



Pharmacokinetic properties of single doxycycline dose orally administered in the African catfish (*Clarias gariepinus*)

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ABSTRACT

African catfish (*Clarias gariepinus*) aquaculture has experienced widespread production and has lately gained considerable interest in Egypt. Doxycycline (DOX) is used to control certain common fish's bacterial diseases, such as Septicemia, Fin rot, Columnaris, and Tail Rot. Therefore, our experiment was conducted to assess the pharmacokinetic properties of single doxycycline dose (20 mg/kg BW) orally administered in the African catfish. DOX plasma levels were measured using HPLC with a limit of detection nearly 0.035 µg/ml, and then were undergoing compartmental analysis; a one-compartment model was detected. The doubled-peak phenomenon was identified after oral administration and the 1st peak concentration (C_{max1}) and the 2nd peak concentration (C_{max2}) in plasma were 2.29 ± 0.46 and 1.68 ± 0.33 µg/mL at 1st and 8th h respectively, the absorption half-life ($t_{1/2ka}$) was 0.045 h, the elimination half-life ($t_{1/2ke}$) was 5.81 h, systemic total body clearance (Cl) was 0.72 mL/h/kg, volume of distribution of the central compartment (V_d/F) was 5.74 ± 1.11 L. These findings suggested that DOX was to some extent rapidly absorbed, widely distributed, and slowly excreted; moreover, it could be subjected to enterohepatic recycling.

INTRODUCTION

Nowadays, fish supplies more than one billion poor people with the higher portion of their daily animal protein. Fish provides nutrients and micronutrients that are vital for cognitive and physical development, particularly in children, and are an important ingredient of a healthy diet. African catfish is a substantial species that are cultured worldwide. Nigeria is the topmost producing country after that come Holland, Brazil, Hungary, the Republic of Kenya, Syria, South Africa, Cameroon, and the Republic of Mali. It is characterized by rapid growth, elevated market demand and the acceptability by consumers and farmers (Dauda *et al.* 2018). African sharptooth catfish aquaculture has experienced an expanded production also it has lately earned considerable interest in Egypt, diverting it from only a bothersome fish in tilapia fish ponds or a 'police-fish' to control unwanted reproduction in mixed-sex tilapia farming to an important fish species for aquaculture (El-Hawarry *et al.* 2016). Numerous illnesses and issues have emerged as a consequence of the aquaculture intensification. The treatment of infectious diseases has been changed after the discovery of antibacterials, resulting in a substantial reduction in morbidity as well as

mortality and leading to marked progress in the general population health (Sekkin and Kum 2011).

Tetracyclines have been widely used in fish aquaculture due to a broad spectrum of activity and low price. Several fish bacteria have developed resistance to tetracycline's 1st generation (Kim *et al.* 2011; Meizhen *et al.* 2011). Doxycycline (DOX), (α - 6- deoxy- 5- hydroxytetracycline), is a long-acting/2nd generation tetracyclines and it is one of the most widely utilized antibiotics throughout the globe for treating a broad spectrum of infectious diseases, as vulnerable intracellular/zoonotic agents. It has a long half-life, which makes convenient twice-a-day dosing possible. It is well absorbed orally even in the presence of food and has excellent tissue penetration (Klein and Cunha 1995). It has good pharmacokinetics properties, including high assimilation rate and wide distribution to different body tissues after oral treatment (Aronson 1980; Riond and Riviere 1988). DOX kinetic properties have been studied in channel catfish and Nile tilapia (Ai *et al.* 2011; Ding *et al.* 2009; Yang *et al.* 2014).

An antibiotic's effectiveness is determined based on evaluating the blood level of antibiotic over time and associating those pharmacokinetic/pharmacodynamic (PK-PD) parameters with the clearance of infection. Despite the recent interest in African catfish and the importance of doxycycline for control of some common bacterial fish diseases, including Septicemia, Columnaris, Fin Rot, and Tail Rot, the kinetics of doxycycline have not yet been studied in African catfish. Therefore, the present work was conducted to assess the pharmacokinetic profile of DOX in African catfish (*Clarias gariiepinus*) after a single dosage administration in the feed using high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Chemicals

Doxycycline hyclate (lot # D9891, purity > 98%) was obtained from Sigma Aldrich Chemical Co. (USA). Methanol and acetonitrile were of HPLC grade and were purchased from BDH Laboratories Supplies (UK). Deionized, Milli-Q water was obtained from Milli-Q Plus system (USA) and was used to prepare the mobile phase and diluent solutions. All the other chemical substances used in the present experiment were of technical grade.

Fish and experimental protocol

A total number of 50 apparently healthy African catfish (100 \pm 5 g) were purchased from fish hatchery, Central Lab for Aquaculture Research, Abbassa, Egypt. They were reared at the wet lab in fiberglass for 2 weeks to be acclimated with the experimental location. Acclimated fish were randomly allocated at a rate of 5 fishes/100 L aquarium. The different quality criteria of the water were checked daily. The pH was about 7.5, and the ammonia and dissolved O₂ levels were about 0.1 mg/L and >7 ppm, respectively. The fish were fed daily on a drug-free pelleted diet. The experimental protocol was carried out following Ethics of Animal Use in Research Committee (EAURC), Zagazig University.

The fish were fasted for 24 h pre and post-DOX administration to exclude the impact of nutrient on the absorption of DOX. Fish received one dose of doxycycline hyclate (20 mg kg⁻¹ BW) (Ai *et al.* 2011) in the feed and fish witnessed for 5 min for probable regurgitation. If the feed was vomited, the fish was removed from the study and replaced. Samples of blood were taken under anesthesia from the tail sinus at 0.25, 0.5, 1, 2, 4, 8, 10, 12, 24, 48, 60, 72, 96, 120, 144, 168, 192, and 216 h post-

drug administration. The serum samples were prepared by centrifugation at 3000 rpm for 15 minutes, then transferred immediately to sterile tubes and preserved at -80°C until further analysis using HPLC.

Method validation

The validation was performed by laboratory studies to ensure that the performance characteristics of the method met the requirements for the intended analytical application. This technique is validated according to the International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH). Precision is the closeness of agreement among a set of results. It was conducted using six replicates of DOX standard solutions. The percent of relative standard deviation (% RSD) for peak responses was calculated. Linearity was performed by preparing a minimum of six different concentrations of drug standard at a squared correlation coefficient of 0.99 (r^2) according to ICH. The serum samples were spiked by adding known quantities of DOX. Those samples were analyzed against standard solutions of the same concentrations. The accuracy was then calculated from the test results as a percentage recovery (Senyuva *et al.* 2000). Limit of detection (LOD) is the concentration which gives a signal to noise ratio 3:1 according to ICH. Limit of quantification (LOQ) is the concentration which gives a signal to noise ratio 10:1 according to ICH.

Doxycycline determination

Serum DOX levels were examined by HPLC with an ultraviolet detector. In short, following thawing at room temperature, 200 μl of serum previously spiked with doxycycline, 2 μl (10.0 $\mu\text{g}/\text{ml}$) of a stock standard solution of doxycycline for preparation of 0.1 $\mu\text{g}/\text{ml}$ spiked serum sample, then 200 μl of acetonitrile were put. The blend was mixed for 30 seconds and then centrifuged for 10 min at 12,857 Xg and 4°C . Then by using nitrogen evaporator at 40°C , a 200 μl of supernatants was evaporated. Dry precipitates were restructured by 200 μl of mobile phase and 25 μl aliquot samples were injected to HPLC. Isocratic mobile phase formed from 25% acetonitrile, 55% acetic acid (5%) and 20% methanol. A reversed-phase column C18 (4.6 mm, i.d., 250 mm, 5 μm) represented the chromatographic column and was adjusted at 15°C . A flow rate of 1 ml/min Injection volume: 25 μL . Detection and quantitation: via UV detector at 347 nm, quantitation was assimilated by HPLC 2D Chemstation software interfaced to a personal computer (Ruz *et al.* 2004).

Pharmacokinetics analysis

After drug administration, the mean DOX concentrations at every time point were assessed, and then average concentrations and time data were undergoing to compartmental analysis using Kinetica program (5.0; Thermo Electron Corporation, USA) for obtaining the pharmacokinetic parameters of doxycycline. A linear trapezoidal method was used to calculate the areas under the concentration-time curve from 0h to ∞ ($\text{AUC}_{0-\infty}$) and from 0 h to 24 h ($\text{AUC}_{0-24\text{ h}}$). After oral administration, the peak concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly estimated using the obtained data.

RESULTS

The feasibility of the detection method: The DOX separation method was precise as the RSD of six replicates of the DOX standard solution was 2%. The DOX retention time (RT) was 5.212 min (Fig.1).

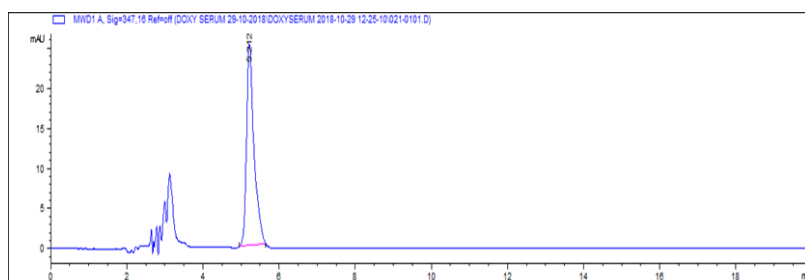


Fig. 1: Chromatograms of doxycycline standard (0.5 µg/ml) determined automatically using HPLC chromatogram system

The DOX mean recovery rate in plasma was 97.26%. LOD and LOQ of the doxycycline in serum were 0.035 µg/mL and 0.108 µg/mL, respectively. Doxycycline standard concentrations of 0.1, 0.2, 0.4, 0.8, 1 and 2 µg/ml and their respective peak responses are shown in Fig. (2). A linear relationship was shown through the calibration curve with a range of 0.1 to 2 µg/ml, with an elevated correlation coefficient indicating linearity ($r^2 = 0.99938$). The samples with drug concentrations above the upper limit of calibration were diluted with blank serum.

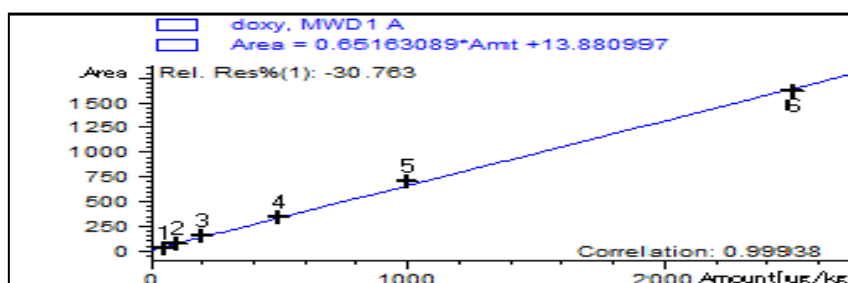


Fig. 2: Standard curve of doxycycline determined automatically using HPLC chromatogram system.

Pharmacokinetics

Plasma doxycycline concentrations against time curves following single doxycycline (20.0 mg /kg BW) dose through medicated feed are shown in Figs. (3 & 4).

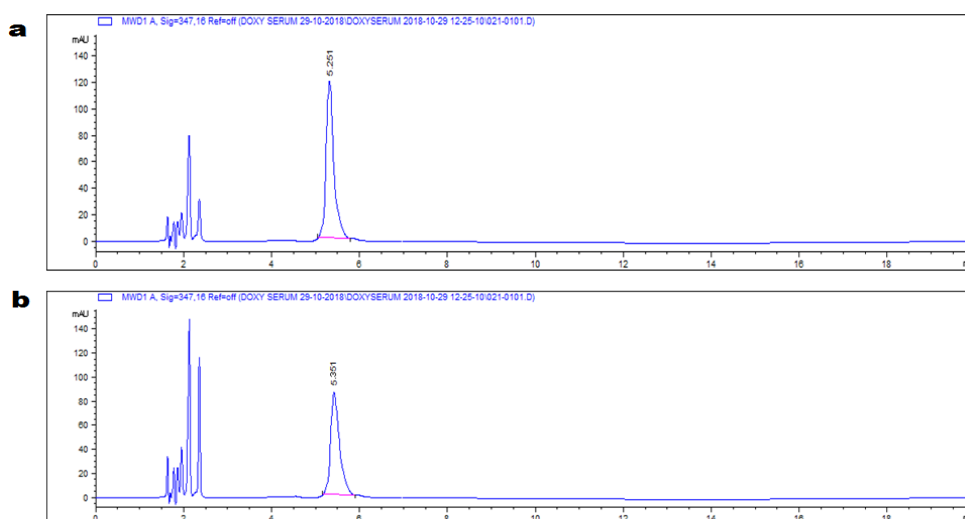


Fig. 3: Chromatograms of doxycycline extract of catfish serum at (a) 1st h (2.29 µg/ml) and (b) 8thh ((1.68 µg/ml) following oral dose (20 mg/kg BW) in feed determined automatically using HPLC chromatogram system.

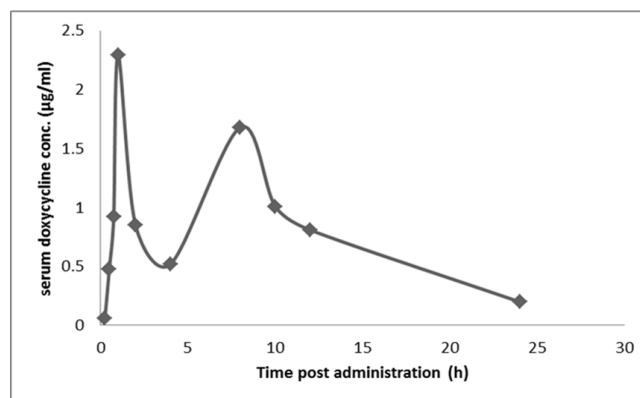


Fig. 4: Doxycycline levels (mean \pm SD) in serum of African catfish after a single 20 mg/kg oral dose.

After a single administration of doxycycline (20.0 mg /kg BW) via medicated feed, the plasma concentration peaked at 2.29 μ g/ ml at 1 h and then reduced rapidly from 1 h to 5 h post-treatment. A second absorption peak (1.68 μ g/ ml) was recorded at 8 h post-administration, after which the drug concentration decreased slowly and continuously. The absorption rate constant was 15.45, the elimination rate constant was 0.12, the half time of absorption was 0.045h (2.7 min), the half time of elimination was 5.81h and the total body clearance was 0.72 μ l/h (Table 1).

Table 1: The pharmacokinetic parameters of doxycycline (mean \pm SD):

Parameter		unit
K_a	15.45 \pm 1.95	1/h
K_e	0.12 \pm 0.08	1/h
$T_{1/2ka}$	0.045 \pm 0.02	h
$T_{1/2ke}$	5.81 \pm 1.84	h
T_{max1}	1	h
C_{max1}	2.29 \pm 0.46	μ g/ml
T_{max2}	8	h
C_{max2}	1.68 \pm 0.33	μ g/ml
Cl	0.72 \pm 0.15	μ L/h/kg
V_d/F	5.74 \pm 1.11	L
AUC	27.95 \pm 2.5	(μ g/ml) h
AUMCtot	522.5 \pm 12	μ g/mL*(h) ²
MRT ₁	17.95 \pm 2.51	h
T_{abs}	0.22 \pm 0.04	h
T_{lag}	0.68 \pm 0.16	h

K_a , Absorption rate constant; K_e , Elimination rate constant from the central compartment; $T_{1/2ka}$, Absorption half-life; $T_{1/2ke}$, Elimination half-life; C_{max} , Calculated maximum plasma concentration; T_{max} , Time of maximum plasma concentration; Cl, systemic total body clearance; V_d/F volume of distribution in the central compartment, AUC, Area under the concentration-time curve; AUMC, Area under the first moment of the concentration-time curve; MRT, Mean residence time.

DISCUSSION

Despite the recent interest in African catfish as characterized by rapid growth, high market demand and the acceptability by consumers and farmers, and the importance of doxycycline for control of some common bacterial fish diseases, including Septicemia, Columnaris, Fin Rot and Tail Rot the kinetics of doxycycline have not yet been studied in African catfish. Therefore, the present work was conducted to assess the doxycycline pharmacokinetic profile in African catfish after a single dose administration in the feed.

The peak concentration time, the time required for the drug to reach the peak plasma level after administration is displayed in hours and helps estimate the rate of absorption. Our results revealed that, after treatment with a single doxycycline (20 mg/kg) dose orally via medicated feed, doxycycline showed a relatively rapid absorption with the doubled-peak phenomenon and the first peak plasma concentration ($C_{\max 1}$) and second peak plasma concentration ($C_{\max 2}$) were 2.29 and 1.68 $\mu\text{g/mL}$ at 1st and 8th h respectively. Ding *et al.* (2012) and Yang *et al.* (2014) stated the same phenomenon in both Channel catfish and Nile tilapia. Our study revealed that $C_{\max 1}$ was greater than that in both channel catfish (0.58 ± 0.094) with earlier $T_{\max 1}$ (30 min) and in Nile tilapia ($1.99 \pm 0.43 \mu\text{g/mL}$) which indicates rapid absorption. Enterohepatic recycling might lead to the second peak for DOX in different species (Ding *et al.* 2012; Gibaldi 1967; Riond and Riviere 1990; Yang *et al.* 2012). Enterohepatic reprocessing resulted from biliary elimination plus intestinal resorption, sometimes with hepatic conjunction and intestinal deconjunction.

Sjodahl and Wetterfors (1974) and Barza *et al.* (1975) reported that the double peaks and the longer apparent disposal half-life in plasma were due to recycling. Past examinations demonstrated that doxycycline had a greater affinity with the bile (ten to fifteen folds) than the serum. Therefore, physiological and repeated intermittent elimination through bile happened (Fabre *et al.* 1968; Pedersen and Miller 1980).

Yang *et al.* (2012) suggested that lower oral bioavailability of DOX and the larger extent of its fecal excretion may result in its reabsorption from pond water which might result in the second peak.

The apparent volume of distribution of the central compartment (V_d/F) is an accurate indication of the diffusion of the drug in the body. In this work, V_d/F of DOX in African catfish was evaluated to be 5.74 L/kg which was higher than that recorded for oxytetracycline in Rainbow trout (0.151L/kg) (Yang *et al.* 2014). These results reflected that DOX was vastly distributed in African catfish. For oxytetracycline, the volume of distribution extended from 1.31 to 2.10 L/kg in various fish (Bjorklund and Bylund 1991; Grondel *et al.* 1987; Uno 1996). This distinction may be because of varieties in tested medications, fish species, fish estimate, water temperature, or test plan.

The drug's elimination half-life is represented as the time taken to reduce its concentration in the plasma or the total amount in the body by 50 %. Our findings revealed that doxycycline was excreted rapidly from African catfish with $T_{0.5ke}$ of 5.81 h. While longer $T_{0.5ke}$ of 38.63 h and 36.527 h were detected in channel catfish orally administered 20 mg doxycycline /kg and cultured in freshwater at 28 as well as 26 °C (Ai *et al.* 2011; Ding *et al.* 2012). Furthermore, $T_{0.5ke}$ of 77.2 h was reported in Nile tilapia orally administered 20 mg doxycycline /kg and cultured in freshwater at 24 °C (Yang *et al.* 2014). These results revealed that DOX was removed slowly at low temperature.

Clearance reflects the elimination of the drug from the body. In the current research a systemic total body clearance (Cl) of 0.72 $\mu\text{l/h/kg}$ was determined in African catfish which was faster than that of doxycycline in Nile tilapia (0.041 $\mu\text{l/h/kg}$) (Yang *et al.* 2014) and those of oxytetracycline in rainbow trout (0.006–0.016 $\mu\text{l/h/kg}$), chinook salmon (0.007 $\mu\text{l/h/kg}$) (Abedini *et al.* 1998), carp (0.01 $\mu\text{l/h/kg}$), channel catfish (0.022 $\mu\text{l/h/kg}$) and ayu (0.017 $\mu\text{l/h/kg}$) (Grondel *et al.* 1987; Plakas *et al.* 1988; Uno 1996). Our results specified that the DOX removal in African catfish was faster than other fish species.

CONCLUSION

To our knowledge, the pharmacokinetics profile of doxycycline was evaluated for the first time following a single oral dose in feed to African catfish. The pharmacokinetics profile of doxycycline demonstrated relatively rapid assimilation, wide distribution and rapid elimination in African catfish. Furthermore, doxycycline may be undergoing enterohepatic recycling in African catfish.

Conflicts of interest

The researchers state that they have no conflicts of interest.

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