RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences
(P-ISSN 1595-093X/ E-ISSN 2315-6201)
Okoroafor *et al*. /*Sokoto Journal of Veterinary Sciences* (2017) 15(1): 11-19.
http://dx.doi.org/10.4314/sokjvs.v15i1.2

Pharmacokinetics of ciprofloxacin in bath medicated hybrid tilapias using enzyme linked immunosorbent assay (ELISA)

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Abstract

The apparent absence of established dosage regime for medicating tilapia with fluoroquinolones in the country necessitated investigating the absorption and tissue distribution of ciprofloxacin, since dosage regimes conform to accepted standards of pharmacokinetic studies. Ciprofloxacin pharmacokinetic studies were conducted on 76 hybrid tilapia weighing between 100 and 200 grams divided into two groups with each subjected to bath administration of ciprofloxacin at two different concentrations. The fishes in groups 1 and 2 were exposed by bath to 50 mg and 25 mg respectively of ciprofloxacin per litre of water. Tissue and blood samples were collected at 0.5h, 1h, 2h, 4h, and 8h during medication and drug withdrawal at 24h, 48h, and 72h, to quantify their ciprofloxacins by Enzyme Linked Immunosorbent Assay (ELISA). Peak serum concentration of ciprofloxacin in group 1 was 2,251±877 µg/L at 8 h while for group 2; it was 2,226±174 µg/L at 4 h exposure time. The t- test comparing the means of the groups 1 and 2 showed that there was no significant statistical difference ($p > 0.05$) on group sera, liver and kidney, except skeletal muscle ciprofloxacin concentration. The elimination half-lives in groups 1 and 2 were determined on serum ciprofloxacin values, after withdrawal of drug and were 27.75 h and 31.8 h, with rate constant of elimination being 0.025 h^{-1} and 0.022 h^{-1} respectively. Sera Area under Curve, {$AUC_{(0.5\text{-}8h)}$} values were 12,159.3 and 13,194 µg/L respectively with 92.2% correlation. Prolonged high ciprofloxacin concentration was observed in tilapia skeletal muscle. High correlation in sera AUC values is suggestive of similar therapeutic action hence; it is cheaper to use the lower dose. While a prolonged high ciprofloxacin concentration in skeletal muscle has therapeutic advantage for fish, it remains a public health concern because the muscle is an edible tissue.

Keywords: Bath, Ciprofloxacin, ELISA, Pharmacokinetics, Tilapia
Received: 26-06- 2016 Accepted: 01-11-2016

Introduction

The drive by the Nigerian Federal Government to encourage food production especially animal protein has led to an increase in tilapia production. It is expected that production pressure will be associated with outbreaks of diseases especially those of bacterial origin. Tilapias are teleosts of family Cichlidae having 3 genera namely *Tilapia, Sarotherodon* and *Oreochromis*. Like other farmed species of fish, they are susceptible to bacterial infections. Commercial fish culture in Nigeria had been in existence since early 50s from the onset of Panyam farms in Plateau state Wokoma (1987). Tilapia culture is currently gaining ground due to increased demand and use of improved breeds and application of "all-male" tilapia farming which promote better feed conversion ratio. The controlled rearing of fish under high stocking density in comparison with the situation in the natural habitat definitely puts some pressure on fish in captivity

increasing their susceptibility to diseases, especially if the pond water quality is not well managed.

Cabello (2006) reported that because of unpredictable high mortalities on fish farms, farmers are compelled to frequently administer antibacterials to their fish. Amongst common antibacterials are the quinolones or more strictly the 4-quinolones which are a group of chemically related synthetic antibacterial agents, all being carboxylic acids. Despite their name, they do not all have a 4-quinolone ring molecular structure. Nalidixic acid which was the first to be developed is a naphthyridine carboxylic acid. Only a limited number of the drugs have been studied for use in fish medicine, and among these, oxolinic acid, which is a true 4-quinolone, is of particular interest and important as it was originally developed in Japan, specifically for fish Treves- Brown (2000).

Flourine atom at position 6 in the 4-quinolone molecule significantly enhances antibacterial activity and this has led to the development of a sub-group called fluoroquinolones, of which ciprofloxacin and enrofloxacin are examples Treves- Brown (2000). Fluoroquinolones are important group of synthetic antimicrobials with a broad-spectrum antibacterial activity, good absorption after oral administration, and extensive tissue distribution Yang *et al*. (2005). They act by inhibition of bacterial DNA-gyrase and because gyrase is an essential enzyme in prokaryotes, but not found in eukaryotes, bacteria are an ideal target for these antibiotics Booth (1998); Yorke & Froc (2000). Fluoroquinolones have a widespread application in the prevention and treatment of diseases in food-producing animals Marta *et al.* (2007).

Studies by Ashiru *et al.* (2011) showed that 100% of *Aeromonas* spp isolated from skin and intestinal lining of tilapia were susceptible to ciprofloxacin. In their work, *Aeromonas caviae*, *Aeromonas sobria,* and *Aeromonas hydrophila* were all resistant to tetracycline, nitrofurantoin and Augmentin® (Amoxicillin +clavulanate) with average zone of inhibition of 9 mm, 10 mm, and 8 mm respectively but were susceptible to pefloxacin, ofloxacin and ciprofloxacin with an average zone of inhibition of 17 mm, 21 mm and 24 mm respectively. Ciprofloxacin, (a metabolite of enrofloxacin) is a potent fluoroquinolone used in treating bacterial infections in both human and animals and has been studied in different species of fish Nouws *et al.* (1988).

This study was designed to determine and compare the extent of absorption, distribution and pattern of elimination of ciprofloxacin in tilapia subjected to bath treatment in two different bath concentrations of ciprofloxacin. This is in view of the fact that when inappetence or anorexia occurs in the midst of bacterial disease outbreak in fish, the best option for medication would be bath method as medicated feed might not be consumed.

Materials and Methods

Experimental fish and study conditions

This study was carried out at the Department of Veterinary Medicine, University of Ibadan, Nigeria. A total of 76 freshwater tilapias (hybrid of *Sarotherodon galilaeus* x *tilapia zillii*) weighing between 100 and 200 grams each were purchased from department of fishery, University of Ibadan. They were equally stocked at one fish per 7½ litres of water in two plastic containers of capacity, 380 litres each. Fishes were acclimatized for five days and fed on 2 mm floating fish feed (which had 46% crude protein level) at the rate of 1.3% of total biomass.

Water quality assessment

Analysis of water chemistry, involving such parameters as total ammonia nitrogen, unionized ammonia, pH, nitrite, ferrous iron, total hardness, calcium hardness and magnesium hardness, were done as described by Chattopadhyay (1998).

Enzyme linked immunosorbent assay (ELISA) kit

The Ridascreen® enro/cipro ELISA kit from R-Biopharm AG, Germany, is designed for quantifying enrofloxacin residues in edible tissue. This was adapted for pharmacokinetic studies of ciprofloxacin in Tilapia subjected to two different bath concentrations of the drug.

Drug administration and sample collection

Ciprotril® a brand of 10% ciprofloxacin, (manufactured by VAPCO, Jordan) was used in this experiment. From the source, the fishes had not been medicated for over two weeks prior to purchase. Tilapias were subjected to bath administration of ciprofloxacin at two different concentrations. The fishes in groups 1 and 2 were exposed to 50 mg and 25 mg respectively of ciprofloxacin per litre of water for 8 hours after which there was withdrawal of medicated water and replacement with fresh water.

At different time intervals (0.5h, 1h, 2h, 4h, 8h, 24h, 48h and 74h) after drug administration, blood samples (from two randomly selected tilapia in each group per time interval) were collected from the

caudal vein of fish and dispensed into eppendorf tubes and labeled.

The same fish samples were immobilized by transecting the vertebral column and bled to death. Dead fish were then placed on lateral recumbency to incise the abdomen and expose the viscera. Liver and kidney samples were excised. Portions of muscle from the incised abdominal wall were also taken and all were labeled according to groups and time taken.

Blood, liver, kidney and muscle samples were collected at 0.5h, 1h, 2h, 4h, 8h, 24h, 48h and 72 h (after drug administration). Note that twenty-four hours after drug administration means 16 hours post-drug withdrawal since fishes were exposed to the drug for 8 hours before the water change.

Sample preservation and preparation

All samples were preserved at 0°C in a freezing compartment of a refrigerator before processing for drug extraction, dilution and quantification.

Sample preparations and methanolic extraction of ciprofloxacin from tissues were done as described by Nizamlioglu & Aydin (2012) who used the Ridascreen® enro/cipro ELISA kit. Their procedure was modified in this work for pharmacokinetic studies of ciprofloxacin in tilapia tissues and sera. Blood samples collected in eppendorf tubes were spun, using a centrifuge (Haraeus Biofuge Primo Centrifuge from Thermo Electron Corporation, Germany) at 10,000 rpm for 10 minutes, to obtain the sera. The separated sera were aspirated (using new pipette tips for each), dispensed into new eppendorf tubes and re-labeled before preservation at 0°C. Liver, kidney, and muscle samples collected at each time interval from the fishes were separately pooled and labeled.

Tissue samples were also stored at 0°C before weighing and processing for extraction of ciprofloxacin by use of 70% methanol. Each gram weighed was taken into a sterile plastic universal bottle, containing 4 ml of 70% methanol and then homogenized in the universal bottle, using a plastic spatula and left for 15 minutes for extraction of ciprofloxacin.

The methanolic extract from the tissues thus became a 1:5 dilution of the original concentration of the drug in the tissue. One microlitre of extract of each tissue sample was taken and further diluted in 599 microlitre of 70% methanol (1:600 dilution), thus increasing the dilution to 1:3000. Optical density readings of tissue samples were extrapolated on standard curve to obtain the corresponding ciprofloxacin concentrations. The serum samples were diluted, using 1 microlitre of serum to 599 microlitre of 70% methanol to obtain a dilution of 1:600. These further dilutions were done to ensure that optical densities of samples fell within the 0 to 18 ppb (µg/kg or µl/L) range of standards of ciprofloxacin, before multiplication by dilution factors. All diluted samples were thoroughly and individually mixed using a multichannel pipette (Eppendorf®) fitted with pipette tips. Fifty (50) microlitre of each sample was used in the assay.

Enzyme linked immunosorbent assay (ELISA) procedure

The assay was done according to the guidelines of the Ridascreen kit (r-biopharm®, Germany). The required numbers of wells (i.e. 32 wells for serum, 48 wells for tissues and 6 wells for standards) were inserted into the microwell holder for standards and samples to be run. The well positions and corresponding samples or standards were recorded. Fifty microlitres of samples (diluted serum and tissue extracts) or standards were dispensed in specific wells using new pipette tips for each sample. This was followed by addition of 50 µL of enzyme conjugate (peroxidase conjugated ciprofloxacin) as described by kit manufacturer (r-biopharm®) and a modified form of method by Nizamlioglu & Aydin (2012) who used similar kits from (r-biopharm®).

After completion of the procedures, the wells were read photometrically at 450 nm, using an ELISA microtitre reader, (Elx800ms from Biotek, USA). Optical densities generated on the computer screen were recorded. The optical densities for the ciprofloxacin standards were plotted against their corresponding concentrations and the inverse proportion relationship reflected in the nature of the curve. By extrapolation from the standard curve, optical densities of samples (serum, liver, muscle and kidney) were used in generating the values of specific concentrations which were multiplied by corresponding dilution factors (600 for serum samples, 3000 for liver, kidney and muscle) to obtain the respective concentrations of ciprofloxacin in serum and tissues at the various times post- drug administration.

Determination of elimination half-life, rate constant of elimination(β) and area under the curve (AUC).

A cartesian plot of serum ciprofloxacin concentration versus time, after withdrawal of the drug, was done for the two groups. Graphs of both groups 1 and 2 tilapias in this study after drug withdrawal, fitted a two compartment open model from which the

elimination half-life ($t_{1/2}$) of ciprofloxacin was derived, for the two bath concentrations (Figures 5 and 6). The formula for rate constant of elimination (β =0.693 /t½) as described by Brander *et al*. (1991) was used in determining 'β' for the two bath concentrations.

The (AUC) values of the drug (between 0.5 to 8 hours of exposure) for serum and tissue samples were determined by the trapezoidal method as described by Riviere (1999) and Prawez *et al.* (2007).

Statistical analysis

Student *t*-test to compare the means of the values of sera and tissue concentrations of both groups was done, using Graphpad Instat3 software for statistical analysis.

Results

From the result of the water analysis shown in table 1 there were no need for adjustment, as the values of the parameters analyzed were within the tolerance limit for fish culture as described by Swann (2006).

The results as shown in tables 2 and 3 indicate the levels of ciprofloxacin in sera and tissues of hybrid tilapias, subjected to bath treatment over a period of 8 hours at two different drug concentrations. Table 2 shows the average serum concentration of ciprofloxacin in the two fish samples at each time recorded. The maximum serum concentrations were 2251.2 ± 877 µg/L in group 1 at 8 h exposure time and 2226 ± 174µg/L in group 2, at 4 h exposure time. Also, at 30 minutes exposure, serum concentration in groups 1 and 2 were 1479 ± 21µg/L and 814.2 ± 385.8µg/L, respectively. The maximum concentration (Cmax) of ciprofloxacin in groups 1 and 2 liver samples (separately pooled pairs of liver) were 12,300 µg/kg and 13,071µg/kg at 4 h exposure time and at 1 h exposure time, respectively (table 3). The ciprofloxacin Cmax for kidneys in groups 1 and 2 were 12,210 µg/kg and 11,130 µg/kg at 8 h exposure and 4 h exposure time, respectively, while those of skeletal muscles of groups 1 and 2 were 14,785µg/kg and 11,010µg/kg at 4 h and 2 h exposure time, respectively. Tables 2 and 3 were subjected to statistical analysis to test for significant difference between means of groups 1 and 2 ciprofloxacin concentration in sera, liver, kidney and muscle using t – test. The result of the t-test in table 5 showed that there were no significant difference ($p>0.05$) between means of ciprofloxacin concentration in sera, livers and kidneys of the two groups but it was significant in skeletal muscles ($p<0.05$). Figures 1 to 4 compared between the groups 1 and 2 on sera, liver, kidney and muscle ciprofloxacin concentration versus time.

The area under curve ($AUC_{0.5\text{-}8\ h}$) values for sera and

Table 1: Water quality test of hybrid tilapias medicated with ciprofloxacin

Parameters	pH	TH (mg/L)	Ca2+ (mg/L)	Mg2+ (mg/L)	NO-2 (mg/L)	Fe2+ (mg/L)	TAN (mg/L)	UIA (mg/L)
Value	8	125	200	25	0.05	0.222	1	0.042

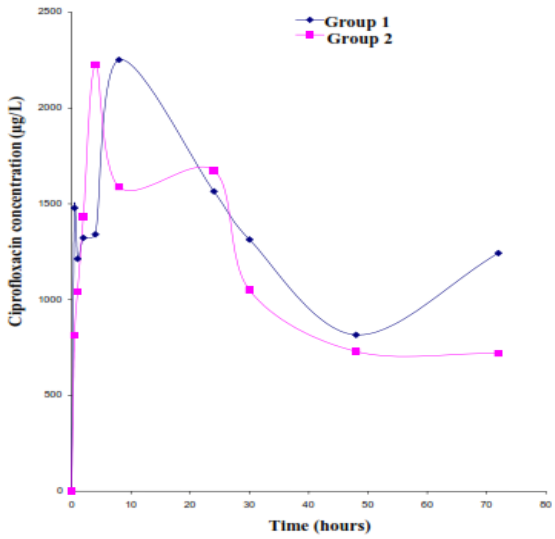

Figure 1: Time comparison with serum ciprofloxacin concentration (µg/L) of tilapias in 50mg/L and 25mg/L of ciprofloxacin

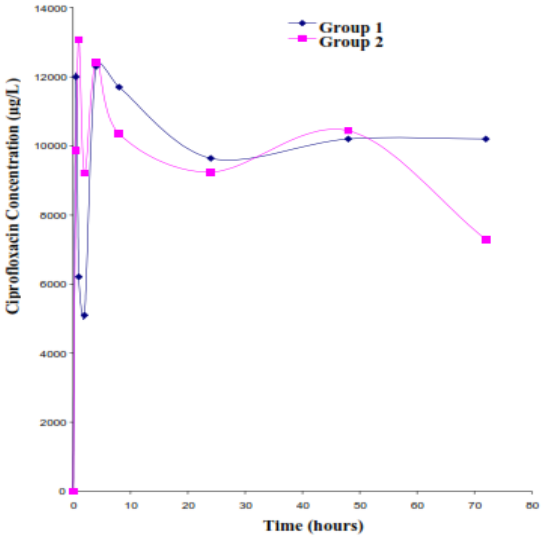

Figure 2: Time comparison with liver ciprofloxacin concentration (µg/L) of tilapias in 50mg/L and 25mg/L of ciprofloxacin

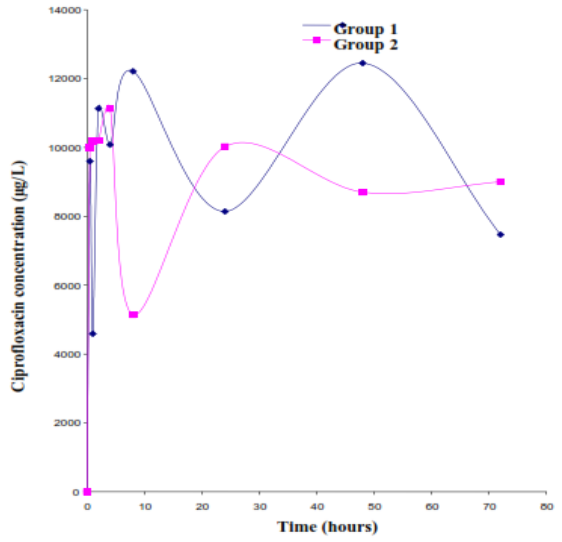

Figure 3: Time comparison with kidney ciprofloxacin concentration (µg/L) of tilapias in 50mg/L and 25mg/L of ciprofloxacin

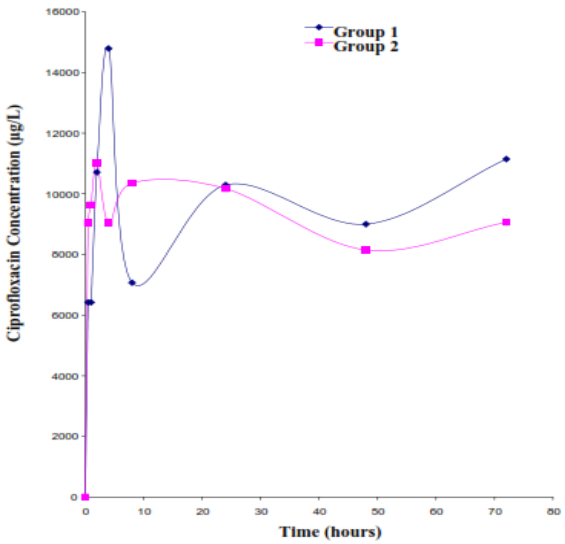

Figure 4: Time comparison with muscle ciprofloxacin concentration (µg/L) of tilapias in 50mg/L and 25mg/L of ciprofloxacin

Table 2: Ciprofloxacin concentration in hybrid tilapia serum during drug medication and after drug withdrawal

Time (hour)	Group 1 (50 µg/L)	Group 2 (25 µg/L)
0.5	1,479± 21	814±385.8
1	1,213±184	1,041± 98.7
2	1,323±123	1,434± 24
4	1,341±709	2,226±174
8	2,251±877	1,588±216
	Post drug withdrawal	
24	1,565± 451	1,674±240
48	816± 42	729±396
72	1,243± 85.2	720 ±282

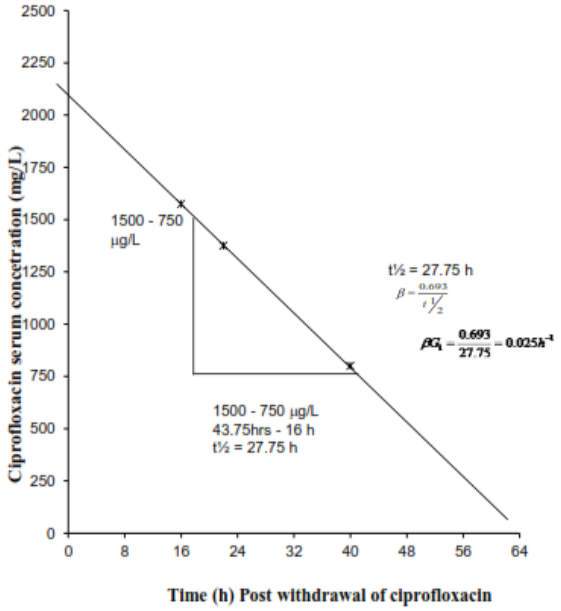

Figure 5: Elimination half-life of serum ciprofloxacin post withdrawal of tilapias in 50 mg/L of ciprofloxacin

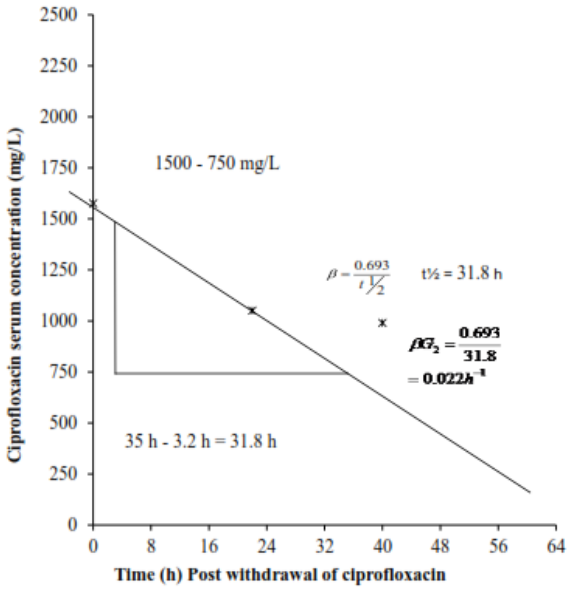

Figure 6: Elimination half-life of serum ciprofloxacin post withdrawal of tilapias in 25 mg/L of ciprofloxacin

Table 3: Ciprofloxacin concentration in hybrid tilapia tissues during drug medication and after drug withdrawal

	Group 1 (50 µg/L)			Group 2 (25 µg/L)		
Time (hour)	Liver (pooled sample)	Kidney (pooled sample)	Muscle (pooled sample)	Liver (pooled sample)	Kidney (pooled sample)	Muscle (pooled sample)
0.5	12,000	9,600	6,420	9,858	10,000	9,030
1	6,210	4,590	6,420	13,071	10,170	9,630
2	5,100	11,130	10,710	9,210	10,200	11,010
4	12,300	10,080	14,785	12,420	11,130	9,030
8	11,700	12,210	7,071	10,350	5,142	10,350
		Post Drug	Withdrawal			
24	9,642	8,142	10,290	9,240	10,020	10,170
48	10,200	12,450	9,000	10,440	8,700	8,142
72	10,200	7,470	11,142	7,290	9,000	9,060

Table 4: Distribution of ciprofloxacin in sera and tissues of hybrid tilapias using AUC and T/S ratio

	Group 1 (50 µg/L)		Group 2 (25 µg/L)	
Source	$AUC_{0.5\text{-}8\,h}$ Values µg/L	T/S (AUC) Values	$AUC_{0.5\text{-}8\,h}$ Values µg/L	- Values
Sera	12,159.3	-	13,194.0	-
Liver	78,607.5	6.46:1	86,506.75	6.56:1
Kidney	79,597.5	6.55:1	71,641.5	5.43:1
Muscle	81,297.0	6.69:1	76,042.5	5.76:1

Key
AUC - Area under curve

Table 5: Mean ± S.D distribution of ciprofloxacin in sera and tissues of hybrid tilapias

Sample	Group	N	Mean ± S.D	Diff in Mean	T-value With 14 Df	P-value (Two Tailed)	Comment
Sera	1	8	1403.80 ± 408.4	-125	0.523	0.236	Not sig
	2	8	1278.80 ± 542.4				P >0.05
Liver	1	8	9669.00 ± 2669.2	565.88	0.494	0.174	Not Sig
	2	8	10235.00 ± 1840.9				P >0.05
Kidney	1	8	9459.00 ± 2648.3	-248.25	0.219	0.167	Not Sig
	2	8	9210.80 ± 1806.0				P >0.05
Muscle	1	8	9479.80 ± 2874.1	73	0.068	0.0038	Significant
	2	8	9552.80 ± 919.9				P<0.05

tissues are shown in table 4. During the 8 h exposure in group 1 sera, liver, kidney and skeletal muscle $AUC_{(0.5\text{-}8\ h)}$ values were 12,159.3µg.h/L, 78,607.5 µg.h/kg, 79,597.5 µg.h/kg and 81,297 µg.h/kg respectively. In group 2, $AUC_{(0.5\text{-}8\ h)}$ values for sera, liver, kidney and skeletal muscle were 13,194 µg.h/L, 86,506.75 µg.h/kg, 71,641.5 µg.h/kg and 76,042.5 µg.h/kg respectively. The ratios of tissue to sera $AUC_{0.5\text{-}8\ h}$ for group 1 shown in table 4 were 6.46:1, 6.55:1 and 6.69:1 for liver, kidney and muscle, respectively. In group 2, the ratios were 6.56:1, 5.43:1 and 5.76:1 respectively. From a Cartesian plot (figures 5 and 6) of serum ciprofloxacin values, post withdrawal of drug (i.e. over a period of 64 hours) for groups 1 and 2 tilapia, elimination half-lives ($t_{1/2}$) determined were 27.75 h and 31.8 h, respectively with rate constant of elimination (β) for groups 1 and 2 being 0.025 h^{-1} and 0.022 h^{-1} respectively.

Discussion

The results showed clearly that administration of ciprofloxacin to hybrid tilapia (*Sarotherodon galilaeus* x *Tilapia zillii*) by bath method in slightly hard water resulted in significant and measurable level of the drug in serum and tissue samples of the two groups of fish. In this study, high ciprofloxacin concentrations were observed in tissues (liver, kidney and muscle) than in sera for both groups 1 and 2 (50 mg/L and 25 mg/L bath concentrations respectively). Higher level of ciprofloxacin concentration in the tissues than serum samples is an indication of significant level of drug distribution and this was further demonstrated by the high ratio of tissues to sera $AUC_{0\text{-}8h}$ values which ranged between 5 and 6:1.

At 30 minutes of exposure to the medicated water, the serum concentration of ciprofloxacin in group 1

Tilapia was 1479µg/L while in group 2, it was 814.2 µg/L. Higher serum level of the drug in group 1 tilapia exposed to higher ciprofloxacin bath concentration was observed at most of the sample collection time up to the 8th hour of exposure to the drug when compared with group 2. This may be associated with the higher bath ciprofloxacin concentration which the fishes were subjected to. Oladele *et al.* (2011) observed higher serum level of ciprofloxacin in catfishes, exposed to lower ciprofloxacin bath concentration (25mg/L) up to the 8th hour when compared with those that were exposed to 50mg/L concentration. This phenomenon (observed in African catfish) can occur as a result of some degree of tissue saturation (Treves-Brown, 2000).

After withdrawal of drug at 8 hours post-exposure, it was observed (particularly in group 1 tilapia) that liver and muscle ciprofloxacin concentrations increased. This unusual phenomenon (increasing tissue drug concentration post-withdrawal) was also observed in the use of flumequine (a 4-quinolone) in juvenile halibut after exposure to the drug had been terminated at 72 hours (Samuelson & Lunestad, 1996).

Although bath treatment is a popular method of administering antibiotics, more quantity of drug is required to achieve the desired result as compared to feed medication or injections. In some cases, large amount of antibiotic in the water is not a guarantee that enough of it will get into the fish to attain a chemotherapeutic level. This is because certain factors like variation in absorptive capacities of different fish species and chemical parameters of water like pH, salinity, Ca^{2+} and Mg^{2+} ions, can strongly influence the uptake and hence, bioavailability of drugs administered to fish by bath. Influence of pH on absorption of flumequine (a quinolone) in bath-medicated brown trout was shown by O'Grady *et al.* (1988) who reported a progressive decline in uptake of flumequine as pH increased from 6.4 to 9, such that no drug was absorbed from 50mg/L bath concentration at pH 8 or at 100mg/L at pH 9.2 . Blasiola *et al.* (1980) reported that channel catfish readily absorb oxytetracycline, while common carp (*Cyprinus carpio*) do not absorb therapeutic concentrations of it, nor ampicillin or nitrofurazone despite continous exposure to high concentrations for up to 24 hours. Bath administration of drugs is of importance, especially because sick fishes often manifest anorexia. However, it is important to take the hardness of bath water into consideration when medicating fish via this route.

Slightly hard and alkaline water (pH = 8 and total hardness as 100 mg/L) was used in this experiment in view of the fact that very hard water (rich in calcium and magnesium ions) and high pH (i.e., alkaline medium above 9) could result in reduced bioavailability of fluoroquinolones, hence a reduced serum and tissue area under curve (AUC) values which are reflections of extent of drug absorption and tissue distribution, respectively. Bath administration of fluoroquinolones via very hard and alkaline water can result in low tissue distribution and correspondingly have reduced systemic bactericidal effect. Effect of hardness (up to 412.5ppm) was demonstrated by Oladele *et al.* (2010), in a study of the uptake of ciprofloxacin by *Clarias gariepinus* in medicated water at different levels of calcium hardness.

Attaining a therapeutic tissue level is very important when administering antibiotics through any recommended route. This is because the curative effect of such drugs will only be of relevance when they are able to reach and saturate tissues harboring the target susceptible pathogen.

Kirkan *et al.* (2006) demonstrated the minimum inhibitory concentration (MIC_{50}) and maximum inhibitory concentration (MIC_{90}) values of ciprofloxacin and other antimicrobials for 39 pathogenic bacterial strains that affect fish. While using two methods, i.e., the agar dilution (AD) and e-test method, the minimum inhibition concentration (MIC) range of ciprofloxacin for *Enterococcus seriolicida* was 0.13-4 mg/L (=130 to 4000 µg/L) with 0% resistance for both methods, while for *Aeromonas salmonicida* and *Yersinia ruckeri* range of MIC by agar dilution method was 0.5- 1 mg/L and 0.5-8 mg/L respectively. These findings suggest that under bacteriaemic conditions in fish, the maximum ciprofloxacin values for serum in groups 1 and 2 tilapia (2,251.2 µg/L and 2,226 µg/L, respectively) would be sufficient to exert antimicrobial action against most of the bacterial pathogens.

Similarly, higher concentrations obtained in fish tissues in the present study, were also bactericidal. According to Kirkan *et al.* (2006), the MIC_{50} value of ciprofloxacin was approximately 1 mg/L (i.e. 1000µg/L) for all pathogenic bacterial isolates of fish tested. Considering their submission, this would also suggest that tilapias exposed to both bath concentrations (50 mg and 25 mg ciprofloxacin/L) had tissue samples (liver, kidney and muscle) maintaining above 1000 µg/kg ciprofloxacin

concentration (i.e. over 7,000 µg/kg) even for more than 30 h after withdrawal of the drug.

Comparatively in group 1, the muscles had the highest ciprofloxacin concentration followed by the liver and then the kidneys. It was observed that group 2 tilapias attained maximum ciprofloxacin concentration faster than group 1 tilapias in both serum and tissues. However, these high concentrations in the tissues even to the end of the experiment, indicated that ciprofloxacin depletion in tilapia follows a much slower trend compared with observations from earlier studies on the African catfish, *Clarias gariepinus.* In this present study, the group 1 with higher bath concentration, had a shorter serum elimination half-life of ciprofloxacin, hence a faster elimination rate, with a rate constant of elimination of $0.025h^{-1}$, in comparison with group 2 ($0.022h^{-1}$) which were subjected to a lower bath concentration of ciprofloxacin. This elimination rate in group 1 is expected when tissues are saturated with a drug. Issues of drug uptake and depletion in fish could be influenced by many factors such as unique physiology of the fish species, pH and solubility of the drug, water temperature and route of administration. In this experiment, ciprofloxacin accumulated in tilapia skeletal muscles, unlike previous observations made in African catfish (Oladele *et al.,* 2011). It may be necessary to further investigate if the difference in muscle density, compactness, vascularization and composition (fat to water ratio) between the two species of fish, can be factors associated with prolonged high ciprofloxacin concentration in tilapia muscle. It is also important to see the merits and demerits of the ciprofloxacin retention in skeletal muscle of tilapia. From the therapeutic and economic point of view and comparing the AUC values of groups 1 and 2, it suggests that the lower bath concentration of group 2 (25 mg of ciprofloxacin/L of water) could exert similar effect as that of group 1 (50 mg of ciprofloxacin/L of water) hence a lower cost of medication. It may be necessary to consider this in situation where polyculture is practiced. Since the skeletal muscle remains the major edible portion of fish, high retention of ciprofloxacin in it (post-treatment) is of public health importance and suggests a need to adhere strictly to withdrawal periods which are often temperature-dependent. It is suggested that further studies of this drug in tilapia at lower bath concentrations, longer duration of exposure and much longer post-exposure monitoring of skeletal muscle residue should be determined via the muscle ciprofloxacin half-life and estimate the right withdrawal time needed to achieve tissues concentrations below the internationally recognized MRL (maximum residue level) in fish muscle.

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