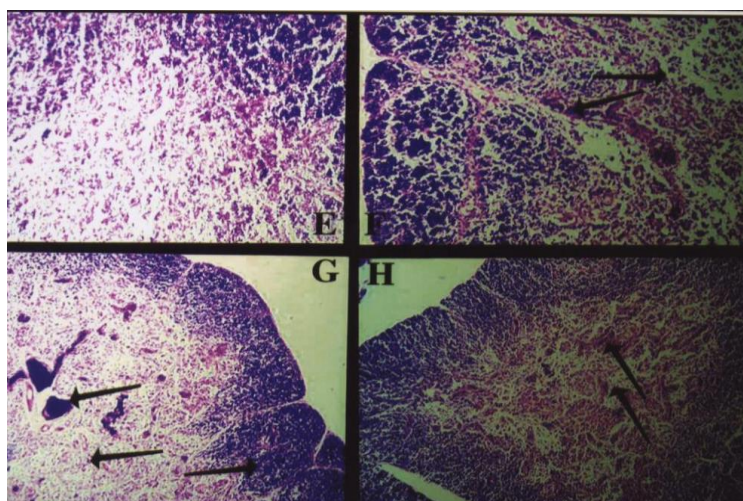

Fig. (7): E) Thymus glands (TG) of chickens in uninfected-untreated group showing normal histological structure without any histopathological alterations (H&E X40). F) TG of *E.coli*-infected untreated chickens showing depletion of lymphoid cells in both cortex and medulla with hyperemia in the medullary portion (H&E X40). H) TG of *E.coli*-infected and pefloxacin treated group in the drinking water showing hyperemia and depletion in the medulla (H&E X16). G) TG of *E.coli*-infected and pefloxacin treated chickens by I/M injection showing depletion in the lymphoid cells in both cortex and medulla with hyperemic medulla (H&E X16).
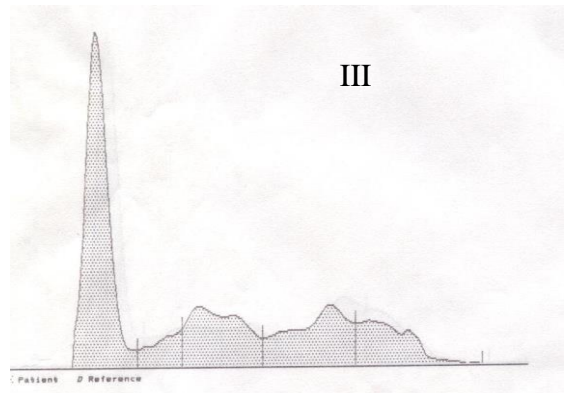
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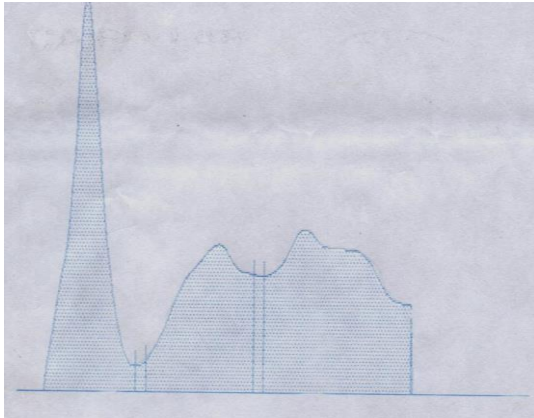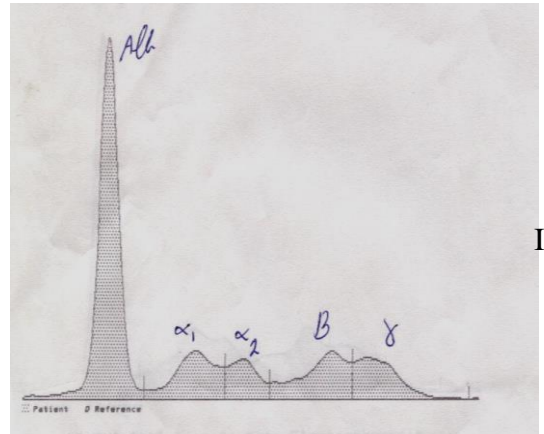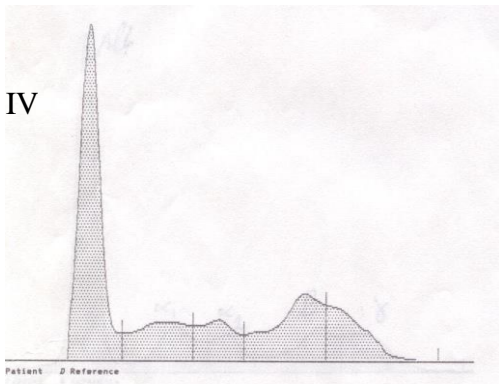
Serum protein fractions separated by electrophoresis

Fig. (8): Serum protein fractions separated by electrophoresis in uninfected-untreated group (I), in infected-untreated group (II), in pefloxacin-treated group in the drinking water (III) and in pefloxacin treated group by I/M injection (IV).
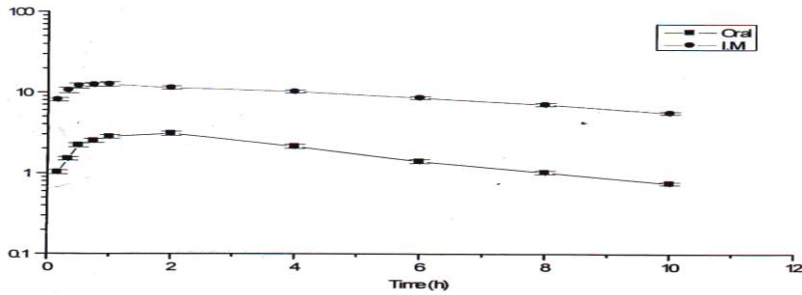
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Fig. (9): Semi-logarithmic graph depicting serum concentration-time course of pefloxacin following oral and I/M administration in a single dose (10 mg/kg b.wt) in *E.coli* experimentally infected broiler chickens.

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