Bioavailability of Orbifloxacin in African sharptooth catfish, *Clarias gariepinus*, and its efficacy in control of induced Edwardsiellosis

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Abstract: This study was conducted to investigate the Pharmacokinetics of Orbifloxacin in African sharptooth catfish, *Clarias gariepinus*, and its efficacy in control of induced Edwardsiellosis caused by *Edwardsiella tarda* (*E. tarda*), and to estimate its tissue distribution. Safety test, *in vitro* determination of the minimum inhibitory concentration (MIC) of orbifloxacin against *E. tarda* isolate; in addition to; the *in vivo* efficacy of orbifloxacin in treating Edwardsiellosis at 2 stages; the early stage 7 days and late stage 15 days post infection. The results showed that orbifloxacin is safe for Catfish at concentrations up to 50 mg/L in water. The minimum inhibitory concentration (MIC) of orbifloxacin against *E. tarda* isolate was 0.016 mg/L with MIC_{50} and MIC_{90} equal to 0.5 and 1.0 mg/L respectively. Almost 100% of the infected fish recovered after treatment with Orbifloxacin for 72 hours in early stage of the disease with complete disappearance of clinical signs. No *Edwardsiella* could be isolated from second group 96 hours post treatment; although the treated fish showed unhealed skin lesions, results of liver dysfunction and tissue alterations were recorded. Orbifloxacin residues in Catfish muscles decreased gradually after cessation of treatment and disappear by day 10 post-treatment in the first group. In conclusion orbifloxacin can be awaited as effective antibacterial agent for control of edwardsiellosis caused by *E. tarda*. The treatment is much more successful when initiated at the earliest time of infection. [Journal of American Science 2010;6(6):236-244]. (ISSN: 1545-1003).

Keywords: Bioavailability, Edwardsiella tarda, African sharptooth catfish, Liver function tests, histopathology.

1. Introduction

Bacterial diseases are significant setback for successful aquaculture (Austin and Austin2007). *Edwardsiella* is among the most important pathogens in aquatic environment (Mohanty and Sahoo 2007). Human intestinal and extra intestinal infections with *Edwardsiella tarda* are well documented (Walton et al., 1993, Coutlee et al., 1992, Castro et. al., 2006 and Mohanty and Sahoo 2007). *Edwardsiella tarda* has been infrequently isolated from variety of warm blooded animals including birds (Thune et al., 1993). Edwardsiellosis is an important disease in various fish species; clinical manifestation of *Edwardsiella tarda* infections was first and well described by Meyer and Bullock (1973) in channel catfish, in tilapia (Kubota et al., 1981), in turbot, *Scophthalmus maximus (L.)* (Padros et al., 2006), Korean catfish (Yu et al., 2009 & 2010), Mullets (*Mugil cephalus*) (Kusuda et al., 1976). Among the most important strategies for control of aquaculture infectious diseases is to alleviate the predisposing causes of the disease (Noga, 2000), and to improve health management practices. For successful treatment of fish exhibiting signs of edwardsiellosis; immediate diagnosis and treatment are recommended while the majority of the fish are still feeding (Samuelsen et. al., 1998 and McGinnis et al., 2003; in this respect the use of antibacterial agents of low inhibitory concentrations and effective systemic distribution is the best choice (Samuelsen 2006). Currently, the antibiotics approved for use with fish food according to the Food and Drug Administration (FDA) in USA were oxytetracycline and sulphadimethoxine and ormetoprim combination. However, there are reports of bacterial resistance to these antibiotics (Plumb et al., 1995 and Smith et al., 1994 & Xiao et al., 2009). In addition, palatability problems have been reported with sulphadimethoxine and ormetoprim combinations (Poe and Wilson 1989). Therefore, search for an alternative, effective and save remedy for edwardsiellosis is essential. Quinolones are important group of antibacterial agents used to treat bacterial diseases in fish (Samuelsen, 2006). Fluoroquinolones are antimicrobial drugs that generally have very good activities against a broad spectrum of bacteria and are used for the treatment and prevention of diseases in fish (Hannan et al., 1997; Samanidou and Evaggelopoulou 2007 & Zhu et al., 2009). Some

Fluoroquinolones were licensed in Europe and United states for use in fish and companion animals to control gram-negative and gram-positive bacteria, in addition to Mycoplasma species (Schrieder et al., 1996, Schrieder et al., 2004 and Albarellos et al., 2005). Enterofloxacin, the first veterinary fluoroquinolone in the market, soon was joined by orbifloxacin, Marbofloxacin and others. Marbofloxacin, a fluoroquinolone, showed effectiveness in aquaculture of carp (Fungke et al., 2006 and Zhu et al., 2009).The main target site for their bactericidal action is the DNA-gyrase, an enzyme required for uncoiling of DNA to allow spatial arrangement of DNA in the bacterial cell. (Samanidou and Evaggelopoulou 2007). Orbifloxacin is a new synthetic fluoroquinolone antimicrobial drug that has been developed especially for use in veterinary medicine (Nakamura, 1995). Sensitivity of *Edwardsiella* to antimicrobial agents was reported by Muyerube et al., 1973, Reinhardt et al., 1985, Waltman et al., 1986, Clark et al., 1991. Although there is a conclusion that strains of *E. tarda* have uniform susceptibility to about 22 antibiotics, there are differences linked to the source of strains. The presence of various R- plasmids that mediate antibiotic resistance were reported in *Edwardsiella* by Aoki et al., 1977, Lobb and Rhoades 1987, Libb et al., 1993. Because little data is available about registration of orbifloxacin as a drug to control Edwardsiellosis in catfish, the current study aimed to investigate the Pharmacokinetics of Orbifloxacin in African sharptooth catfish, *Clarias gariepinus*, and its efficacy in control of induced Edwardsiellosis at two levels; the early stage of infection (7 days post infection) and the late stage of infection (15 days post infection).

2. Material and Methods

2.1. Orbifloxacin

A fluoroquinolone antibiotic, 1-cyclopropyle-7-[(C3s,5r)-3,5-dimethylpiperazin-1-y1]–5,6,8-trifluro-4-oxo-1,4-dihydroquinoline-3 carboxylic acid (IUPAC), $C_{19}H_{20}F_3N_3O_3$, of molecular mass 395.37g/mol.,was obtained as water soluble white powder in pure form; supplied by Dainippon Pharmaceutical Co, Ltd., Osaka, Japan.

2.2. Fish

A total of 720 sharptooth Catfish (*Clarias gariepinus*) weighting 50±2g were obtained alive from semi intensive fish farm. The fish were apparently healthy; transferred and maintained for acclimatization in glass aquaria (30× 40×80 cm^3); according to the recommended biomass for each aquarium; supplied with dechlorinated tap water, according to Best et al. (2002). Samples from the catfish were used for isolation of *E. tarda* infection and found negative for isolation. Both sexes of fish were used and no attempt was made to determine gender or sexual maturity.

2.3. Edwardsiella tarda strain

Edwardsiella tarda strain was locally isolated and identified from clinically diseased African sharptooth Catfish (*Clarias gariepinus*) using MacConky's agar, Edwardsiella isolation agar (Shotts, and Waltman 1990) and the biochemical reactions according to Quinn et al. (2002). The selected isolate was tested for its pathogenicity and was found to be pathogenic after intra-peritoneal injection (IP) in a concentration of (2.4×10^4 CFU /ml).

2.4. Assay procedures

2.4.1. Safety testing

To investigate the safety of orbifloxacin to catfish, a total of 210 apparently healthy catfish were used; two groups each of 40 in 3 replicates were treated by application of one of two doses of (0.0 mg^{-1} and 50 mg^{-1} /day) of orbifloxacin in water according to Paget and Barnes (1964). The treated groups were kept under observation for 7 consecutive days. The safety of orbifloxacin was justified by recording mortalities; alteration on the behavioral patterns and clinical picture in the two groups following the Code of American Federal Regulation (1985).

2.4.2. In vitro determination of minimum inhibitory concentration of orbifloxacin versus E. tarda:

The minimum inhibitory concentrations (MICs) testing against the E. tarda was performed in accordance with the NCCLS approved performance standards guideline for the agar dilution susceptibility test, with adjustments necessary for testing E. tarda. A stock solution containing 1,280 mg/ ml of orbifloxacin was prepared. Orbifloxacin was then serially diluted in sterile distilled water at concentrations of 640, 320, 160, 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 mg/ml. Each dilution was then poured into Mueller–Hinton agar (MHA) with 5% sheep blood to final concentrations of 0, 0.002, 0.004, 0.008, 0.016, 0.03, 0.06, 0.125, 0.250, 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/ml. Two plates of each concentration were poured. Edwardsiella tarda colonies were suspended in sterile broth media to the density of McFarland 0.5 barium sulfate turbidity standard (approximately 1×10^8 to 2×10^8 colony-forming units/ml) with a sterile cotton swab. One to two microliters of the inoculated broth was placed on the surface of duplicate blood agar plates from the lowest concentration (0 mg/ml on a control plate) to the highest concentration (64 mg/ml). A second control agar plate (0 mg/ml) was inoculated last to ensure that there was no contamination or antimicrobial agent carryover from the inoculation. Plates were then incubated at 25 ± 2 C for 2 days and observed to determine which concentration completely inhibited the growth of E. tarda. The control bacteria used were E. coli ATCC 25922. Controls were incubated and read at 16 to 20 hours according to the NCCLS guidelines. Results were determined as (++) no visual inhibition of bacterial growth, (+)scant bacterial growth observed,

and (-) no growth observed Korsholm and Sogaard (1987).

2.4.3. In vivo orbifloxacin

A total of 600 apparently healthy catfish were grouped in to 5 groups each of 40 fish (I, II, III, IV and V); used for the treatment trials by orbifloxacin as follows; all treatment trials were repeated in triplicate.

2.4.3. 1. Group I

Forty catfish were individually inoculated intraperitoneally (I.P.) with 0.2 ml of Edwardsiella tarda (2.4×10^4 CFU /ml) (Austin and Austin, 2007). The onset of treatment with orbifloxacin 50 mg^{-1} water /day started 7 days post infection.

2.4.3. 1. Group II

A second 40 catfish were assigned for the second group, inoculated intraperitoneally (I.P.) with 0.2 ml of E. tarda (2.4×10^4 CFU /ml). The onset of treatment with orbifloxacin 50 mg^{-1} water /day started 15 days post infection.

2.4.3. 1. Group III

Fish of group III consists of 40 catfish were kept without infection and treated daily with orbifloxacin in water in the same dose and method as the previous groups.

2.4.3. 1. Group IV

Forty catfish were experimentally infected by E. tarda and not treated by orbifloxacin.

2.4.3. 1. Group V

Forty catfish were kept none infected and none treated.

2.5.1. Aquaria and management

The temperature in the experimental aquaria was 24 ±1 C when measured by digital thermometer; dissolved oxygen was maintained at a range from 6.0 to 8.0 mg/L during treatment. The pH of experimental aquarium water ranged from 7.70 to 8.32 during dosing. Alkalinity and hardness were measured titrimetrically; alkalinity ranged from 112 to 132 mg/L as CaCO3 and hardness ranged from 152 to 168 mg/L as CaCO3. Fish were kept under an approximately 12-hour light: 12-hour dark photoperiod regime throughout the study. A commercial diet of 35% protein was offered in a feeding rate of 2% body weight daily.

2.5.3. Anaethesia and sampling

Tricaine methane sulphonate (MS222) was used to anesthetize fish in a dose of 50 mg/ml buffered by 100 mg/ml sodium bicarbonate to adjust water alkalinity to exceed 50 mg/L as CaCO3 according to Davis et al,, (2008).

For blood sampling, blood were collected from the caudal vein; using a 2 ml syringe. Blood was stored in heparinized tubes and centrifuged at 4000 g for 20 min at 15 C. The supernatant (plasma) was frozen and stored at -80 C.

Tissue samples of muscles and kidney were taken daily from Two Catfish/ group for re-isolation of E. tarda.

For histopathological examination; by the end of the treatment trials; five catfish from each treated group was sacrificed; liver, spleen, skeletal muscles, cardiac muscle and gills were preserved using formalin buffered saline for tissue alteration studies.

2.5.4. Orbifloxacin -Tissue distribution

The residue of orbifloxacin in Catfish muscles was accomplished by a modified agar diffusion bioassay method reported previously by Bennett et al. (1966), Bo¨ttcher et al. (2001) and Albarellos et al. (2005) and Althaus et al., (2009) using Escherichia coli (ATCC 10536) as the reference organism. The medium was prepared by dissolving 9.5 g Mueller–Hinton agar in 250 mL distilled water in a 0.5 L flat-bottomed flask, which was autoclaved for 20 min. After cooling to 50 C in a water bath, 0.4 mL of the diluted suspension of reference organism was added to the media. After the medium was poured (25 mL) and solidified, six wells were cut at equal distances in the solidified bioassay plates. Triplicate tissue samples and a known standard concentration of the drug in tissue (0.01 to 50 mg/kg) were placed directly into the wells without any clean-up step. The standard (in tissue) was included in each assay plate in order to compensate for any plate-to-plate variations. The plates were kept at room temperature for 2 h before being incubated at 37 C for 18 h. Zones of inhibition were measured using micrometers, and the results from the standards were used to calculate the concentration in each sample. A linear relationship existed between the zone of inhibition and the logarithm of orbifloxacin concentrations 0.015 and 50mg/kg, with a correlation coefficient of 0.995. The concentration of Orbifloxacin in tissue samples was calculated by linear regression analysis of the corresponding zones of inhibition to the standard curve.

2.5.5. Liver function tests:

Plasmatic activity of AspAT and AlaAT were determined in the collected plasma at l ¼ 340 nm (37 C) with Bio Merieux kits –France according to the manufacturer's instructions. Results were expressed in U l/1, where 1 U is the amount of enzyme that converts 1 m mol substrate per min under the specific conditions of the procedure.

2.5.6. Histopathological study:

Tissue samples were taken from liver, spleen, skeletal muscles, cardiac muscle and gills of the 4 fish groups. The samples were prepared following the method of Bancroft, et-al., (1996).

2.6.1. Data recording

The tested Catfish under experimentation as well as control ones were clinically examined daily for morbidity, mortality according to Kimberley (2004), the behavioral patterns during the treatment periods were daily observed and recorded according to Tsubokawa et al. (2009). The plasmatic activities of AspAT and

AlaAT were recorded**;** in addition to the results of the tissue alterations.

2.6.2. Statistical analysis

Data were expressed as mean ± S.D. Statistical analysis was carried out using computerized SPSS program (version 8.0, Chicago, IL, USA) with one way ANOVA test for significance according to Snedecor and Cochran (1986).

2.6.3. The conflict of interest

The study was approved by the Bioethics Committee of the Faculty of Veterinary Medicine, Cairo University. No conflict of interest is known. All phases of this study were conducted in compliance with US Food and Drug Administration guidelines for Good Laboratory Practice Standards.

3. Results

3.1. Safety test

Orbifloxacin proved to be safe when applied in water for Catfish at a concentration of 50 mg^{-1} /day for 7 days in comparison to the control group (0.0 mg^{-1} concentration of orbifloxacin). The treated fish did not display any concentration-related changes regarding mortalities, clinical and behavioral abnormalities,

3.2. In vitro evaluation of Orbifloxacin versus E. tarda

The minimum inhibitory concentration (MIC) of orbifloxacin against E. tarda isolate was 0.016 µ/ml with MIC50 equal to 0.5 and MIC90 equal to 1.0 µ/ml.

3.3. The in vivo efficacy:

Treatment of the induced edwardsiellosis by orbifloxacin in Group I resulted in complete recovery, the results was proved by the negative isolation for the E. tarda from the liver kidney and muscles of the infected fish 72 hour post-treatment. The treated Catfish held in appropriate management and water quality expressed complete recovery from the clinical signs within one week post -treatment. In the Group II the onset of treatment with orbifloxacin was 15 days post induced infection, no bacterial growth could be detected 96 hours post –treatment; although the treated fish showed unhealed skin lesions, despite the fact that the treated fish were kept in appropriate management conditions.

3.3.1. Behavioral Observations during treatment trials

The experimentally induced infected fish Group III; expressed severe behavioral changes manifested by lethargy, podding, and accumulation on one side of the aquaria. Fish exhibited abnormal flight/fright response to the stimulus in comparison to Group V. In Group I; a marked improvement in the behaviours was recorded, in Group II; no clear changes in the adverse behavioral alterations were seen as a result of treatment.

3.3.2. Clinical picture, Morbidity and mortality rates

During the early stage of experimental infection with *E. tarda*, (Group I), revealed loss of appetite, skin depigmentation with some external petechial hemorrhage and small cutaneous ulcerative lesions. Internally, the infected fish organs showed general signs of septicemia with some edematous fluids in the abdominal cavity. During the time of induced infection by *E. tarda* and before the onset of treatment, a total of 20% mortality rates were recorded in the infected groups (Group IV) starting from day 10 tell the 15 days post infection. In the experimentally infected and treated groups; no dead fish were recorded in Group I and in Group II. Groups IV and V; no mortalities were recorded along the corse of the experiments.

3.3.3. The tissue distribution

In the first 2 days post treatment; the level of orbifloxacin in muscles reached up to 0.03 mg/g tissues. At the 7^{th} day post treatment traces of orbifloxacin (0.015 mg/g tissue) were detected. At the 10^{th} day post treatment, no tissue residues of orbifloxacin were found in the muscles sampled.

3.3.4. The liver function tests

In the present study; the recorded values for AspAT and AlaAT activities in group V were (143±0.2 and 17±0.2 U/L). Induced infection in catfish with *E. tarda* resulted in significant increase in plasma AspAT and AlaAT activity (230±0.11and 35±0.11 respectively); after 15 days post infection the values of plasma AspAT and AlaAT increased to reach 220±0.2 and 60±0.2 respectively. The values of the liver function tests decreased upon treatment with orbifloxacin nearly to the normal values 6-8 days from the start of treatment. In Group III; the values of AspAT and AlaAT were 353±0.1 and 30±0.5 as shown in table (1).

Table 1: Effect of orbifloxacin on plasma liver enzymes of African sharptooth Catfish.

3.3.5. Histopathological examination

The results of histopathological examination revealed that; in Group I, liver sections showed apparent normal histological picture, the spleen showed slight lymphocytic depletion (Fig 4). The cardiac muscle showed intra cellular vacuolation and myositis as well as some leucocytic infiltrations (Fig. 6). No pathological changes were observed in skeletal muscles sections. The examined gill sections revealed slight necrosis of gill lamellae associated with slight leucocytic infiltration (Fig. 8). In the second group; liver showed vacuolar degeneration of the hepatocytes and dilatation with congestion of the hepatic sinasoids (Fig. 1 and 2). The spleen cleared a marked hemorrhages associated with lymphocytic depletion (Fig. 3). The heart muscle showed focal necrosis of myocytes completely replaced by leucocytic infiltrations (Fig. 5), meanwhile the muscles showed focal myolysis replaced by fibrinous connective tissue proliferation (Fig. 7). The gills showed clear lamellar oedema, focal necrosis associated with massive leucocytic infiltration (Fig. 9). Group III, On histopathological examination, E. tarda infection causes

hypertrophy of the liver cells and enlargement of their nuclei. Bacteria-laden phagocytes are found in the sinusoids of the anterior kidney, liver and spleen. Liquefaction and gaseous necrosis is seen in body musculature leading to ulcer formation. The gills show hyperplastic changes, the spleen accumulates haemosiderin pigments along with the presence of hyperaemia and necrotic changes. Group V exhibited normal histological sections in all the organs.

4. Discussions

It is important that therapeutic regimes are designed to maximize efficacy, and minimize the risk of development of resistant pathogens. In this respect, the study of the pharmacokinetic properties of drugs, in combination with susceptibility test, is an important tool for the establishment of optimal dosage regimes and thus the promotion of their correct use Samuelsen, (2006). Bath treatment, was the method of choice in the present study as it is suitable to carry out with agents of high solubility in water in addition to its privilege in treatment of fish suffering from systemic and skin infections (Samuelsen and Lunestad, 1996; Samuelsen, 2003 and Samuelsen, 2006).; which is the case in edwardsiellosis. Orbifloxacin proved to be safe when applied in water for Catfish at a concentration of 50 mg/L for 15 days. Fish, (2001) reported improved pharmacokinetic properties and more acceptable safety profile of fluoroquinolones, as norfloxacin and ciprofloxacin in fish. Immediate diagnosis and treatment with antibiotics has previously been recommended (McGinnis et al., 2003). The minimum inhibitory concentration (MIC) of orbifloxacin against *E. tarda* isolate was 0.016 µ/ml with MIC50 and MIC90 equal to 0.5 and 1.0 µ/ml respectively. The MIC for orbifloxacin against *E. tarda* was comparable to other fluoroqinolones as no available literature concerning MIC of orbifloxacin in fish could be obtained; against Enterobacteriaceae (0.03-0.5), Pseudomonas aeruginosa (0.12-2.0), Aeromonas hydrophilia (0.03-1.0), Acinetobacter calcoaceticus (1.0-2.0), Brucella melitensis (0.5-2.0), staphylococci (0.06-1.0) and enterococci (1.0-2.0) (Qadri et al., 1993). On studying the *in vivo* efficacy; during the experimental infection with *E. tarda*, mortalities started at the 10th day post infection and reached 20% of the total infected fish, Catfish revealed loss of appetite, skin depigmentation with some external petechial hemorrhage and small cutaneous ulcerative lesions. Internally, the infected fish organs showed general signs of septicemia with some edematous fluids in the abdominal cavity. As the disease progress; the clinical signs were severe loss of skin colour with extensive external petechial hemorrhages. Pyogenic reaction and/or ulcerations reached the muscles of the flanks and caudal peduncle, the PM examination showed severe fibrinous peritonitis and organs appeared as one homogenous necrotic mass with white nodules in the liver. The clinical signs of infection reported in the current study were similar to those seen in *Clarias batrachus* (Sahoo et. al. 1998), the Japanese flounder Paralicthys Olivaceus (Miwa and Mana, 2000) and in Indian major carp, *Labeo rohita* (Mohanty et. al. 2007). Similar findings were previously reported by Muratori *et. al.* (2000), Padros *et. al.* (2006) and Mohanty *et. al.* (2007). E. tarda was re-isolated from the infected fish. Treatment of the induced edwardsiellosis by orbifloxacin; Group I resulted in complete recovery, the results was proved by negative isolation for the *E. tarda* from liver, kidney and muscles of the infected fish 72 hour post-treatment. The treated catfish expressed complete recovery from the clinical signs within one week post exposure to orbifloxacin when accompanied by good hygienic practices and good water quality. In Group II, no bacterial isolation could be detected 96 hours post – treatment; although the treated fish showed unhealed skin lesions. The mortalities stopped in the experimentally induced infected fish post treatment. The results are concedes with results of Elliott and Shotts., (1980) who stated that initiation of treatment in the earliest time possible is much more successful than in the late stages of the disease.

This study is among the first studies to measure the in vitro and in vivo activity of orbifloxacin against *E. tarda* in Catfish; its application in water. In mammals, orbifloxacin was an effective, safe, and convenient antibiotic for the treatment of superficial and deep staphylococcal pyoderma in dogs (Scott et al., 2006), and for the treatment of a variety of infections, including skin infections, urinary tract infections, respiratory infections and wound infections caused by susceptible bacteria (Nakamura, 1995). Regarding behavioral observations during treatment trials; the improved responses of catfish in the first post treatment with orbifloxacin could be attributed to the over all improvement of health condition as the fish was in the recovery stage. In the second group, the infected fish expressed severe behavioral changes; this may be explained by the decreased amount of orbifloxacin accidentally absorbed orally since fish could not eat as the disease progress (Samuelsen et. al. 1998). Immediate diagnosis and treatment with antibiotics while the majority of the fish are still feeding has been previously recommended (McGinnis et al., 2003).

The tissue distribution in muscles was measured; knowing the fact that muscles are the end of the systemic distribution and it is the edible part of the fish; so it is important to evaluate the drug in muscles and its withdrawal time for public health safety. In the first 2 days post treatment; the level of orbifloxacin in muscles reached to 0.03 mg/g tissues. At the 7th day post treatment traces of orbifloxacin (0.015 mg/g tissue)

were detected. At the 10th day post treatment, no tissue residues of orbifloxacin were found in the muscles sampled. The limits of *in vitro* detection of orbifloxacin were 10 to 30µg/kg in the tissue. Blood chemistry is the mirror for the internal reaction of the body to certain stimulus and it largely explains the external clinical signs and the internal tissue alterations related to the intended stimuli. Plasma chemistry studies of specific diseases in fishes are scanty. This makes interpretation of fish blood chemistries from clinical patients very difficult, Stoskope, (1993). Two plasma enzymes, AspAT and AlaAT, are frequently used to determine the toxic effects of varied pollutants (Oruc and Uner, 1998; Malbrouck et al., 2003). An increase of their activity in plasma indicates the development of tissue lesions and is observed primarily when hepatic lesions occur.

In the present study; the recorded values for AspAT and AlaAT activities in control fish were (17±0.2 and 143±0.2 U/L) comparable to those previously reported (17.5 and 95 U/L for AspAT and AlaAT respectively in channel catfish; Stoskope, 1993), AspAT and AlaAT are present in low concentrations in plasma, probably as a result of normal cell degradation (Schmidt and Schmidt, 1974).

Our data shows that injection of *E. tarda* in a dose of 0.2 ml of 2.4×10^4 CFU/ml induces significant increase in plasma AspAT and AlaAT activity in Catfish . Yu et al., (2010) reported also a significant increase in AspAT and AlaAT in plasma of the Korean catfish, Silurus asotus, after infection with E. tarda. This can be attributed to the induced hepatic damage as cleared by significance elevation of plasma liver enzymes and histopathological picture.Treatment with orbifloxacin had returned plasma liver enzymes nearly to the normal values 6-8 days from the start of treatment. This effect may be due to eradication of infection. In catfish received orbifloxacin alone a marked increase in liver enzymes was recorded, this result may suggest that orbifloxacin may have a hepatotoxic effect in catfish when used in prevention. In this respect in mammals, other fluroquinolones caused no additional hepatotoxicity when they were used by patients with hepatitis (Ho et al., 2009) and did not present a risk to patients receiving DX-619 (a new fluroqinolone) in clinical trials (Sarapa et al., 2007). The different predilection of body organs and tissues could be an important aid for following up treatment and prognosis of diseases. From this point of view, experimentally inoculated and treated Catfish was subjected to histopathological examination 72 hour post treatment. It is clear that catfish in the first group expressed very mild and non significant histopathological changes in comparison to those seen in the second group. In this concern, some workers have recently revealed the relationship between the kinetic change of apoptosis in lymphoid organs and the inflammatory process. The extensive apoptosis in the thymus and spleen suggests that *Edwardsiella* septicemia generates systemic immunosuppression via lymphocyte apoptosis (Pirarat *et al*, 2007). Moreover, Wilson and Macmillan, (1989) reported that fluoroquinolones therapy in channel Catfish may induce a minimal to mild dose-dependent decrease in hematopoietic/ lymphopoietic tissue of spleen.

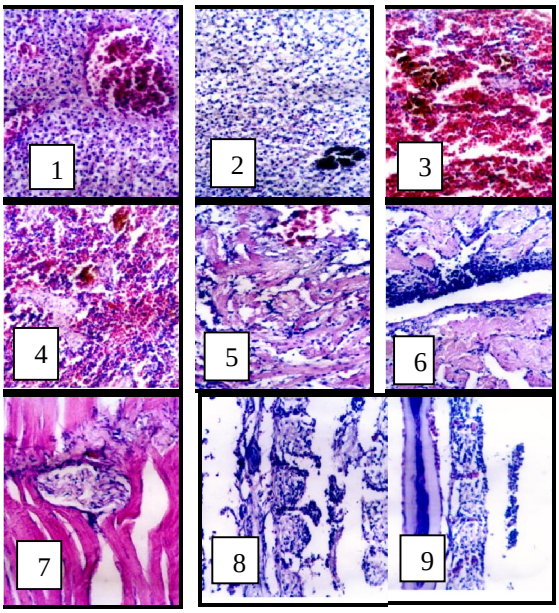

FIGURE 1. Liver from group 2 showing congestion of central veins and vacuolar degeneration of hepatocytes (H&E ×200).

FIGURE 2. Liver from group 2 showing vacuolar degeneration of hepatocytes (H&E ×200).

FIGURE 3. Spleen from group 1 showing marked hemorrhages and slight lymphocytic depletion (H&E ×200).

FIGURE 4. Spleen from group 2 showed marked heamorrhages associated with lymphocytic depletion (H&E×200).

FIGURE 5. Heart muscle from group 1 showed vacculation of some cardiac myositis as well as some leucocytic infiltrations (H&E ×200).

FIGURE 6. Heart muscle from group 2 showed focal necrosis of myocytes completely replaced by leucocytic infiltrations (H&E ×200).

FIGURE 7. Muscles of fish from group 2 showed focal myolysis replaced by fibronous connective tissue proliferation (H&E ×200).

FIGURE 8. Gills from group 2 showed necrosis of gill lamellae associated with leucocytic cell infiltration (H&E ×200).

FIGURE 9. Gills from group 2 showed clear lamellar oedema, focal necrosis associated with massive leucocytic infiltration (H&E ×200).

Table 1: Effect of orbifloxacin (50mg/ml) treatment on some plasmatic activities (AspAT and AlaAT) of catfish.

Treatment	Time post treatment	AlaAT	AspAT
	Infected	35±0.11	230±0.11
First stage	2days	30±0.2	126±0.1
(7 days)	4 days	31±0.11	230±0.3
	6 days	30±0.2	335±0.3
	8 days	28±0.1	231±0.1
	Infected	60±0.2	220±0.2
Second stage	2days	67±0.11	294±0.4
(15 days)	4days	126±0.1	1009±0.2
	6days	33±0.3	151±0.1
	8days	37±0.2	71±0.3
Control (Non infected treated)	-	30±0.5	353±0.1
Control (Non infected non treated)	-	17±0.2	143±0.2

*Significant at <0.05

5. Conclusion Orbifloxacin is potentially an effective antibacterial agent for the treatment of edwardsiellosis caused by *E. tarda* by water born method. In addition, control of orbifloxacin is much more successful when initiated at the earliest time of infection.

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