

## FLUOROQUINOLONE RESIDUES IN CATFISH

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### ABSTRACT

Sixty six catfish weighing about 200 gm each were used in the present study. Six fish were kept as a control, the others were classified into two groups in the present study each of 30 and were given enrofloxacin and ciprofloxacin (10%) daily for 5 successive days (5 mg/kg B wt.) in aquarium respectively. Blood and tissue samples were taken from six fish from each group at 24, 48, 72, 96 and 120 hours for studying some biochemical and hormonal parameters as well as their residues. The residues of these drugs were detected microbiologically using *Bacillus subtilis* organisms on nutrient agar medium. The effects on some biochemical parameters as well as reproductive hormones were also investigated. It was observed that enrofloxacin and ciprofloxacin residues could be detected in muscle samples up to 96 hours and 120 hours respectively (1.1 mm and 2.2 mm). The residues could not be detected after 120 hours for both drugs. Enrofloxacin and ciprofloxacin induced a significant decrease in serum total proteins. The drugs also showed a significant increase in serum creatinine, AST and ALT blood urea nitrogen. Testosterone and oestrogen levels decreased significantly after 4 days from exposure to enrofloxacin and ciprofloxacin. The gonadotropins did not show any significant difference. It is concluded that residues of enrofloxacin and ciprofloxacin lasted for 6 days in fish muscles. Prolonged exposure to these drugs resulted in a decrease in the oestrogen and testosterone levels while gonadotropins were not altered.

### INTRODUCTION

The production of meat is not proportional to the large increase in the world population especially in the developing countries. So much efforts are directed towards the production of fish and poultry to overcome the problem of animal protein supply.

Antibacterial agents are widely used for prophylaxis and treatment of various diseases in animals. Enrofloxacin and ciprofloxacin the quinolone carboxylic acid derivatives are chemotherapeutic agents with the antibacterial activity for both oral and parenteral administration in veterinary practice (1).

Enrofloxacin and ciprofloxacin have been proved to be highly effective against bacterial infections in cattle, rabbits, dogs, poultry and fish (2). Ciprofloxacin is a broad spectrum fluoroquinolone antibacterial agent, with clinical efficacy and low potential for adverse effects (3).

The purpose of the present work was thus initiated to throw the light on enrofloxacin and ciprofloxacin residues in fish tissues. Special emphasis has been drawn to study their effects on reproductive hormones specially oestrogen, testosterone and gonadotropins. Some biochemical parameters were also investigated to declare the side effects of these drugs on fish.

### MATERIAL AND METHODS

**Drugs :** Enrofloxacin (Baytril®) and ciprofloxacin were obtained from Bayer A.G., Germany.

**Animals:** Sixty six catfish (*Clarias lazera*) weighing about 200 gm each were used as experimental animals throughout this study.

### Detection of the residues:

The method described by Boagaerts and Wolf Brussels (4) was used for detection of the antibiotic residues using *Bacillus subtilis* BGA strain on nutrient agar media at pH 6.8.

### Interpretation of the results :

Inhibition zone > 2mm is considered +ve

Inhibition zone 1 : < 2mm is considered suspicious

Inhibition zone < 1mm is considered -ve

### Hormonal assay :

Testosterone and oestrogen hormones were estimated by enzyme linked immunoassay kits (5).

Gonadotropin hormones (GTH) were assayed by radio immunoassay. Standards and antisera of ovine FSH and LH were used for GTH assay (6).

### Liver and kidney function tests :

Kits for liver and kidney function tests were obtained from bioMerieux (France).

Determination of serum aspartate aminotransferase; (AST) and alanine aminotransferase (ALT) were determined colorimetrically (7).

Determination of serum urea was done according to Trinder (8) and creatinine according to Husdam and Rapoport (9).

Total proteins were also estimated by Henry (10)

Analysis of variance (ANOVA) and Students (t) test were carried out for comparing the different groups with each other (11).

### RESULTS

Table (1) revealed that enrofloxacin and ciprofloxacin disappeared from the muscles after 120 hours of drug treatment

Table (2 and 3) showed the levels of oestrogen, testosterone and gonadotrophic hormones (GTH) at the end of the experiment in mature female catfish after exposure to enrofloxacin or ciprofloxacin. GTH showed non significant changes. Significant decrease in the testosterone level was achieved after 96 hour of exposure to enrofloxacin or ciprofloxacin and the level of oestrogen was also decreased significantly after 120 hours of exposure to either drugs.

#### Biochemical effects of ciprofloxacin:

Ciprofloxacin significantly decreased the serum level of total proteins in all exposure periods. Blood urea nitrogen (BUN) and creatinine were significantly

Table (1) : Mean inhibition zones in mm. of enrofloxacin and ciprofloxacin (5 mg/kg b.wt) in muscle of catfish.

Time of exposure (hours)	Enrofloxacin		Ciprofloxacin	
	PH6	PH8	PH6	PH8
Zero	0	0	0	0
24	7.3	7.6	6.8	7.2
48	5.2	5.6	4.8	5.3
72	3.5	3.6	4	4.2
96	2.2	2	3	3.2
120	1.1	0	2.2	2.2

Table (2) : Effect of enrofloxacin (5 mg/kg b.wt) on testosterone, oestrogen and gonadotrophic hormone (GTH) n = 6

Time of exposure (hours)	Testosterone pg/ml	GTH mg/ml	Oestrogen ng/ml
Control	55.2±2.5	32.1±2.2	80.0±2.7
24	55.4±2.9	28.8±1.8	79.1±1.9
48	57.3±3.5	30.5±1.8	81.5±3.1
72	55.6±2.1	31.8±2.3	83.1±2.9
96	49.8±2.2*	33.2±1.2	77.0±3.1
120	47.8±2.1*	34.8±1.1	70.8±2.1

\* Significant at P<0.05

increased up to 72 hours, of exposure to ciprofloxacin. Serum aspartate amino transferase (AST) and serum Alanine aminotransferase (ALT) were also affected by ciprofloxacin table (4).

Results illustrated in table (5) indicated that enrofloxacin caused significant decreases in serum total proteins of Nile catfish in all exposure periods. Blood urea nitrogen (BUN) was increased up to 72 hours of exposure to enrofloxacin

Creatinine was significantly increased up to 48 hours of exposure, to enrofloxacin. Serum AST & ALT were significantly altered by enrofloxacin table (5).

Table (3) : Effect of ciprofloxacin 5 mg/kg b.wt on testosterone, oestrogen and gonadotrophic hormone (GTH) n = 6

Time of exposure (hours)	Testosterone pg/ml	GTH mg/ml	Oestrogen ng/ml
Control	55.2±2.5	32.1±2.2	80.0±2.7
24	57.2±2.4	29.0±2.9	78.9±2.9
48	58.4±2.8	27.5±1.2	80.5±2.5
72	55.6±3.5	30.1±2.1	82.2±2.1
96	50.8±2.1*	31.4±2.3	78.0±2.1
120	48.2±2.3*	33.1±1.9	73.1±2.5*

\* Significant at P<0.05

### DISCUSSION

The residues of antibiotics in the human food of animal origin cause many troubles to the human. Antibiotic residues may cause allergic reactions, sensitization and antibiotic resistance. Our results revealed that the residues of enrofloxacin or ciprofloxacin (5 mg/kg B.wt.) for 5 successive days disappeared from the fish muscle after 5 days of drug administration. Our results are in accordance with that reported by Bogorditskaya et al.<sup>(12)</sup> who detected the residues of enrofloxacin in muscles till 72 hours after last administration. Enrofloxacin residues disappeared completely after 72 hours in healthy chicks given the drug orally (10 mg/kg B.wt.) for five consecutive days<sup>(13)</sup>. Our result were not consistent with this result and may be due to difference in dose and species used.

The increased serum AST and ALT activities of catfish treated with fluoroquinolone for 5 successive days are compatible with those before obtained<sup>(14)</sup>.

Table (4): Effect of ciprofloxacin on liver and kidney functions Nile catfish (*Clarias lazera*). (Means + S.E) n = 6

Parameters	Control	Exposure time (hours)				
		24	48	72	96	120
Total protein (g/dl)	7.35 <sup>a</sup> ± 0.28	4.6 <sup>cd</sup> ± 0.09	5.68 <sup>c</sup> ± 0.10	5.06 <sup>c</sup> ± 0.10	4.28 <sup>dc</sup> ± 0.12	4.03 <sup>c</sup> ± 0.10
Blood urea nitrogen (g/dl)	8.33 <sup>c</sup> ± 0.40	14.96 <sup>b</sup> ± 0.60	14.83 <sup>b</sup> ± 0.10	17.71 <sup>a</sup> ± 0.70	9.33 <sup>c</sup> ± 0.31	8.68 <sup>c</sup> ± 0.26
Creatinine (g/dl)	4.43 <sup>c</sup> ± 0.09	5.16 <sup>b</sup> ± 0.06	5.45 <sup>a</sup> ± 0.07	5.6 <sup>a</sup> ± 0.10	4.6 <sup>c</sup> ± 0.10	4.48 <sup>c</sup> ± 0.10
AST (I.U)	78.13 <sup>d</sup> ± 1.6	76.9 <sup>d</sup> ± 1.0	91.18 <sup>c</sup> ± 2.35	165.25 <sup>a</sup> ± 4.2	130.48 <sup>b</sup> ± 2.32	83.15 <sup>d</sup> ± 1.3
ALT (I.U)	18.81 <sup>c</sup> ± 0.65	16.85 <sup>c</sup> ± 1.92	17.1 <sup>c</sup> ± 1.5	52.38 <sup>a</sup> ± 2.5	38.35 <sup>b</sup> ± 1.30	35.53 <sup>b</sup> ± 1.70

Mean values that have different letters are significantly different from each other vice versa and the highest mean value is represented with the letter (a) followed by the letters (b, c, ....etc). P<0.05 level was taken as a level of significance.

Table (5): Effect of enrofloxacin on liver and kidney functions Nile catfish (*Clarias lazera*) (Means + S.E) n = 6

Parameters	Control	Exposure time (hours)				
		24	48	72	96	120
Total protein (g/dl)	7.35 <sup>a</sup> ± 0.28	4.5 <sup>bc</sup> ± 0.02	4.13 <sup>bc</sup> ± 0.20	3.98 <sup>bc</sup> ± 0.04	4.51 <sup>b</sup> ± 0.17	3.6 <sup>c</sup> ± 0.13
Blood urea nitrogen (g/dl)	8.33 <sup>c</sup> ± 0.40	14.41 <sup>a</sup> ± 0.50	14.86 <sup>a</sup> ± 0.24	11.1 <sup>b</sup> ± 0.23	9.03 <sup>c</sup> ± 0.25	9.01 <sup>c</sup> ± 0.36
Creatinine (g/dl)	4.43 <sup>c</sup> ± 0.09	6.16 <sup>b</sup> ± 0.12	6.66 <sup>a</sup> ± 0.12	4.3 <sup>c</sup> ± 0.10	4.45 <sup>c</sup> ± 0.10	4.28 <sup>c</sup> ± 0.15
AST (I.U)	78.13 <sup>d</sup> ± 1.6	110.0 <sup>b</sup> ± 1.3	121.83 <sup>c</sup> ± 1.43	120.16 <sup>a</sup> ± 3.8	76.61 <sup>b</sup> ± 0.96	103.75 <sup>b</sup> ± 1.20
ALT (I.U)	18.81 <sup>c</sup> ± 0.65	39.5 <sup>b</sup> ± 1.40	22.1 <sup>d</sup> ± 1.2	30.86 <sup>c</sup> ± 2.2	56.6 <sup>a</sup> ± 1.5	58.29 <sup>a</sup> ± 1.9

Mean values that have different letters are significantly different from each other vice versa and the highest mean value is represented with the letter (a) followed by the letters (b, c, ....etc). P < 0.05 level was taken as a level of significance.

They observed a slight increase in serum ALT and AST resulting from some liver damage produced by drugs. The obtained results showed that serum creatinine level was elevated. Enrofloxacin and ciprofloxacin revealed a significant increase in blood urea nitrogen which could be attributed to affection of the kidney along the course of the treatment. The elevated creatinine may be due to nephrotoxic effect of the drugs (15).

Our result revealed a hypoproteinemia which might be due to amino acids utilization as a defense against the pathogens and renal damage provoked by bacteria or drugs (16). The decreased serum of levels total

protein in the present data might be explained by the renal damage provoked by the drug as reported by (17) who stated that hypoproteinemia can occur with renal diseases. Hypoproteinemia is most commonly associated with lack of proper diet or poor absorption of dietary constituents from the intestinal tract. Excessive loss of protein resulting from burn, draining wounds, renal disease or increased protein breakdown required for gluconeogenesis may result in decrease in total proteins.

The testosterone and oestrogen levels decreased after exposure to enrofloxacin or ciprofloxacin, this reduction might be due to stress reaction induced by

antibiotics. Also alterations in the circulating levels of reproductive steroid hormones and in reproductive function which have been demonstrated in several teleost species after exposure to physical and chemical stress. Our results coordinated with those previously reported<sup>(18)</sup>. They stated that reproduction in teleost is a complex process involving considerable physiological coordination that is largely controlled by reproductive hormones secreted by the endocrine glands.

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### بقايا الفلوروكينولون في الأسماك

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تم اعطاء دوائى الأتروفلوكساسين والسبروفلوكساسين (١٠٪) يومياً لمدة ٥ أيام متتالية بجرعة ٥ مجم / كجم من وزن الجسم إلى ٦٦ سمكة قرموط ( كلاريز لازيرا) بوزن ٢٠٠ جم للواحد. وتم وضع ستة سمكات كمجموعة ضابطة. وأمكن قياس بقايا الأدوية ميكروبيولوجيا باستخدام عطرة الباسليس ستلز. وأمكن معرفة تأثير ذلك الأدوية على بعض وظائف الكبد والكلى وعلى الهرمونات التكاثرية ومن تلك الدراسة أتضح أن بقايا الأتروفلوكساسين والسبروفلوكساسين فى العضلات تظل حتى اليوم الرابع إلى الخامس والبقايا هى ١ر١ جزء فى المليون ، ٢ر٢ جزء فى المليون ، وتختفى البقايا بعد ستة أيام .

وأوضحت الدراسة أن الأتروفلوكساسين والسبروفلوكساسين أديا إلى نقص ذا دلالة إحصائية فى مستوى البروتين زيادة فى مستويات الكرياتينين ، أنزيم الأستريت أمينوترانسفيريز والالانين أمينوترانسفيريز وذلك بالمقارنه بالمجموعة الضابطة . أيضا أثبتت الدراسة زيادة معنوية فى اليوريا بينما أظهرت النتائج نقص معنوى فى هرمون التستوستيرون والأستروجين بعد التعرض لفترة طويلة بينما لم يتأثر هرمون الجونادوتروپين . ومن هذه الدراسة يتضح أن بقايا الأتروفلوكساسين والسبروفلوكساسين تحتفى بعد ٥ أيام فى عضلات السمك ويمكن أستهلاكه بعد هذه الفترة من العلاج وأن تلك الأدوية لها تأثير مثبت لهرمون الأستروجين والتستوستيرون بينما لم يتأثر هرمون الجونادوتروپين .