Int.J.Curr.Microbiol.App.Sci (2020) *9*(12): 3197-3210

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 12 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.912.381

Comparative Pharmacokinetics of the Orally Administered Sub-lethal Doses of Miconazole Nitrate in *Labeo rohita*

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Keywords

miconazole nitrate, *Labeo rohita*, Pharmacokinetics, Elimination

Article Info

Accepted:
20 November 2020
Available Online:
10 December 2020

A B S T R A C T

The present study examined the pharmacokinetics of imidazole antifungal drug Miconazole nitrate (MCZ) in one of the most economically important aquaculture carp *Labeo rohita.* The pharmacokinetics study of MCZ was conducted after the single time administration of three sub-lethal doses (T1-6.30 mg $kgBW^{-1}$, T2- 12.61 mg $kgBW^{-1}$ and T3-25.22 mg $kgBW^{-1}$) in the feed. Plasma drug concentrations of MCZ were determined using high performance liquid chromatography (HPLC) with a limit of detection 0.032 µg ml^{-1}. The calibration curves were observed to be linear in the investigated areas of 0.5-25 µg ml^{-1} of MCZ and the recovery rates were 88.70 to 99.03 %. The peak plasma concentrations (C_{max}) in rohu varied significantly ($p<0.05$) in all the three doses at 6 to 8 h (T_{max}) and the order of magnitude for maximum concentrations were T3 (20.28 µg ml^{-1}) > T2 (9.44 µg ml^{-1}) > T1 (5.35 µg ml^{-1}). The calculated half-lives ($t_{1/2\beta}$) and the area under the plasma concentration-time curve (AUC) was observed to be varied from 30 h to 77 h and 263.11 µg*h ml^{-1} to 1048.99 µg*h ml^{-1} respectively. The mean residence time and the half value duration of the drug in plasma also varied significantly ($p<0.05$) in all the three orally administered doses. These findings support the potential of MCZ being absorbed and eliminated rapidly with a marked drug concentration in the blood of rohu, hence, can be recommended as a suitable candidate for the treatment of fungal infections in carps based on the observed pharmacokinetics data.

Introduction

Oomycetes are some of the most damaging pathogens responsible for diseases in freshwater fish; in particular, *Saprolegnia* is an aquatic oomycete negatively affecting the aquaculture industry (van West, 2006). Saprolegniasis is the disease caused by the etiologic agent *Saprolegnia* characterized by white or gray patches of mycelium growing on the epidermis of fish and enters the blood vascular system in the case of severe infections. This disease condition is predominantly a result of secondary infection incited by the compromised immune system of fish in illness or adverse environmental conditions (Sarowar *et al.*, 2013). The respiratory and osmoregulatory functions of

the fish are severely affected during the gill invasion of *Saprolegnia* inciting acute respiratory failure leading to death (Sarowar *et al.,* 2013).

In the earlier days, *Saprolegnia* infections were treated successfully with the chemical dye malachite green (Olah and Farkas, 1978; Srivastava and Srivastava, 1978; Alderman, 1985) until this was banned globally in 2002 for its known carcinogenic, mutagenic, and teratogenic properties (Hu *et al.,* 2019; Van West, 2006; Stammati *et al.,* 2005; Srivastava *et al.,* 2004). Besides this, other chemical compounds such as formaldehyde, hydrogen peroxide, sodium chloride (Rach *et al.,* 2005; Barnes *et al.,* 2003), copper sulphate (Straus *et al.,* 2009), bronopol (Pottinger and Day, 1999) and ozone (Forneris *et al.,* 2003) were also utilized to eradicate the fungal pathogen but none were as effective as malachite green and the environmental impacts of these chemicals are also apprehensive (Song *et al.,* 2020; Pottinger and Day 1999). The unregulated and extensive use of these chemicals in aquaculture farms gave rise to some serious concerns like the development of the resistant strains of fungus and their potential harm to human health (Phillips *et al.,* 2008; Stammati *et al.,* 2005). To address these situations and to help farmers encountering the deadly winter kill pathogen, the need arises to develop an alternate safe and cost-effective treatment for *Saprolegnia* for preventing significant economic loss in the aquaculture industry.

To counter and control these pathogens, miconazole nitrate (MCZ), a synthetic broad-spectrum imidazole antifungal agent (Godefroi *et al.,* 1969; Van Custem and Thiepont, 1972) that has been used for nearly 40 years to treat infections effectively and safely in human and veterinary animals (Vazquez and Sobel, 2012) was utilized in fish (Singh *et al.,* 2018a). It was observed and reported that the orally administered miconazole in guinea-pigs, was more potent and promptly effective against fungus than any other established antifungal like nystatin, amphotericin B and pimaricin (VanCutsem and Thienpont, 1972). MCZ was reported as a very effective and promising drug to be used as therapeutic (Singh *et al.,* 2018a) as well as a prophylactic agent (Singh *et al.,* 2018b) against *Saprolegnia* in rohu fingerlings. The discoveries correlating novel mode of action through multifarious clinical trials confirm the pharmacological efficacy of the drug against diverse fungal strains (Barasch and Griffin, 2008) with a wide safety margin in various animal species (Robert A. and Fromtling, 1988; Van Custem and Thiepont, 1972; Moriello, *et al.,* 2017)

The preferences of administering the antifungal drug and dosing regimen are among the factors which have to be stated by a fisheries clinician, though at times the drug concentration at the site of infection is widely influenced by the pharmacokinetic variability and may contribute to the treatment failure. The drug exposure with the lack of proper pharmacokinetic data can sometimes even exceed the probable extent resulting in toxicity. The antifungal drugs display the discrete variation in the drug plasma concentrations due to their inconsistency in the absorption and elimination rate. An understanding of the pharmacokinetics of these drugs has been observed to be a crucial factor in optimizing drug dosage and administration. The most convenient mode of detecting drug exposures for the effective medication in fisheries is the monitoring of drug concentration in fish plasma. This study was undertaken to comprehend the pharmacokinetics of MCZ in *L. rohita* after single oral administration of three sub-lethal doses (Singh *et al.,* 2018a) for better insight into the drug action in the fish.

Materials and Methods

Chemicals

Miconazole nitrate (white powder form, purity > 98%) was obtained from Sigma Aldrich Chemical Co. (USA). HPLC-grade acetonitrile and water were purchased from Himedia Laboratories Supplies (UK). Sodium acetate was purchased from Sigma Aldrich Chemical Co. (USA). All other chemicals used were of HPLC grade.

The stock solution was prepared at a concentration of 1000 mg l^{-1} MCZ in acetonitrile and stored at -20°C until further need. Six reference samples containing 0.5, 1, 5, 10, 15, 20, 25 µg ml^{-1} of MCZ were prepared by diluting this stock solution with drug-free pooled fish plasma. These samples were used to assess accuracy and precision. For routine use of the assay, a single-point working standard plasma containing MCZ at 1.0 µg ml^{-1} in drug-free plasma was prepared and stored in 1 ml aliquots at -20°C.

Chromatographic conditions

The HPLC system employed for the study was a Merck-Hitachi Lachrom system (Tokyo, Japan) equipped with a quaternary pump L-7100, an integral degasser, an interface D-7000 and a UV detector module L-7400,. For data acquisition and processing, the chromatography software ChromQuest 5.0 was used. A manual injector system (Rheodyne, Cotati, CA, USA) equipped with a 20 µl sample loop and 100 µl syringe (Hamilton, Microliter 710, Bonaduz, Switzerland) was used. The HPLC column was a ZORBAX Eclipse XDB-C18 (4.6mm×150mm, 5µm particle size), Agilent Technologies. The chromatographic separation was performed following Hosotsubo (1988) using a mobile phase consisting of 80: 20 (v/v) mixture of acetonitrile and 0.05 M sodium acetate buffer (pH 7.4) in a flow rate of 1.5 ml/min which produces a column pressure of about 70 bars (1,000 p.s.i.). Solvents were filtered through 0.45µm pore Nylon filter membrane before use. The column was maintained at 25°C and an injection volume of 10µl was used. The detection was performed at 232 nm.

Calibration curves and recovery studies

The calibration curves for MCZ were obtained by reference samples with standard solutions of fish plasma to yield 0.5, 1, 5, 10, 15, 20, 25 µg ml^{-1} of MCZ. Triplicate samples were used. The recovery rates were determined by comparing the results of the analysis of the reference samples with those of standard solutions. The linearity of the standard curves for MCZ in fish plasma was tested using peak-height measurements. Precision is the measurement of the closeness of individual data sets or agreement among a set of results. It was conducted using six replicates of MCZ standard solutions. The percent of relative standard deviation (% RSD) for peak responses was calculated. Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of response and slope of the mean analytical curve and were expressed as a signal to noise ratio 3:1 and 10:1 for LOD and LOQ, respectively (ICH, 2005).

Experimental fish

Healthy *L. rohita* (24.2 ± 1.5 g) was collected from the farm of the College of Fisheries, CAU, Lembucherra. Acclimatization of collected fingerlings was done for about 20 days in circular fibre reinforced plastics (FRP) tanks (Plasto Craft, Mumbai) of 500 l capacity and was fed with a routine amount of pelleted feed twice daily. Proper aeration was provided and feeding was done adlibitum. A

completely randomized design (CRD) was followed throughout the experiment. The different quality criteria of the water were checked daily. The temperature of the experimental tanks was maintained at 27.5 °C-28.5 °C, pH was about 7.5, and the ammonia and dissolved O_2 levels were about 0.1 mg l^{-1} and >7 ppm respectively.

Dosing and Sampling

The fish were divided randomly into three treatment groups and a control group in triplicate and were acclimatized with the experimental control feed. A total of five hundred and forty (540) acclimatized rohu fish were distributed uniformly in 12 FRP tanks. The fish were famished for 24 h pre-medicated feed administration, for immediate feed consumption and to avoid leaching of the MCZ from the feeds. Four iso-nitrogenous (35.18-35.99% crude protein) and iso-caloric (357-365 kcal 100 g^{-1}) diets were prepared (Table 1) as per treatment doses control- 0.0 mg $kgBW^{-1}$, T1- 6.30 mg $kgBW^{-1}$, T2- 12.61 mg $kgBW^{-1}$ and T3- 25.22 mg $kgBW^{-1}$ (Singh *et al.*, 2018a).

Fish received one dose of MCZ feed as per the above-mentioned doses as 1% of their body weight and were witnessed for 5 minutes for probable regurgitation. The feeding percentage was selected for the avoidance of residual experimental feeds as well as to maintain the certainly required dose of application. The feed intake was observed to be instant and the entire experimental diet was consumed by the fish within 5 min of feeding. Samples of blood were taken under clove oil anaesthesia from the caudal vein of three-four fish each at 0.25, 0.5, 1, 4, 6, 8, 12, 24, 48, 96, 120 and 240 h post-drug administration. Blood was stored on ice and the plasma samples were prepared by centrifugation at 3500g for 5 min, then transferred immediately to sterile tubes and preserved at -20°C until further analysis using HPLC.

Sample preparation

A 100 µl volume of plasma standard or sample was pipette into a 2-mL micro-centrifuge tube, and protein precipitation was initiated by the addition of an equal volume of acetonitrile. The stopped tube was vortexed for 30 sec, kept standing for 5 min at room temperature and then centrifuged at 12,000 g for 2 min. Finally, a 10 µl aliquot of the supernatant was injected into the HPLC system.

Pharmacokinetic analysis

The determination of pharmacokinetic parameters of MCZ was done by the non-compartmental pharmacokinetic model based on the statistical moment theory. The area under the concentration-time curve (AUC) was determined using the trapezoidal method. The elimination rate constant (β) was the slope of the linear regression equation on log transferred MCZ concentration (ln C) against time, and the elimination half-life ($t_{1/2\beta}$) was calculated from the equation $t_{1/2} = 0.693/\beta$ for each treatment. After oral administration, the peak concentration (C_{max}) and time to reach C_{max} (T_{max}), half-value duration (HVD) and mean residence time (MRT) were directly estimated using the pharmacokinetic software Kinetica program (5.0; Thermo Scientific Corporation, USA).

Statistical analysis

Statistical analysis was done using SPSS-16.0 (SPSS Inc., Chicago IL, USA). Results are presented as mean± standard error. Comparisons of mean were done using one-way analysis of variance (ANOVA) and Duncan's test. Probability levels of 0.05 were used to find out the significance in all cases.

Results and Discussion

Detection limit and recovery percentage

Using the described conditions, miconazole was found to have a retention time of 8.175 min. A linear relationship was shown through the calibration curve with a range of 0.5 to 25µg ml^{-1}, with an elevated correlation coefficient indicating linearity (R^2 = 0.995) in fish plasma (Fig.1). The recovery rate for MCZ from fish plasma was also calculated and is shown in Table 2. The extraction procedures were validated and showed good recovery of MCZ. The recovery of MCZ varied from 88.70 % to 99.03% for fish plasma. The precision of these recovery studies varied from 0.34-1.97%. The limit of detection (LOD) and limit of quantification (LOQ) of MCZ in plasma was 0.032 µg ml^{-1} and 0.109 µg ml^{-1} respectively. No interference was seen during analysis, when calibrating the curves, or when performing recovery studies.

Pharmacokinetics

The mean concentrations of MCZ versus time in plasma of *L. rohita* after administration are shown in Fig. 2. MCZ Plasma concentration of lowest dose in first treatment (T1) reached 1.45µg ml^{-1} at 1 h and the maximum plasma concentration (5.35 µg ml^{-1}) was reached at 6 h after feeding. After this, the drug level declined rapidly and reached 0.07 µg ml^{-1} close to the limit of detection at 240 h. Plasma concentration of second dose treatment (T2) reached 2.31µg ml^{-1} at 1 h and the maximum plasma concentration (9.44 µg ml^{-1}) was reached at 6 h after feeding. After this, the drug level declined rapidly and reached 0.54 µg ml^{-1} at 240 h. Plasma concentration of third dose treatment (T3) reached 8.32 µg ml^{-1} at 1 h and the maximum plasma concentration (20.28 µg ml^{-1}) was reached at 8 h after feeding. After this, the drug concentration declined rapidly and reached 1.02 µg ml^{-1} at 240 h. T_{max} of all the doses varied from 6 to 8 h and with the increase in dose, the T_{max} also gets delayed. However, the pharmacokinetic analysis showed that the C_{max} in the study varied from 5.35 to 20.86 µg ml^{-1} and the concentration increases with each time interval of sampling till the highest peak in plasma was obtained. The total area under the curve (AUC) was varied from 263.11 to 1048.99 µg*h ml^{-1} and subsequently, the mean residence time also varied between 52 ±0.13 to 98 ±0.23 h. The R square (R^2 value) from the pharmacokinetic model used by the Kinetica 5.0 varied from 0.87 to 0.98. The statistical analysis showed that the parameters like T_{max}, C_{max}, $t_{1/2\beta}$, AUC and MRT of all the experimental groups fed with MCZ based feed are significantly ($p<0.05$) different from each other. The HVD value of MCZ in plasma of rohu was observed to be 5.53±0.02 h, 8.53±0.07 h and 36.71±0.05 h respectively for all three doses. The elimination of MCZ in rohu after three sub-lethal oral dosing was rapid and the half-life ($t_{1/2\beta}$) was estimated to be 30 h, 71 h and 77 h respectively in plasma. All the pharmacokinetic parameters are shown in Table 3.

Despite the immense interest in *L. rohita* as characterized by its good flesh quality, high market demand and acceptability by consumers, the kinetics and dosing study of any potent antifungal drug have not been very well established. Therefore, the present work was conducted to assess the MCZ pharmacokinetic profile of three sub-lethal doses (Singh *et al.*, 2018a) in *L. rohita* after a one-time feed administration in the fish. The stock and the reference solutions had separation and good peak symmetry as obtained by using the mobile phase of 80: 20 (v/v) mixture of acetonitrile and 0.05 M sodium acetate buffer. The retention time of MCZ was found to be 8.175 min. DeZan *et*

al., (2009) during the method validation of MCZ has observed the retention time of 8.3 min on using a mobile phase of water, methanol, and acetonitrile for 15min which was extremely stable among injections. The serum MCZ analysis using a mobile phase of 85:15 methanol and aqueous 0·05 M ammonium phosphate buffer gave a lower retention time of 6.05 min at 230 nm UV detection (Puranajoti *et al.*, 1999). Some reports have differences in the retention time of MCZ resulting from slight variations in the composition of the mobile phase, wavelength and the flow rate of the mobile phase (Hermawan *et al.*, 2017).

Table.1 Formulation of three sublethal doses of experimental diets (100g) containing MCZ in the required quantity for the pharmacokinetic study

Ingredients	Quantity (in g)			
	Control diet	Diet T1 (6.30 mg kg BW^{-1})	Diet T2 (12.61 mg kg BW^{-1})	Diet T3 (25.22 mg kg BW^{-1})
Casein	16.0	16.0	16.0	16.0
Gelatin	8.0	8.0	8.0	8.0
Dextrin	30.0	30.0	30.0	30.0
Fish meal	16.0	16.0	16.0	16.0
CMC	2.0	2.0	2.0	2.0
Veg oil	4.5	4.5	4.5	4.5
Cod liver oil	4.5	4.5	4.5	4.5
Vit-min mixture*	6.0	6.0	6.0	6.0
BHT	0.2	0.2	0.2	0.2
Cellulose	12.80	12.74	12.67	12.55
Drug	0.0	0.06	0.12	0.25

*Composition of vitamin-mineral premix (PREEMIX PLUS) (quantity2.5 kg^{-1}): Vitamin A, 5500000 IU; Vitamin D_3, 1100000 IU; Vitamin B_2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Vitamin B_6, 1000 mg; Vitamin B_{12}, 6 mcg; Calcium Pantothenate, 2500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450mg; L- lysine, 10 g; DL- Methionine, 10 g; Selenium, 50 ppm**.**

Table.2 Recovery of MCZ from the plasma of *L. rohita* with the described HPLC conditions (n=3)

MCZ added (µg ml^{-1})	MCZ found (mean ± SE) (µg ml^{-1})	Recovery (mean± SE) (%)
0.5	0.46± 0.01	92.03±2.71
1.0	0.93±0.02	93.10±2.98
1.5	1.32±0.01	88.70±0.91
2.0	1.87±0.04	93.50±1.78
3.0	2.89±0.02	96.33±2.54
4.0	3.96±0.02	99.03±0.88
5.0	4.88±0.01	97.60±1.98
	mean± SE	94.32±1.96

Table.3 Pharmacokinetic parameters for MCZ were calculated in plasma of *L. rohita* after oral administration with three different sub-lethal doses at different sampling hours. Control: fish fed with a basal diet without MCZ. T1, T2 and T3: fish fed with diets containing MCZ at the inclusion levels of 6.30 mg kgBW^{-1}, 12.61 mg kgBW^{-1} and 25.22 mg kgBW^{-1} respectively

Parameters	**T_{max}**	**C_{max}**	**HVD**	**$t_{1/2\beta}$**	**AUC**	**MRT**	**AUMC**	**R^2**
Unit	(h)	(µg ml^{-1})	(h)	(h)	(µg*h ml^{-1})	(h)	(µg*h^2 ml^{-1})	
Control	N/A*	N/A*	N/A*	N/A*	N/A*	N/A*	N/A*	N/A*
T1	6±0^a	5.35±0.29^a	5.53±0.02^a	30±0.51^a	263.11±9.92^a	52±0.62^a	13943±21.98^a	0.87^a
T2	6±0^a	9.44±0.30^b	8.53±0.07^b	71±0.92^b	472.96±13.76^b	66±0.73^b	31458±32.09^b	0.91^b
T3	8±0^b	20.28±0.48^c	36.71±0.05^c	77±0.83^c	1049.99±18.03^c	98±0.23^c	66653±27.80^c	0.98^c

*N/A: not available

Data are presented as mean ± SE. a, b and c indicate statistically significant difference ($p < 0.05$) when compared with the control group. Data is Tmax, Time of maximum plasma concentration; Cmax, Calculated maximum plasma concentration; HVD, Half value duration; $t_{½\beta}$, Elimination half-life; AUC, Area under the concentration-time curve; MRT, mean residence time; AUMC, Area under the first moment of the concentration-time curve.

Fig.1 Mean analytical curve obtained from MCZ standard and reference solutions (0.5 to 25.0 µg ml^{-1}) using the HPLC chromatogram system

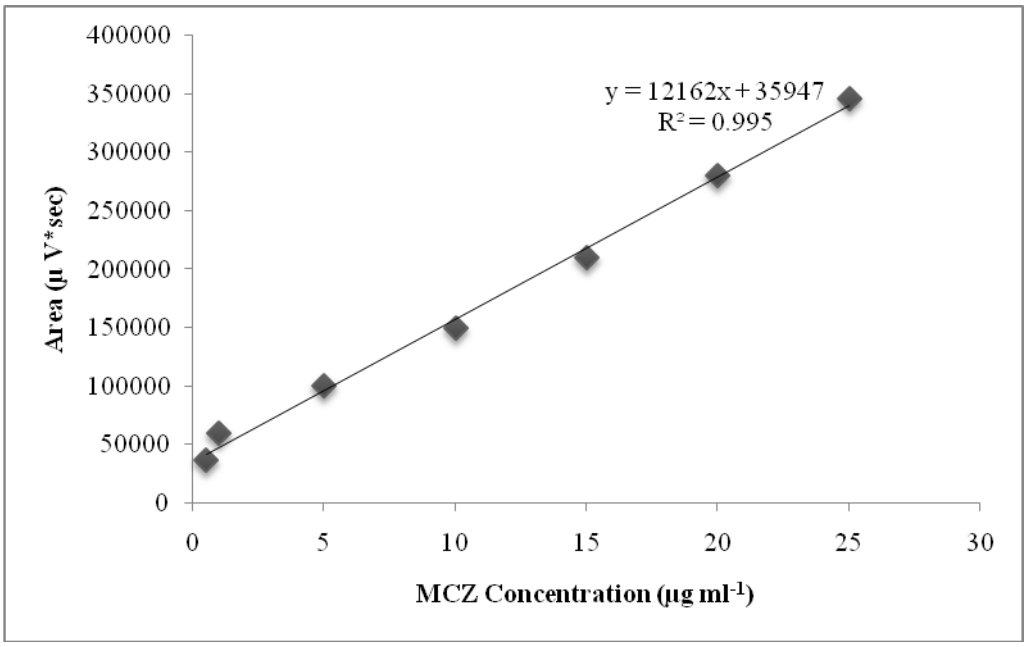
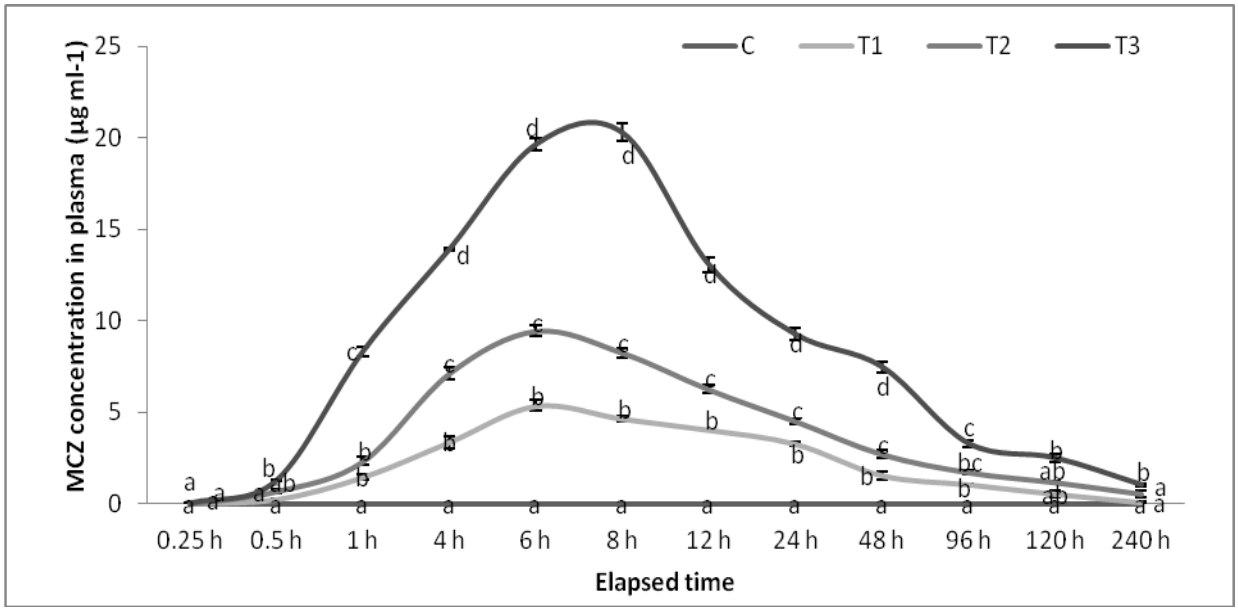


Fig.2 MCZ levels (mean ±SD) determined in the plasma of *L. rohita* after single oral administration with three different sub-lethal doses at different sampling hours. Control: fish fed with a basal diet without MCZ. T1, T2 and T3: fish fed with diets containing MCZ at the inclusion levels of 6.30 mg $kgBW^{-1}$, 12.61 mg $kgBW^{-1}$ and 25.22 mg $kgBW^{-1}$, respectively.



The calibration curve for a range of 0.5 to 25µg ml^{-1} has shown a linear relationship with an elevated correlation coefficient indicating linearity (R^2 = 0.995) in fish plasma similar to the case of Puranajoti *et al.,* (1999) reporting R^2 value more than 0·99 with serum samples

in the range of 0.5-100 µg ml^{-1} MCZ. The precision and recovery rate of MCZ was observed as varying from 0.34 to 1.97% and 88.70 % to 99.03% respectively for fish plasma. In the pharmaceutical preparations the recovery percentage of MCZ varying from 99-102% was reported by DeZan *et al.*, (2009). The limit of detection and limit of quantification was 0.032 µg ml^{-1} and 0.109 µgml^{-1} of MCZ in plasma respectively. The low value of LOD and LOQ signifies that the HPLC condition maintained in the analysis is sensitive and sufficient to determine the quantity of MCZ in plasma and pharmaceutical samples. In the pig plasma, the LOD and LOQ of MCZ were observed to be 0.013 mg ml^{-1} and 0.044 mg ml^{-1} respectively (Barillaro *et al.*, 2005). Hermawan *et al.*, (2017) observed the LOD and LOQ of MCZ pharmaceutical preparations as 2.24 mg l^{-1} and 7.47 mg l^{-1}, respectively and a linear calibration curve with an R^2 value of 0.9983. The experimental results demonstrated the specificity and sensitivity of the adopted methodology with good accuracy and recovery percentage. Furthermore, this procedure can be considered as convenient and quick to apply in routine analysis for the quantification of MCZ in fish plasma.

For assisting in early and appropriate fungal therapy of fish by MCZ medicated feed, it is crucial to understand the pharmacokinetic characteristics as they are the key treatment considerations. The variability in drug absorption is the most vital pharmacokinetic consideration that could limit the efficacy and oral availability during the initial curing phase (Lewis and Russell, 2012). Pharmacokinetics study is a mathematical model for estimating the effects and the time course of drugs inside the body. Furthermore, the pharmacokinetic properties of drugs in fisheries differ significantly between the species as well as on the mode of administration. Hence, the disposition of a drug should be inspected in the particular fish species in which it is expected to be utilized (van der Heiden *et al.*, 1994, Kleinow *et al.*, 1994, Martinsen *et al.*, 1994). In the present study, we determined the plasma pharmacokinetics of *L. rohita* fed with a single dose of MCZ medicated semi-purified feed. The three sub-lethal doses when incorporated orally in fish were detected in the fish plasma immediately within 30 minutes of feeding.

The T_{max} value, i.e., the maximum plasma concentration-time is displayed in an hour and is defined as the time required for the drug to reach the peak plasma level after administration. It helps to estimate the rate of absorption whereas C_{max} is the observed maximum concentration of drug in the plasma. Our results revealed that after one-time administration of three different sub-lethal doses of MCZ orally, it showed a relatively rapid absorption with the immediate detection of drug in plasma after 0.5 h and the T_{max} value varying from 6 to 8 h in all the three doses. C_{max} value was recorded as 5.35 µg ml^{-1} and 9.44 µg ml^{-1} in the dose T1 and T2 respectively at the 6^{th} h after feeding. The third and the highest sub-lethal dose of MCZ recorded peak plasma level concentration at 8 h after feeding as 20.28 µg ml^{-1}. Aljaeid and Ibrahim (2016) have reported the T_{max} value of 4 h reaching the maximum plasma concentration in albino rabbits as 13.71 ng ml^{-1} on being fed with 150 mg of miconazole. Cartwright (1975) has recorded human serum levels up to 1.75 µg ml^{-1} and 1.2 µg ml^{-1} on oral administration of 1.5 g per day and 1 g at 8 h interval of MCZ intake respectively. Boelaert *et al.*, (1976) reported peak plasma levels reaching 0.3µgml^{-1} after single oral doses of miconazole and peak plasma levels of 6 µg ml^{-1} after a single intravenous dose in four hours. Studies also reported maximum MCZ plasma levels in the range of 0.5 to 1µgml^{-1} following 1 gm of oral administration

and 1.6 µg ml^{-1} on 200mg of intravenous injection in humans (Boelaert *et al.*, 1976). The study conducted in pigs recorded the mean miconazole plasma peak concentrations varying from 0.10 to 0.59 mg ml^{-1} on the oral administration of 10 mg miconazole/kg body weight at 19.30 minutes (Barillaro *et al.*, 2005). The oral administration in the form of MCZ tablets in adult humans gave a single peak concentration at 6 h after application with a mean value of 15.1 mg ml^{-1} and 39.1 mg ml^{-1} for the 50 and 100 mg doses respectively (Cardot *et al.*, 2004). Although, the pharmacokinetic study data of azoles drugs in fish is very limited to be compared, the plasma levels in humans and the other animals shown promising results. Furthermore, there can be enormous plasma level concentration variations among the different fish species which may exist because of fish being the poikilotherms, their pharmacokinetics vary significantly depending on the mode of administration, difference in species or some other factors like alterations in water temperature and minor variations or a time lag in collecting blood samples.

The area under the curve (AUC) is a parameter used as an indicator of the drug exposure of the body and reflects the actual exposure of the drug in the body after single-dose administration of the drug. The AUC value is dependent closely on the amount of drug that enters the systemic circulation and on the ability of the system to eliminate the drug (Urso *et al.*, 2002). The AUC value was recorded to be significantly different in all the three treatment doses with the value ranging from 263.11 to 1048.99 µg*h ml^{-1} in MCZ fed fish plasma. Following MCZ oral administration, the mean area under the plasma concentration curve (AUC) for miconazole was found to be 95.0 ±55.8 mg*min ml^{-1} as observed in pig plasma at the dose of 10 mg miconazole/kg body weight (Barillaro *et al.*, 2005). The researchers have reported the AUC value of 231 to 294 ng*h ml^{-1} in albino rabbits in an *in vivo* drug absorption study fed with formulations composing 150 mg of miconazole (Aljaeid and Ibrahim, 2016).

The mean residence time (MRT) of a drug is the specific time until the drug molecule is remained in the plasma before its elimination from the body and is calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$. The MRT value for all the three sub-lethal doses under study was found to be significantly different ($p<0.05$) from each other varying from 52±0.13 to 98±0.23 h. The half-value duration (HVD) of a drug is the time at which the plasma drug concentrations were higher than half of the Cmax. HVD value of MCZ in rohu was estimated for all three doses and was recorded to be 5.53±0.02 h, 9.91±0.07 h, and 36.71±0.05 h respectively. In the present study, the elimination of MCZ in rohu after three sub-lethal oral dosings was rapid and the elimination half-life ($t_{1/2\beta}$) was estimated to be 30 h, 71 h and 77 h respectively in plasma. The $t_{1/2\beta}$ values of 51.80 min to 57.72 min were found in the plasma of sheep after IV administration by Piel *et al.*, (1990). In healthy human plasma, MCZ was observed to have a half-life ($t_{1/2\beta}$) of about 20 to 25 hours (Lewis *et al.*, 1976; Stevens *et al.*, 1976). Heel *et al.*, (1980) also confirms the elimination half-life in human plasma to be about 20 to 25 hours.

According to Daneshmend (1983), MCZ serum pharmacokinetics was observed to have a short initial half-life of less than 1 hour and a terminal half-life of 20 hours in human plasma. The rate of elimination of the drug from fish plasma is mainly influenced by the surrounding temperature (Bjorklund and Bylund, 1990). Furthermore, Chung *et al.*, (2019) and Ellis *et al.*, (1978) suggested that every 1°C change in water temperature results

in a 10% change in the metabolic and elimination rate in the case of fish. Thus we can understand that the delayed elimination of the drug from fish and variations in the other pharmacokinetic parameters are broadly influenced by its poikilothermal behavior and can oscillate among the species depending on their surrounding environment.

In conclusion, this study exhibits that the plasma concentration of the antifungal drug MCZ in fish fed with medicated feed can be determined by a very simple assay with a good precision level. To our knowledge, the pharmacokinetic parameters of MCZ were evaluated for the first time in fish and the relevant data on antifungal pharmacokinetics in fish is scarce.

The result demonstrated rapid absorption and elimination of MCZ in fish plasma and the high drug plasma concentration is an excellent property from a therapeutic stance. However, due to the rapid elimination, consecutive dose administration is necessary to maintain effective concentration levels. Apart from this, taking into consideration the effect of temperature and metabolic rate, pharmacokinetic studies in the other fish species concerned should be assessed to ensure the proper utilization of the drug.

Competing interests disclaimer

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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How to cite this article:

Mukta Singh, Himadri Saha and Ratan Kumar Saha. 2020. Comparative Pharmacokinetics of the Orally Administered Sub-lethal Doses of Miconazole Nitrate in *Labeo rohita*. *Int.J.Curr.Microbiol.App.Sci.* 9(12): 3197-3210. doi: https://doi.org/10.20546/ijcmas.2020.912.381