

Pefloxacin Residues in Tissues and Organs of Treated

Rabbits

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ABSTRACT: Thirty balady rabbits of both sexes (weighing 1.5-2 Kg wt) were used for studding pefloxacin residues. The effect of heat treatment and freezing on the presence of these residues were also studied. The drug was injected intramuscularly (10 mg/Kg body weight) for 5 successive days. Animals were slaughtered at different intervals, samples from shoulder, thigh, back muscle, liver, and kidneys were examined. Parts of samples from animals slaughtered at 12 hr, 24 hr, 48 hr, 72 hr, and 96 hr after treatment, were examined for presence of drug residues and then boiled for 45 minutes and tested while the rest of these samples were frozen and examined weekly for presence of tested drug using HPLC. The result showed that pefloxacin highest concentration level was detected in kidneys at 12 hr from last dose ($28.32 \pm 2.261 \mu\text{g/g}$) and decreased till not detected at 144hr followed by liver ($26.32 \pm 2.31 \mu\text{g/g}$) then shoulder muscle ($18.21 \pm 1.011 \mu\text{g/g}$) and its level showed significant decrease at ($p < 0.05$) till not detected at 72 hr. concentration level showed in shoulder muscle, thigh and back muscles was (18.21 ± 1.11 , 13.5 ± 1.023 , $12.6 \pm 1.031 \mu\text{g/g}$). Regarding the effect of boiling of the drug in kidney sample it is evident that pefloxacin concentration was decreased from 28.32 ± 2.261 to $16.516 \pm 0.421 \mu\text{g/g}$. Such decrease was detected in the samples examined at the following hours until disappeared. Meanwhile frozen kidneys sample at -10°C showed significant decrease after 1st week and decreased till not detected at 5th week (14.240 ± 0.351 , 6.425 ± 0.052 , $1.253 \pm 0.322 \mu\text{g/g}$) followed by liver (4.250 ± 0.33 , 2.872 ± 0.251 , 1.205 ± 0.158 , $0.38 \pm 0.241 \mu\text{g/g}$).

INTRODUCTION

Antibacterial agents are widely used for prophylactic and treatment of various diseases in animals. The first antimicrobials based on the 4-quinolone ring were nalidixic acid and oxolinic acids, which are active in vitro against a wide range of Gram-negative bacteria. The included problems associated with their application were restricted spectrum of activity and the relatively rapid emergence of resistant mutants, which led to the discovery of fluoroquinolones. One of them is pefloxacin (1-ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid. As other fluoroquinolones, Pefloxacin achieves rapid bactericidal

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activity by inhibiting the bacterial DNA gyrase **Chu and Fernandes, (1991)¹ – Marie et al., (2000)²**. The drug possesses good in vitro activity against a variety of pathogens, including Gram-positive and Gram-negative bacteria. Pefloxacin is relatively similar to other fluoroquinolones such as Enrofloxacin, Ciprofloxacin, and Marbofloxacin (**Van-Custen et al., (1990)³ – Spreng et al., (1995)⁴ – Brown, (1996)⁵** in possessing a wide spectrum of activity, a large volume of distribution, and their activity at low concentration. Residues of veterinary medicinal products which are defined by European Union, are pharmacologically active substances (whether active principals, exipients, or degradation products), and their metabolites remain in food stuffs obtained from animals to which the veterinary medicinal products in question has been administrated **Vander Creek, (1984)⁶**. There has been an increased concern among consumer about antibiotic

and other drug or chemical residues **Katz and Brady, (1993)⁷**. The potential problems associated with drug residues may be aesthetics, allergic reactions, direct toxic effects, and change in the resistance patterns of bacteria exposed to antibiotic **Weaver. (1992)⁸**. The drug is metabolized in the animal's body and broken down or excreted, within the directed withdrawal time. Organs such as the kidney and liver remove residual drug and greatly reduce the content present in red meat or milk. These organs are the tissues tested for residues but content in red meat are less by many times **Glott et al., (1979)⁹**. The aim of this study is to detect the light pefloxacin residues in different tissues as well as the effect of heat treatment (boiling) and freezing on tissue residues.

MATERIAL AND METHODS

Material

i. Drug:

Pefloxacin (pefloadad 10% solution)

Structural formula

The chemical name: (1-ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid.

1) Chemical Formula: $C_{17}H_{20}FN_3O_3$

2) Dose: Pefloxacin is available in an injectable solution to be given intramuscularly at a dose of 10 mg/kg body weight.

ii. Experimental Rabbits:

Thirty healthy rabbits (Balady bread) of both sexes weighing about 1.5-2 Kg Body weight were used as experiment animals throughout this study.

They were kept for 15 days under well hygienic conditions and fed on concentrated mixture of barseem barley in a pellet form free from any antibacterial agents and water ad. Lid.

Methods

- 1) Two rabbits (control) were slaughtered and tested for the absence of any antibacterial residues.
- 2) The rest of rabbits were given pefloxacin intramuscularly (10 mg/Kg

body weight) for 5 successive days.

- 3) Two rabbits were slaughtered at 12, 24, 48, 72, 96, 120, and 144 hours after the last treatment.
- 4) Shoulder, thigh, back muscle, liver and kidneys were obtained from each rabbit at slaughtering time.

☒ Effect of Heat:

Part of samples (12 hours post treatment) were boiled in distilled water for 45 minutes and used to detect the drug residues.

☒ Effect of Freezing:

The rest of samples (12 hours post treatment) were kept at -10°C in freezer and examined weekly for detecting residues for one month.

Detection of Residues:

Pefloxacin residues were determined using High Performance Liquid Chromatography (HPLC) Knoure, Ink Germany, according to the method described by **Groeneveld and Brouwers, (1986)¹⁰**. Pefloxacin was extracted from

samples with dichloromethane and 0.1 M sodium phosphate buffer pH 7.4. Chromatography was performed on an amino-exchange column with the mobile phase and tested using UV detector, UV absorbance was monitored at 278 nm. Into a 10 ml extraction tube of 1 g of homogenized tissue (shoulder, thigh, back muscle, liver, and kidney) and 1 ml of 0.1 M phosphate buffer pH 7.4 were added. After adding 5 ml dichloromethane, the tube was stoppered and gently shaken at 100 cycle/min for 10 minutes and centrifuged at 4000 rpm for 10 minutes at room temperature. After removing the aqueous layer, the organic layer was transferred into another tube and dried under nitrogen at -50°C. The residues were dissolved in 1 ml mobile phase using Vortex mixer and sonication, before HPLC analysis. Depending on concentration, 5-20µl were injected.

Standard preparation

Pefloxacin standard solution was

prepared from drug pure 100% by dissolving a weight amount of drug in distilled water to make stock solution

Statistical analysis

It was carried out according to **Snedecor and Cochran (1967)**¹¹

RESULTS AND DISCUSSION

Nowadays antibiotic was used continuously in poultry farms and rabbit batteries for prophylaxis and treatment purposes of various bacterial diseases as well as for growth promotion. Some of these antibiotics leave residues in the animal tissues and may cause hazards to human beings consuming such tissues. Residues in meat of animals and poultry form a great problem facing food hygienists and constitute a real hazard to human consumers in the recent years. These residues are responsible for inducing allergic reactions such as urticaria, eczema, and other forms of dermatitis as well as increasing resistance of pathogenic micro-organisms in man, in addition to their harmful effects on the microflora and

consequently the produced vitamins (**Mol, 1971**)¹². Growth promoters are mostly given during the whole life time, while prophylactic and therapeutic regimens of antibiotics are given only for a short period (**Van Schothors et al., 1978**)¹³. Organs such as liver and kidney remove residual drug and greatly reduce the content in meat. These organs and tissues are tested for detecting drug residues (**Glott et al., 1979**)⁹. In the present study, pefloxacin administered intramuscularly to rabbits at dose of 10 mg/Kg body weight for 5 successive days. Table (1) revealed that the highest concentration level of pefloxacin was detected in kidneys at 12 hours from administration of last dose (28.32±2.61 µg/g) and its level showed significant decrease ($p<0.05$) at (24 hr, 48 hr, 72 hr, 96 hr, and 120 hr till not detected at 144 hr. (20.34±1.721, 16.04±0.013, 9.03±0.012, 4.02±0.01, and 1.44 ±0.670 µg/g), respectively. The effect of boiling (100°C for 30 minutes) showed significant

decrease (16.516±0.421, 13.610 ±0.198, 8.513±0.198, 4.931 ±0.03, 1.12 ±0.06, and 0.72±0.05 µg/g) and not detected at 144 hr. The effect of frozen storage (table 2) showed significant decrease at 1st week (14.240±0.351 µg/g) and its level decline till not detected at 5th week (6.425 ±0.052, 1.253±0.322 µg/g) then followed by liver, shoulder and thigh (26.32 ±2.231, 18.21±1.011, and 13.5±1.023 µg/g) and its level decreased till not detected at 5th week. Pefloxacin concentration level in liver decline at 24 hr till not detected at 144 hr from drug administration (13.65±1.401, 8.451±0.69, 4.03 ±1.021, 1.98±0.81, and 0.89 ±0.003 µg/g). Regarding the effect of boiling liver concentration level was (4.720±0.320, 6.612±1.631, 3.72±0.051, 2.13±0.52, 0.06 and ±0.041 µg/g) till not detected at 144 hr. Pefloxacin concentration level showed significant decrease in frozen liver sample (4.250±0.331, 2.872 ±0.251, 1.205 ±0.158, and 0.38±0.241 µg/g) and not detected at

5th week. Boiled shoulder muscle was (11.456±0.778, 6.82±0.721, 3.310±0.671, and 1.08 ±0.01 µg/g) it was decreased till not present after 96 hr. Frozen shoulder muscle showed a decrease from the 1st week (13.65±0.672, 9.723±0.832, 1.475±0.832, and 0.4±0.051 µg/g) at 2nd, 3rd and 4th weeks and not detected at 5th week. Pefloxacin concentration level in thigh decreased (8.21 ±0.521, 4.25 ±1.032, and 1.62 ±0.011 µg/g) and not detected at 72 hr (5.273 ±0.822, 2.350 ±1.783, and 0.32 ±0.015 µg/g). The lowest drug concentration was found in back muscle (12.6 ±1.031 µg/g) and decreased at 24 hr till not detected at 48 hr. (12.6 ±1.031, 7.54 ±0.432 µg/g) as a result of freezing in back muscle antibiotic concentration level was (4.605 ±0.531 µg/g) at 1st week then decreased till not detected at 4th week. (2.93 ±0.634, 1.33 ±0.121, and 0.03 ±0.04 µg/g), respectively at 2nd and 3rd week. Pefloxacin level in frozen back muscle, liver, and kidneys revealed significant

decrease ($p < 0.05$) till not detected at 5th week Table (2). lowest pefloxacin level was detected in Table (1) in thigh muscle followed by back muscle (13.5 ±1.023 and 12.6±1.031 µg/g) and showed significant decrease ($p < 0.05$) till not detected at 96 hr. Mean while in boiled thigh muscles the concentration level was 5.273 ±0.822 µg/g and decreased till not detected at 72 hr and in boiled back muscle the concentration level was 4.231 ±0.0654 µg/g at 12 hr and decline till not detected at 48hr from first dose administration. Pefloxacin residues were gradually disappeared with time elapsed from onset of freezing till complete disappearance after the 4th week in thigh and back muscle Table (2). From the recorded data (Tables 1 and 2), it was observed that the high incidence of antibiotic residues in slaughtered rabbits were recorded in kidneys and liver samples then followed by tissues. This may be attributed to the fact that liver is responsible for metabolism and

detoxication of the drug by its microsomal enzyme but kidneys are responsible for filtration and clearance of the blood from any undesirable constituents. These results were greatly in accordance to those detected by **Daoud (1991)¹⁴**, **Daoud (1995)¹⁵**, **Hassan (1995)¹⁶**, **Daoud (1996)¹⁷**, **and Hassan (1998)¹⁸**. Our results are in agreement with **Anadon et al., (1995)¹⁹**. They found that norfloxacin was administrated orally the concentration in breast, fat, and liver was 0.05 µg/g on the second day after the end of dosing. **Bergeron et al., (1985)²⁰** reported that the concentration of norfloxacin in kidney parenchyma was 4-12 times of the serum concentration. **Scheer (1987)²¹** reported that intravenous injection of Baytril showed highest concentration in liver and kidney. **Alesting (1990)²²** found that the highest concentration in liver, breast and muscle. **Mankorios (1999)²³** found that liver contains the highest concentration of ciprofloxacin followed by fat and muscles

where the residues disappeared at 10th day of administration of the last dose. **Elinstein et al., (1994)²⁴** reported that all of fluoroquinolones are well absorbed after oral administration. Fluoroquinolones (FQs) are minimally protein bound and widely distributed in body tissues. **Scheibner (1972)²⁵** concluded that high concentration of penicillin G and oxytetracycline were completely inactivated by an hour heating at 90°C. Available literatures are lacking any figures concerning the effect of boiling and freezing of pefloxacin.

Pefloxacin is fluoroquinolones and are similar to antibiotics in their distribution and activity. **Scheibner (1969)²⁶** stated that heating of meat for 60°C for 60 minutes had no effect on antibiotic residues but heating at 90°C minimized to some extend the antibiotic activity. **Chuna (1972)²⁷** mentioned that normal methods of cooking destroyed aureomycin and terrormycin. **Katz et al., (1972)²⁸** reported that boiling of tissues and organs containing

chlorotetracycline residues converted the residues to isochlorotetracycline which had no known biological activity. **Vandenbrande et al., (1972)**²⁹ stated that cold storage of meat reduced the activity of penicillin residue. **Jukes (1973)**³⁰ concluded that cooking temperature destroyed chlorotetracycline residues in meat. **Inglis and Katz (1978)**³¹ recorded that heating may cause some loss of the antibacterial activity of aminoglycosides, depending on the system in which heating was studied. **O'brein et al (1981)**³² studied the effects of cooking and cold storage on antibiotic residues in meat. They recorded that cooking and cold storage caused degradation of antibiotic. **Nashwa (1995)**³³ reported that oral administration of tylosine 25mg/Kg b.wt. twice daily for 5 successive days and boiling of chicks tissues and organs for 30 minutes completely degraded tylosine residues in all tissue samples and at 5th week of freezing tylosine residues were completely disappeared from gizzard

and heart and at 6th week from all tissue samples. **Haagsma (1993)**³⁴ concluded that the content of residues of many veterinary drugs decreased not only as a result of food preparing and processing, but also at cooled and frozen storage. **Amer et al., (1992)**³⁵ stated that gentamycine or netilimicine at dose of 6 mg/kg b.wt. intramuscularly daily for 7 successive days disappeared by boiling the muscle samples for 45 minutes and freezing for one week. Moreover boiling of kidney and liver samples for 45 minutes did not destroy the residues completely. Freezing for 3 months also failed to destroy the residues in kidney completely. **Gyhan (1997)**³⁶ found that apramycin sulphate residues in chicken tissues after boiling at 100°C for 45 minutes failed to detect in liver, kidney, gizzard and fat after 48 hours from drug administration and were failed to detect in breast and thigh muscles after 72 hours from last oral and residues disappeared from breast and thigh

muscles, liver, kidney, gizzard and fat after the 3rd day from freezing and disappeared from skin after 2 days from freezing samples. **Pouliques and Morvan, (2002)**³⁷ determined the residues of oxolonic acid (OA) and flumequine (Flu) in freeze-dried salmon muscle with attached skin, using reversed-phase high performance liquid chromatography. They concluded that, the limits of detection were 3.2 and 16 ng/g wet weight tissue respectively. Mean extraction recoveries of OA and Flu freeze-dried tissue were 85.5 and 85.2%, respectively. **Abd el-Aty and Goudah, (2000)**³⁸ found that after intravenous and intramuscular administration of pefloxacin at dose of 10 mg/Kg body weight at a single dose to lactating goats, drug distributed rapidly and was detected in serum 5 min after injection and its concentration decreased gradually till reading lowest detectable level. **Abou EI-Nil, (2003)**³⁹ revealed that Danfloxacin, Ciprofloxacin, and Flumequine administration orally to chicken at dose of 5 mg/Kg body weight for 5 successive days the highest concentration level was detected in liver at 1st day from drug administration. **Abou EI-Nil, (2006)**⁴⁰ reported that the highest concentration level of Ciprofloxacin detected in chicken liver at 1st day and decreased till not detected at 9th day and the lowest concentration level was detected in thigh muscle 12.6 ± 1.025 $\mu\text{g/g}$. concentration of antibiotic decreased by heat treatment frozen storage of liver, kidney, breast, and thigh muscle resulted in gradual decrease in concentration from 1st week till not detected at 5th week. **Habib et al, (2006)**⁴¹ showed that the effect of cooking on the decomposition and concentration of flumequine and oxolonic was observed. Cooking temperature had no effect but concentration of these quinolones increased by diffusion from the kidney and liver.

Table (1) Pefloxacin residues in tissues and organs, and Effect of heat treatment (boiling u/g) after intramuscular injection with dose of 10 mg/Kg body weight for 5 successive days to rabbits (N = 4)

Time	Tissue	12 hr		24 hr		48 hr		72 hr		96 hr		120 hr		144 hr	
		Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment
	Shoulder muscle	18.21 ^a ±1.011	11.456 ^a ±0.778	13.41 ±2.21	6.82 ^b ±0.721	9.57 ±0.73	3.310 ^a ±0.671	.23 ±0.015	1.08 ±0.01	0.04 ±0.02	0	0	0	0	0
	Thigh	13.5 ^a ±1.023	5.273 ^b ±0.822	8.21 ±0.521	2.350 ^d ±1.782	4.25 ±1.03	0.32 ±0.015	1.62 ±0.011	0	0	0	0	0	0	0
	Back muscle	12.6 ±1.031	4.231 ^b ±0.654	7.54 ±0.432	1.630 ^b ±0.772	0	0	0	0	0	0	0	0	0	0
	Liver	26.32 ±20231	4.720 ^c ±0.320	13.65 ±1.901	6.612 ^c ±1.631	8.451 ±0.69	3.72 ^a ±0.051	4.03 ±1.021	2.13 ±0.52	1.98 ±0.81	0.06 ±0.041	0.89 ±0.003	0	0	0
	Kidney	28.32 ±2.61	16.516 ^d ±0.421	20.34 ±1.721	13.610 ±0.198	16.04 ^d ±0.013	8.513 ±0.198	9.03 ±0.112	4.931 ±0.03	4.02 ±0.01	1.12 ±0.06	1.44 ±0.670	0.72 ±0.05	0	0

Means with different letters are significant at P < 0.05

Table (2) Effect of frozen storage at -10°C on tissue concentration (mg/g) of pefloxacin after injected intramuscularly at dose of 10 mg/ Kg body weight for 5 successive doses to rabbit

Time Tissue	Before freezing	1 st week	2 nd week	3 rd week	4 th week	5 th week
Shoulder muscle	18.21 ^b ±1.011	13.65 ^a ±0.672	9.723 ±0.832	1.475 ^a ±0.832	0.4 ^a ±0.051	0
Thigh	13.5 ^c ±1.023	3.978 ^b ±0.472	2.524 ^c ±0.163	0.341 ^b ±0.139	0	0
Back muscle	12.6 ±1.301	4.605 ^c ±0.531	2.93 ^c ±0.634	1.33 ^b ±0.121	0.03 ^a ±0.04	0
Liver	26.32 ^c ±2.331	4.250 ^c ±0.331	2.872 ^b ±0.251	1.205 ^c ±0.158	0.38 ^b ±0.241	0
Kidney	28.32 ^a ±2.61	14.240 ±0.351	6.425 ±0.052	1.253 ±0.322	0	0

Means with different letters are significant at $P < 0.05$

Conclusions and Recommendations

It is clear that the use of antibiotic for prophylaxis and treatment of some injectionus diseases of poultry and rabbits shortly before slaughter resulted in the presence of their residues in muscles and organs. To safe consumer against the hazards which result from consumption of such in rabbit meat and organs containing antibiotic residues, the following instructions should be recommended:

1. Prohibit administration of antibiotic before slaughter is necessary for withdrawal of any residues (**Garrod, 1964**)⁴².
2. An administration of antibiotics should be done under the supervision of veterinarians.
3. Regular examination of slaughtered animal for detection of antibiotic residues.

4. Carcasses have been proved to contain antibiotics should be condemned.
5. Sufficient heat treatment or cold storage for each slaughtered animal may cause degradation of antibiotic (Waffia, 1989)⁴³.
6. In order to curb the resistance problem, we must encourage the return of the susceptible commensals flora as this way may become the best allies in reversing antibiotic residues (Levy, 2000)⁴⁴.

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