Neuroendocrinology Letters Volume 36 Suppl. 1 2015
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

The effect of the fluoroquinolone norfloxacin on somatic indices and oxidative stress parameters in early stages of common carp (*Cyprinus carpio* L.)

Nina Charvatova [1], Gabriela Zelinska [1], Radka Dobsikova [1], Vlasta Stancova [1], Dana Zivna [1], Lucie Plhalova [1], Jana Blahova [1], Pavla Sehonova [1], Petr Marsalek [1], Marta Bartoskova [1], Miroslav Prokes [2], Ingrid Piskorova [1], Zdenka Svobodova [1]

1 Department of Veterinary Public Health and Animal Welfare, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
2 Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Correspondence to: MVDr. Nina Charvatova
Department of Veterinary Public Health and Animal Welfare
University of Veterinary and Pharmaceutical Sciences Brno
Palackeho tr. 1/3, CZ 612 42 Brno, Czech Republic.
tel.: +420-541562774; e-mail: H14393@vfu.cz

Submitted: 2015-07-18 Accepted: 2015-09-09 Published online: 2015-10-15

Key words: **antibiotics; fish; morphometry; antioxidant enzymes; lipid peroxidation**

Neuroendocrinol Lett 2015; **36**(Suppl. 1):79–87 **PMID:** 26757128 NEL360915A08

Abstract

OBJECTIVES: The aim of this study was to assess the effect of the subchronic exposure of early stages of common carp (*Cyprinus carpio* L.) to norfloxacin using morphometric data and oxidative stress parameters.

METHODS: A subchronic toxicity test was performed on fertilized embryos of common carp according to the OECD Guidelines No. 210. Embryos were exposed to norfloxacin concentrations of 0.0001 (environmental), 0.1, 1.0, 5.0, and 10.0 mg.L^{-1} for 34 days.

RESULTS: At the end of the test (day 34), significant (p<0.05) stimulation of development was observed in all experimental groups, in contrast to the control. Significantly greater (p<0.01) total body length was also observed in the group exposed to 10.0 mg.L^{-1} of norfloxacin compared to the control.

A significant increase in the activity of glutathione S-transferase in all carp exposed to norfloxacin concentrations of 0.1 and 1.0 mg.L^{-1} (p<0.01), and 5.0 mg.L^{-1} (p<0.05) compared to control group was revealed. The activity of glutathione peroxidase was significantly lower (p<0.01) in experimental carp exposed to a norfloxacin concentration of 10.0 mg.L^{-1}. In experimental carp exposed to a norfloxacin concentration of 0.0001 mg.L^{-1}, a significant increase (p<0.05) in glutathione reductase activity was found. Significant (p<0.01) decreases in the content of thiobarbituric acid reactive substances in the groups exposed to norfloxacin concentrations of 1.0, 5.0, and 10.0 mg.L^{-1} were revealed.

CONCLUSION: From the results, we can conclude that norfloxacin has a negative impact on selected biochemical processes related to the production of reactive oxygen species in early-life stages of common carp.

ORIGINAL ARTICLE

Abbreviations

CAT	- catalase
DMSO	- dimethyl sulfoxide
FCF	- Fulton's condition factor
FQs	- fluoroquinolone antibiotics
GPx	- glutathione peroxidase
GR	- glutathione reductase
GSH	- reduced glutathione
GSSG	- oxidized glutathione
GST	- glutathione S-transferase
I	- inhibition of specific growth
OECD	- Organisation for Economic Co-operation and Development
SGR	- specific growth rate
TBARS	- thiobarbituric acid reactive substances
TL	- total length
W	- weight
WWTPs	- wastewater treatment plants

INTRODUCTION

The continuous entry of pharmaceuticals into the aquatic environment, even at low concentrations, can represent potential long-term risks to aquatic and terrestrial organisms (Klavarioti *et al.* 2009). One group of pharmaceuticals frequently detected in the environment in relatively high concentrations are fluoroquinolone antibiotics (FQs). Their ubiquitous presence has been reported in wastewater, surface water, ground water, and even in drinking water (Gros *et al.* 2007; Khetan & Collins 2007).

FQs exhibit a broad spectrum of antimicrobial activities (Fitton 1992). They are widely used for the treatment of urinary, respiratory, and digestive tract infections in humans, and even increasingly in veterinary medicine (Martinez *et al.* 2006). Second-generation quinolones (e.g. norfloxacin) exhibit activity against gram-negative (including *Pseudomonas aeruginosa*), some gram-positive (including *Staphylococcus aureus*), and some atypical pathogens (King *et al.* 2000).

FQs inhibit key bacterial enzymes (DNA gyrase and topo-isomerase IV) involved in unwinding the DNA helix for replication and transcription (Hawkey 2003). The majority of FQs are excreted renally via urine, but some of them (e.g. the fourth-generation quinolone trovafloxacin) are metabolised hepatically (Alghasham & Nahata 1999; Turnidge 1999).

Unfortunately, FQs are not completely removed from wastewater at wastewater treatment plants (WWTPs) and thus contribute to contamination of the aquatic environment. FQs present in water bodies may quickly move into soil and sediments due to its strong adsorption on minerals and organic matter (Golet *et al.* 2002; Lindberg *et al.* 2007).

The aim of this study was to investigate the subchronic effects of the second-generation quinolone norfloxacin on early stages of common carp (*Cyprinus carpio* L.). The parameters glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), catalase (CAT), and lipid peroxidation were investigated to determine the effects of norfloxacin on oxidative stress. In addition, the effect of norfloxacin on somatic parameters was evaluated.

MATERIALS AND METHODS

Experimental protocol

A subchronic toxicity test was performed according to OECD Guideline No. 210 (Fish, Early-life Stage Toxicity Test). Common carp *(Cyprinus carpio* L.) was used as a model organism for embryo-larval toxicity testing. Fertilized carp eggs were obtained from Rybnikarstvi Pohorelice (Czech Republic). The eggs were produced according to standard methods of artificial reproduction (Kocour *et al.* 2005). Twenty-four hours after fertilization, 100 fertilized eggs were separated from unfertilized eggs and randomly distributed into each of 21 crystallization dishes (volume 900 mL). The crystallization dishes were divided equally into 7 groups. Five experimental groups were exposed to concentrations of norfloxacin of 0.0001, 0.1, 1.0, 5.0, and 10.0 $mg.L^{-1}$ (in triplicate). Two groups were used as controls (norfloxacin-free tap water with and without a solvent). Because of the low solubility of norfloxacin in water, dimethyl sulfoxide (DMSO, 0.05%) was used as a solvent. In the control, the concentration of solvent was 0.05%, i.e. the same used in experimental groups. The semi-static method was used, with bath replacement twice daily. Hatching and survival were also observed twice a day, and dead embryos and larvae were recorded and removed. During the test, larvae were fed on freshly hatched *Artemia salina ad libitum* twice a day prior to each bath exchange. The temperature, pH, and oxygen saturation were recorded daily. The beginning of the test was designated as day 1 (one day post fertilization). Hatching began on day 3 and was completed by day 5. Feeding with *A. salina* was initiated on day 6. The test was completed after 34 days. In the course of the test, embryos and larvae were sampled to evaluate developmental stage, length, weight, Fulton's condition factor (FCF), length-weight relationship, and morphological anomalies. Samples from each concentration and from the control were collected on day 6 (before feeding began) and on days 13, 20, 27, and 34.

For the evaluation of somatic parameters and early ontogeny, 10 and 15 specimens, respectively, were taken from each dish on the first four sampling days (day 6, 13, 20, and 27) and at the end of the test (day 34). The samples were fixed in 4% formalin. At the end of the test (day 34), ten samples were taken from each dish, i.e. 30 specimens per group, in order to evaluate antioxidant enzyme activity and lipid peroxidation. The samples were stored at –85 °C until analysis.

Water parameters

The basic physical and chemical parameters of the tap water used in the tests were as follows: acid neutralization capacity ($ANC_{4.5}$) – 1.0–1.2 $mmol.L^{-1}$; chemical oxygen demand (COD_{Mn}) – 1.2–1.5 $mg.L^{-1}$;

total ammonia - below the limit of determination (<0.04 mg.L^{-1}); nitrates – 11.2–13.5 mg.L^{-1}; nitrites below the limit of determination (< 0.02 mg.L^{-1}); Cl^- – 14.5–16.2 mg.L^{-1}, and Σ Ca+Mg – 3.02 mmol.L^{-1}. The water temperature ranged from 19 to 22 °C; pH values were between 7.5 and 8.3, and the level of dissolved oxygen did not fall below 60%.

Morphometric and condition characteristics

FCF was calculated on each sampling day according to the following equation:

$FCF = (W \times 10^5)/L^3$,

where W is the weight in g and L is the total length in mm.

Total length (TL) was measured stereomicroscopically using a micrometer to 0.01 mm; weight (W) was measured to 0.1 mg.

The mean specific growth rate (SGR) was calculated for each experimental group for the period from day 6 to the completion of the test (day 34) according to the following equation:

$SGR = 100 \times ((\ln w_2 - \ln w_1)/(t_2 - t_1))$,

where w_1 is the weight of one fish at time t_1, w_2 is the weight of the fish at time t_2, t_1 is the first sampling time (day 6), and t_2 is the final sampling time (day 34).

The inhibition of specific growth rate (I) for each experimental group was calculated according to the following equation:

$I\,[\%] = (SGR_{control} - SGR_{group}) / (SGR_{control}) \times 100$.

Determination of developmental stages.

Developmental stages were determined according to Penaz *et al.* (1983); nine embryonic (E1–E9), six larval (L1–L6), and one juvenile (J1) stages of common carp were described.

Measurement of oxidative stress parameters

At the end of the test, 30 specimens from each test and control group (i.e. 10 specimens from each dish) were killed with an overdose of the anaesthetic MS 222. They were then homogenised with phosphate buffer (pH 7.2) and stored at –85 °C until analysis. The homogenate was then divided into two parts. The first was used for the measurement of lipid peroxidation using thiobarbituric acid reactive substances method (TBARS); the second was centrifuged (10,500 g at 4 °C for 20 min) and the obtained supernatant fraction was then used to determine the catalytic activities of GPx, GR, GST, and CAT.

To determine lipid peroxidation in fish samples, the TBARS method described by Lushchak *et al.* (2005) was used. TBARS were measured at 535 nm and the concentration was expressed in nmol of TBARS per gram of tissue wet weight.

The catalytic activities of GPx and GR were determined spectrophotometrically at 340 nm by the catalysis conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) in the process involving the consumption of NADPH. The specific activities were expressed as the nmol of NADPH consumption per min per mg of protein (Carlberg & Mannervik 1975).

The total catalytic activity of GST was determined by measuring the conjugation of 1-chloro-2,4-dinitrobenzene with reduced GSH at 340 nm (Habig *et al.* 1974). The specific activity was expressed in the units of nmol of formed product per min per mg of proteinThe catalytic activity of CAT was determined by the spectrophotometrical measurement of H_2O_2 breakdown at 240 nm. The specific activity of the enzyme was expressed as μmol of decomposed H_2O_2 per min per mg of protein (Aebi 1984).

Protein concentrations were determined by a Bicinchoninic Acid Protein Essay Kit (Sigma–Aldrich, St. Louis, MO, USA) using bovine serum albumin as standard (Smith *et al.* 1985). All spectrophotometric measurements were performed using a Varioskan Flash Spectral Scanning Multimode Reader (Thermo Fisher Scientific Inc., USA).

Determination of norfloxacin concentration in water

The measurement of norfloxacin was based on high–performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (LC–ESI–MS/MS). Samples were filtered and used for LC–ESI–MS/MS analysis. A Thermo Scientific UHPLC Accela 1250 system was connected to a Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Instrument (Thermo, San Jose, CA, USA) equipped with a heated electrospray ionization (HESI-II) probe. A Thermo Scientific Hypersil C_{18} (2.1 mm × 50 mm, 1.9 μm) column was used at a constant flow rate of 300 μL.min^{-1}. The mobile phase consisted of water containing 0.1% formic acid (v/v) (solvent A) and acetonitrile containing 0.1% formic acid (solvent B). The full loop injection volume of the tissue extract was set at 20 μL. The heated electrospray ionization was operated in positive-ion mode under specific conditions. The standard of norfloxacin was purchased from Sigma-Aldrich (St. Louis, MO). All solvents were residual analysis purity (Chromservis, Ltd., CZ). The coefficient of variation for between-series was 3.1%. The limit of detection was determined as a signal/noise ratio of 3:1 and was found to be 91 ng.L^{-1}.

Statistical analysis

Statistical analysis was conducted using Unistat 5.6 for Excel (Czech Republic). The Shapiro-Wilk´s test was used to test the normality of all parameters. Data on cumulative mortality, morphometric and condition parameters, hatching, and some indices of oxidative stress (TBARS and GPx) did not exhibit normal distributions and were thus subjected to Kruskal-Wallis test. Individual differences between experimental groups and control were tested using Dunn´s test. Data on oxidative stress parameters which exhibited normal distributions (GR, GST, and CAT) were tested for variance

homogeneity by Levene´s test and then by one-way ANOVA; individual differences between the control and each experimental group were evaluated using Dunnett´s test. The levels of significance of all statistical tests were $p<0.05$ and $p<0.01$.

RESULTS

As the results in control group and control group with a solvent DMSO showed no differences, therefore we used only the results of DMSO group for the following evaluation.

Mortality rate

No significant differences were found in total cumulative mortality between any experimental group and control group. In all experimental groups and control, mortality was below 12%. In the control group (i.e. the control group with a solvent), mortality was 11.7%, which meets the validity criteria of OECD 210 Guideline. The levels of mortality in experimental groups (0.0001, 0.1, 1.0, 5.0, and 10.0 mg.L^{-1}) were as follows: 11.7, 11.0, 11.7, 11.7, and 6.3%, respectively.

Hatching

Hatching began on day 3. The hatching rate on the first day of hatching (day 3) was significantly higher ($p<0.01$) in groups exposed to 0.0001 (60% of embryos hatched), 0.1 (62.0% of embryos hatched), and 10.0 (53.3% of embryos hatched) mg.L^{-1} of norfloxacin compared to the control group (31.0% of embryos hatched). Hatching was completed by day 4 in all groups.

Early ontogeny

The individual development stages of early ontogeny (larval and juvenile stages) are illustrated in Figure 1. Developmental stages were recorded in all groups on each sampling day. The differences in early ontogeny between control and any experimental group were evident at the completion of the test. Stimulated development ($p<0.05$) appeared on day 6 in the group exposed to 1.0 mg.L^{-1} of norfloxacin (by 10.0%) and on day 13 ($p<0.05$) in the groups exposed to 0.1 and 1.0 mg.L^{-1} of norfloxacin (by 26.7% and 40.0%, respectively) compared to the control. The same effect was found on day 27, with significant stimulation ($p<0.01$) found in the group exposed to 10.0 mg.L^{-1} of norfloxacin (by 6.7%) compared to control.

On day 34, i.e. at the end of the test, there was significant ($p<0.05$) stimulation of development in each group exposed to norfloxacin compared to the control group, i.e. by 20.0, 6.7, 15.6, 24.5, and 35.6% in 0.0001, 0.1, 1.0, 5.0, and 10.0 mg.L^{-1}, respectively.

Morphometric and condition characteristics

The effects of norfloxacin on larvae of common carp were observed from day 6. On day 6, total body weight was significantly lower ($p<0.01$) in the group exposed

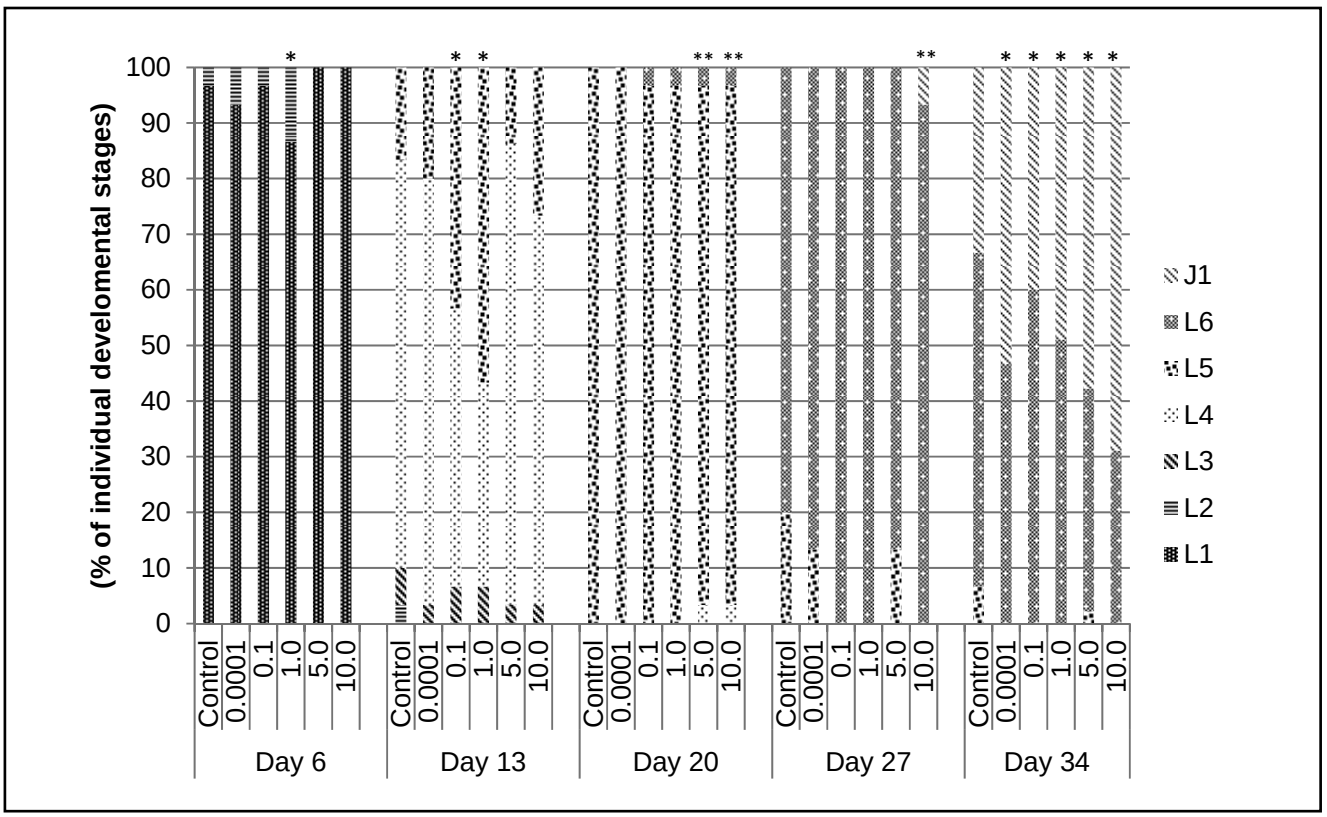

Fig. 1. Development stages in experimental groups exposed to 0.0001, 0.1, 1.0, 5.0, and 10.0 mg.L^{-1} of norfloxacin on days 6, 13, 20, 27, and 34 of the experiment. * and ** mean significance at $p<0.05$ and $p<0.01$

to 5.0 mg.L^{-1} of norfloxacin compared to the control group. On day 13, total body length and weight were significantly higher (p<0.01) in the group exposed to 1.0 mg.L^{-1} of norfloxacin compared to the control. There was no significant difference in total body length or weight on days 20 and 27 compared to control. On day 34, total body length was significantly higher (p<0.01) in the group exposed to 10.0 mg.L^{-1} of norfloxacin compared to the control.

The inhibition of specific growth (I) was found at concentration 0.1 mg.L^{-1} of norfloxacin and the stimulation of specific growth was found at concentrations of 0.0001, 1.0, 5.0, and 10.0 mg.L^{-1} of norfloxacin. With respect to SGR, no significant changes were found in experimental groups when compared to control. The inhibition of specific growth and SGR values are given in Table 1.

On day 13, FCF was found to be significantly lower (p<0.01) in the group exposed to 10.0 mg.L^{-1} of norfloxacin when compared to control. FCF was significantly higher in the group exposed to 1.0 mg.L^{-1} of norfloxacin on day 27 (p<0.01) and day 34 (p<0.05) compared to the control group. Also, on day 27, FCF was significantly higher (p<0.01) in the group exposed to 5.0 mg.L^{-1} of norfloxacin compared to the control group.

Morphological anomalies

In the course of the test, some morphological anomalies were observed in some groups exposed to norfloxacin. These malformations included, for example, axial curvature of the spine (lordosis, kyphosis), that occured in groups exposed to norfloxacin at concentrations of 0.1 and 10.0 mg.L^{-1} (6.7% in both groups) compared to the control group (without spine malformations). Elevated melanin pigmentation was observed in groups exposed to norfloxacin at concentrations of 5.0 (12.7%) and 10.0 (16.0%) mg.L^{-1} compared to the control group (6.0%).

Oxidative stress parameters

Statistical analysis demonstrated significant shifts in most biotransformation and antioxidant enzymes evaluated in common carp (Figures 2–5).

A significant increase was revealed in the activity of GST in experimental carp exposed to norfloxacin concentrations of 0.1 and 1.0 mg.L^{-1} (p<0.01), and 5.0 mg.L^{-1} (p<0.05) compared to control group (Figure 2). GST activities were found to be 103.4 nmol.min^{-1}.mg^{-1} protein in control group and 114.4, 123.7, and 112.8 nmol.min^{-1}.mg^{-1} protein in the 0.1, 1.0, and 5.0 mg.L^{-1} norfloxacin groups, respectively.

The activity of GPx was significantly lower (p<0.01) in experimental carp exposed to a norfloxacin concentration of 10.0 mg.L^{-1} (22.18 nmol.min^{-1}.mg^{-1} protein) compared to the control group (35.43 nmol.min^{-1}.mg^{-1} protein) (Figure 3).

In experimental carp exposed to norfloxacin at the environmental concentration of 0.0001 mg.L^{-1}, a significant increase (p<0.05) in GR activity (8.10 nmol.min^{-1}.mg^{-1} protein) was found when compared to the control (7.04 nmol.min^{-1}.mg^{-1} protein) (Figure 4).

No significant changes in the activity of CAT in any experimental group exposed to norfloxacin was found compared to the control (22.15 μmol.min^{-1}.mg^{-1} protein) (Figure 5).

A significant (p<0.01) decrease in the content of TBARS was found in experimental groups exposed to norfloxacin concentrations of 1.0, 5.0, and 10.0 mg.L^{-1} (6.15, 5.98, and 6.35 nmol.g^{-1} ww tissue, respectively) compared to the control group (8.90 nmol.g^{-1} ww tissue) (Figure 6).

Tab. 1. Morphometric and condition characteristics of carp embryos and larvae on days 6, 13, 20, 27 and 34 of norfloxacin esposure.

Group	Control	0.0001 mg.L^{-1}	0.1 mg.L^{-1}	1.0 mg.L^{-1}	5.0 mg.L^{-1}	10.0 mg.L^{-1}
W_6 (mg)	2.30	2.30	2.50	2.30	2.20**	2.25
TL_6 (mm)	6.78	6.81	6.83	6.74	6.67	6.76
W_{13} (mg)	6.85	6.95	7.00	7.65**	6.70	6.45
TL_{13} (mm)	9.33	9.41	9.50	9.90**	9.46	9.53
W_{20} (mg)	14.70	15.45	13.95	16.90	13.50	16.00
TL_{20} (mm)	11.41	11.58	11.41	12.13	11.41	11.79
W_{27} (mg)	51.25	50.10	42.70	55.10	57.80	51.30
TL_{27} (mm)	16.19	15.82	15.44	15.96	16.00	15.94
W_{34} (mg)	94.30	95.50	85.60	106.10	110.20	107.70
TL_{34} (mm)	18.85	19.22	18.36	19.21	19.83	20.13**
SGR	13.12	13.35	12.90	13.55	13.74	13.78
I (%)	–	–1.75	1.72	–3.26	–4.69	–4.99

W_6, W_{13}, W_{20}, W_{27}, W_{34} - median fish weight; TL_6, TL_{13}, TL_{20}, TL_{27}, TL_{34} - median total body length; SGR – specific growth rate; I - inhibition of specific growth. * and ** mean a significance at p<0.05 and p<0.01, respectively.

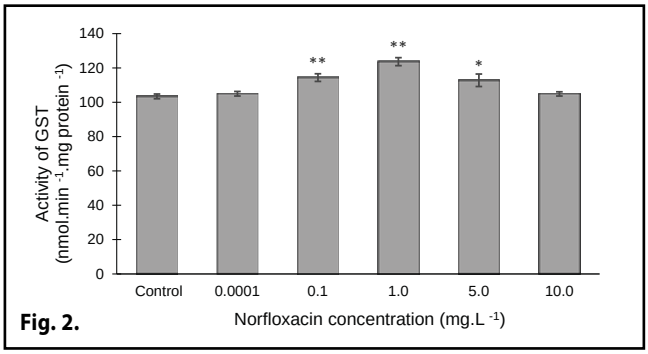

Fig. 2.

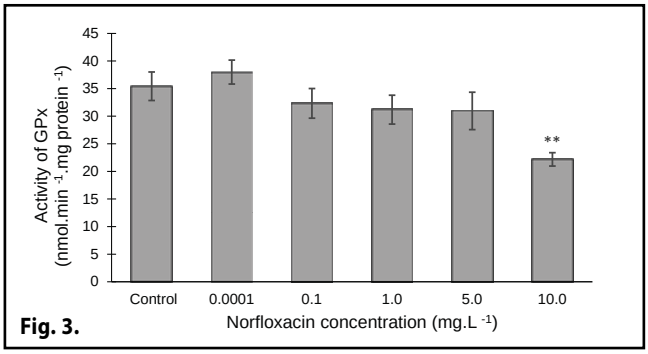

Fig. 3.

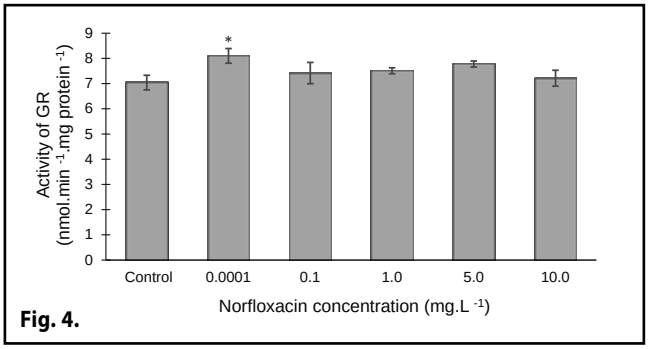

Fig. 4.

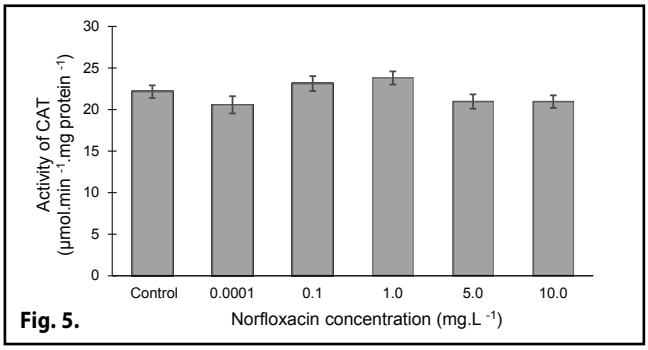

Fig. 5.

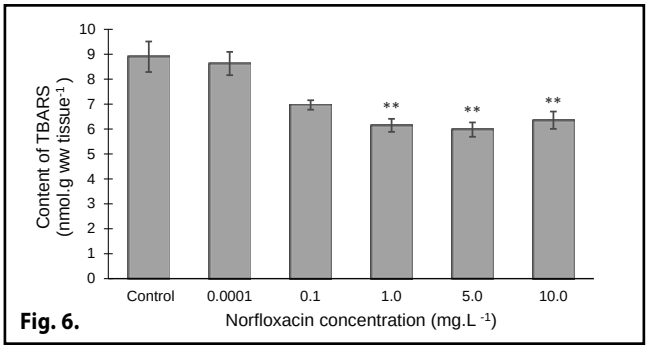

Fig. 6.

Our test met validity criteria required by the OECD Guideline. In the course of the test, norfloxacin concentrations were measured above 80% of the initial level.

DISCUSSION

In the literature, there is little information on the effect of norfloxacin (or other fluoroquinolones) on fish or other water organisms.

In our study, mortality in all groups, including the control group, was below 12%. In the control group, mortality was 11.7%, which meets the validity criteria given by OECD Guideline No. 210. In the study by Bartoskova *et al.* (2014), the mortality of zebrafish (*Danio rerio*) exposed to norfloxacin concentrations of 0.0001, 0.1, 1.0, 10.0, and 30.0 mg.L^{-1} did not exceed 8%. In contrast, Robinson *et al.* (2005) observed 100% mortality of fathead minnow (*Pimephales promelas*) larvae treated with fluoroquinolone clinafloxacin at a concentration of 10.0 mg.L^{-1} which could be explained by using a different fish species and/or a different type of fluoroquinolone as clinafloxacin belongs to the 4th fluoroquinolone generation.

At this time, no other studies on the effects of antibiotics on early stages of common carp are available. In our experiment, we observed a significant (p<0.01) retardation of development on day 20 in groups exposed to 5.0 and 10.0 mg.L^{-1} of norfloxacin compared to the control; however, in contrast, at the end of the test on day 34 we observed a significant (p<0.05) stimulation of development in each group exposed to norfloxacin compared to the control group. We also observed significant (p<0.01) differences in total body length and weight.

In fish exposed to a concentration of 5.0 mg.L^{-1}, FCF was found to be significantly (p<0.01) increased on day 27; however, at the end of the test (day 34), it was comparable to that of the control group. In fish exposed to 1.0 mg.L^{-1}, significantly increased levels of FCF were found at day 27 (p<0.01) as well as at day 34 (p<0.05). In these types of tests, FCF can be increased due to the higher mortality of experimental fish when compared to control resulting from space enlargement in the crystallization dishes. In our study, no increase in mortality was found at a concentration of 1.0 mg.L^{-1} compared

Fig. 2. Glutathione S-transferase (GST) activity in common carp exposed to norfloxacin (mean ± standard error of mean). * and ** mean significance at p<0.05 and p<0.01, respectively

Fig. 3. Glutathione peroxidase (GPx) activity in common carp exposed to norfloxacin (mean ± standard error of mean). ** means significance at p<0.01

Fig. 4. Glutathione reductase (GR) activity in common carp exposed to norfloxacin (mean ± standard error of mean). * means significance at p<0.05.

Fig. 5. Catalase (CAT) activity in common carp exposed to norfloxacin (mean ± standard error of mean).

Fig. 6. Thiobarbituric acid reactive substances (TBARS) concentrations in common carp exposed to norfloxacin (mean ± standard error of mean). ** means significance at p<0.01

to the control group. On this basis we suggest that the increase in FCF was due to exposure to norfloxacin at this concentration.

In contrast, Bartoskova *et al.* (2014) found no significant differences in growth rates in zebrafish exposed to all tested norfloxacin concentrations when compared to the control group. The same results were obtained also by Plhalova *el al.* (2014) in zebrafish exposed to ciprofloxacin at concentrations of 0.0007, 0.1, 0.65, 1.1, and 3.0 mg.L^{-1}.

It has been reported (i.e. Stepanova *et al.* 2013; Zivna *et al.* 2013; Bartoskova *et al.* 2014; Plhalova *et al.* 2014) that xenobiotics (i.e. pharmaceuticals) can induce oxidative stress in fish. In our study, we focused on four oxidative stress enzymes (GPx, GR, GST, and CAT) and lipid peroxidation evaluated using the measurement of TBARS.

GPx plays an important role in the detoxification of various hydroperoxides. It also reduces a wide range of organic peroxides derived from unsaturated fatty acids, nucleic acids, and other important biomolecules (Tappel 1984). In our study, no changes in GPx were found in concentrations below 5.0 mg.L^{-1}, a significant decrease (p<0.01) was found in the activity of GPx in common carp exposed to norfloxacin at a concentration of 10.0 mg.L^{-1}. This can be explained by the reduced ability of the organism to eliminate peroxides, this reduction resulting from oxidative stress. In contrast, in the study by Bartoskova *et al.* (2014), the exposure of zebrafish to norfloxacin at concentrations of 0.1, 1.0, 10.0, and 30.0 mg.L^{-1} caused a highly significant increase (p<0.01) in the activity of GPx. In our study, similar values in all norfloxacin concentrations were found. Plhalova *et al.* (2014) studied the effect of fluoroquinolone ciprofloxacin on zebrafish. They found a significant decrease in the activity of GPx in groups exposed to ciprofloxacin concentrations of 0.0007, 1.1, 3.0 mg.L^{-1} (p<0.01) and 0.65 mg.L^{-1} (p<0.05). It can be explained by using a different species and age of the fish tested or a different fluoroquinolone drug.

GR is an enzyme catalyzing the conversion of glutathione disulfide to GSH, which serves as a substance protecting cells against chemical insult (Chance *et al.* 1979; Di Giulio & Meyer 2008). A decrease in the activity of this enzyme may result in glutathione depletion and in a reduction of antioxidant defense (Stepanova *et al.* 2013). In our study, a significant increase (p<0.05) in GR activity was found in the group exposed to the environmental concentration of norfloxacin (0.0001 mg.L^{-1}) compared to the control group, which can be related to the induction of antioxidant defense system. Bartoskova *et al.* (2014) did not find significant differences in GR activity in zebrafish exposed to norfloxacin concentrations of 0.0001, 0.1, 1.0, 10.0, and 30.0 mg.L^{-1}. In contrast, Plhalova *et al.* (2014) found a significant decrease (p<0.05) in the GR activity in zebrafish exposed to fluoroquinolone ciprofloxacin at concentrations of 1.1 and 3.0 mg.L^{-1}, which can be related to a different type of fluoroquinolone used in the test.

The main function of GST in the endogenous metabolism is the phase II detoxification of xenobiotics. It also provides cellular protection against the toxic effects of a variety of environmental and endogenous chemicals (Di Giulio & Meyer 2008). In our study, a significant increase in GST activity was found in common carp exposed to norfloxacin concentrations of 0.1, 1.0 (p<0.01) and 5.0 mg.L^{-1} (p<0.05) compared to the control group. The highest concentration (10.0 mg.L^{-1}) caused no significant changes in GST activity, which can be explained by the depletion of detoxifying system or disorder in the enzyme synthesis. No changes in GST activity at a concentration of 0.0001 mg.L^{-1} can be explained as an adaptation of the fish organism to a tested substance in the course of the embryo-larval test. Bartoskova *et al.* (2014) found a significant (p<0.01) increase in GST activity in zebrafish exposed to the environmental concentration of norfloxacin (0.0001 mg.L^{-1}); however, only a non-significant increase in GST activity compared to the control group was found at higher tested concentrations in their study. Plhalova *et al.* (2014) also found a significant increase (p<0.05) in GST activity in zebrafish exposed to FQ ciprofloxacin at concentrations of 0.0007 and 0.1 mg.L^{-1}; however at higher ciprofloxacin concentrations (0.65, 1.1, and 3.0 mg.L^{-1}), GST activity exhibited no significant shifts when compared to the control group.

CAT is an enzyme that catalyzes the conversion of a potentially oxidative molecule, H_2O_2, into H_2O and O_2 (Wang *et al.* 2009). In our study, no significant differences were found in CAT activity at all tested concentrations compared to the control group. The same results for CAT activity were found by Plhalova *et al.* (2014) in zebrafish exposed to fluoroquinolone ciprofloxacin at concentrations of 0.0007, 0.1, 0.65, 1.1 and 3.0 mg.L^{-1} compared to the control group. In contrast, Bartoskova *et al.* (2014) found significantly higher CAT activity in zebrafish exposed to norfloxacin in at tested concentrations (0.0001, 0.1, 1.0, 10.0, and 30.0 mg.L^{-1}), which can be explained by using juvenile fish (30-day-old individuals at the beginning of the test) with a developed immune system in comparison to our test, in which fish were mostly in embryonal and larval stages. Wang *et al.* (2009) studied the toxicity of the fluoroquinolone enrofloxacin associated with environmental stress in Tra catfish (*Pangasianodon hypophthalmus*). They tested three different rearing densities (40, 80, and 120 fish m^{-3}). Fish were fed (a dose commonly applied in aquaculture according to rearing density) with pellets containing 1 g.kg^{-1} enrofloxacin for 7 days and then with pellets containing no antibiotic for a further 7 days. On day 7 of the exposure, they observed that CAT activity in the gills of exposed fish increased 1.7 times in a low-density group (40 fish m^{-3}) compared to the control group;however, on days 8 and 10, CAT

activity was significantly lower in the low-density group compared to control.

The measurement of lipid peroxidation levels is considered to be a good indicator of oxidative stress, as lipids easily react with reactive oxygenated species (Wang *et al.* 2009). Lipid peroxidation is a major contributor to the loss of cell function under oxidative stress (Storey 1996). One of the major terminal products of lipid peroxidation is malondialdehyde. To determine the extent of lipid peroxidation the level of TBARS is often used (Hodgson 2004; Lushchak 2011). In our study, we found a significant ($p<0.01$) decrease in lipid peroxidation in groups exposed to norfloxacin concentrations of 1.0, 5.0, and 10.0 mg.L^{-1} compared to the control group. Plhalova *et al.* (2014) found a significant ($p<0.01$) decrease in lipid peroxidation in zebrafish exposed to fluoroquinolone ciprofloxacin at a concentration of 0.1 mg.L^{-1}. Other tested ciprofloxacin concentrations (0.0007, 0.65, 1.1, and 3.0 mg.L^{-1}) induced no significant differences in lipid peroxidation. Similarly, in the study by Bartoskova *et al.* (2014) no significant effect on lipid peroxidation was found in zebrafish exposed to norfloxacin. Wang *et al.* (2009) reported 5times higher lipid peroxidation in gills of fluoroquinolone enrofloxacin-treated fish reared at low (40 fish m^{-3}) and high (120 fish m^{-3}) densities on day 7 of exposure compared to their respective controls. In contrast, they found that lipid peroxidation in the gills of enrofloxacin-treated fish reared at medium density (80 fish m^{-3}) was 3-times lower than in the gills of control fish.

CONCLUSIONS

The results of our study indicate that subchronic exposure to norfloxacin affects somatic indices (i.e. somatic development, body length) and oxidative stress parameters and antioxidants activity (i.e. GST, GPx, GR, and TBARS) in early stages of common carp (*Cyprinus carpio* L.). At the end of the test total body length in the highest tested concentration was significantly higher ($p<0.01$) and a significant stimulation ($p<0.05$) of development in each group exposed to norfloxacin compared to the control group was detected. As for the oxidative stress parameters (GST, GPx, and TBARS), significant shifts were found mostly in higher norfloxacin concentrations. In contrast, a significant increase in the activity of GR was found in the lowest (environmental) concentration.

ACKNOWLEDGEMENTS

This research was financially supported by IGA VFU 3/2014/FVHE and the project "Pharmaco-toxicological evaluation of newly synthesized (isolated) compounds as an integration tool for pre-clinical disciplines at VFU Brno" (CZ.1.07/2.3.00/30.0053). The authors would like to thank Mr. Matthew Nicholls for English proofreading.

REFERENCES

1 Aebi H (1984). Catalase in vitro. Methods Enzymol. **105**: 121–126.
2 Alghasham AA, Nahata M (1999). Trovafloxacin: a new fluoroquinolone. Ann Pharmacother. **33**: 48–60.
3 Bartoskova M, Dobsikova R, Stancova V, Pana O, Zivna D, Plhalova L, *et al.* (2014). Norfloxacin – Toxicity for zebrafish (*Danio rerio*) focused on oxidative stress parameters. Biomed Res Int. **2014:** 6 pages, DOI:10.1155/2014/560235
4 Carlberg I, Mannervik, B (1975). Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem. **250**: 5475–5480.
5 Chance B, Sies H, Boveris A (1979). Hydroperoxide metabolism in mammalian organs. Physiol Rev. **59**: 527–605.
6 Di Giulio RT, Meyer JN (2008). Reactive oxygen species and oxidative stress. In: Di Giulio RT, Hinton EE, editors. The Toxicology of Fishes. Boca Raton: CRC Press, Taylor and Francis Group. p. 273–324.
7 Fitton A (1992). The quinolones. An overview of their pharmacology. Clin Pharmacokinet. **22**: 1–11.
8 Golet EM, Alder AC, Giger W (2002). Environmental exposure and risk assessment of fluoroquinolone antibacterial agents in wastewater and river water of the Glatt valley watershed, Switzerland. Environ Sci Technol. **36**: 3645–3651.
9 Gros M, Petrovic M, Barcelo D (2007). Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (northeast Spain). Environ Toxicol Chem. **26**: 1553–1562.
10 Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. First enzymatic step in mercapturic acid formation. J Biol Chem. **249**: 7130–7139.
11 Hawkey PM (2003). Mechanisms of quinolone action and microbial response. J Antimicrob Chemother. **51**: 29–35.
12 Hodgson E (2004). A Textbook of Modern Toxicology. John Wiley & Sons, Hoboken, New Jersey.
13 Khetan SK, Collins TJ (2007). Human pharmaceuticals in the aquatic environment: a challenge to green chemistry. Chem Rev. **107**: 2319–2364.
14 King DE, Malone R, Lilley SH (2000). New classification and update on the quinolone antibiotics. Am Fam Physician. **61**: 2741–2748.
15 Klavarioti M, Mantzavinos D, Kassinos D (2009). Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. Environ Int. **35**: 402–417.
16 Kocour M, Gela D, Rodina M, Linhart O (2005). Testing of performance in common carp *Cyprinus carpio* L. under pond husbandry conditions I: top crossing with Northern mirror carp. Aquac Res. **36**: 1207–1215.
17 Lindberg RH, Björklund K, Rendhal P, Johansson MI, Tysklind M, Andersson BAV (2007). Environmental risk assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. Water Res. **41**: 613–619.
18 Lushchak VI (2011). Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol. **101**: 13–30.
19 Lushchak VI, Bagnyukova TV, Lushchak OV, Storey JM, Storey KB (2005). Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. Int J Biochem Cell Biol. **37**: 1319–1330.
20 Martinez M, McDermott P, Walker R (2006). Pharmacology of the fluoroquinolones: A perspective for the use in domestic animals. Vet J. **172**: 10–28.
21 OECD Guidelines for testing of chemicals, No. 210: Fish, early-life stage toxicity test. OECD, Paris, 2013.
22 Penaz M, Prokes M, Kouril J, Hamackova J (1983). Early development of the carp, *Cyprinus carpio*. Acta Sci Nat Brno. **17**: 1–39.
23 Plhalova L, Zivna D, Bartoskova M, Blahova J, Sevcikova M, Skoric M, *et al.* (2014). The effects of subchronic exposure to ciprofloxacin on zebrafish (*Danio rerio*). Neuroendocrinol Lett. **35**: 64–70.
24 Robinson AA, Belden JB, Lydy MJ (2005). Toxicity of fluoroquinolone antibiotics to aquatic organisms. Environ Toxicol Chem. **24**: 423–430.

25 Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, *et al.* (1985). Measurement of protein using bicinchoninic acid. Anal Biochem. **150**: 76–85.
26 Stepanova S, Praskova E, Chromcova L, Plhalova L, Prokes M, Blahova J, *et al.* (2013). The effects of diclofenac on early life stages of common carp (*Cyprinus carpio*). Environ Toxicol Pharmacol. **35**: 454–460.
27 Storey KB (1996). Oxidative stress: animal adaptations in nature. Brazilian J Med Biol Res. **29**: 1715–1733.
28 Tappel AL (1984). Selenium-glutathione peroxidase: properties and synthesis. Curr Top Cell Regul. **24**: 87–97.
29 Turnidge J (1999). Pharmacokinetics and pharmacodynamics of fluoroquinolones. Drugs. **58**: 29–36.
30 Wang N, Nkejabega N, Hien NN, Huynh TT, Silvestre F, Phuong NT, *et al.* (2009). Adverse effects of enrofloxacin when associated with environmental stress in Tra catfish (*Pangasianodon hypophthalmus*). Chemosphere. **77**: 1577–1584.
31 Zivna D, Plhalova L, Praskova E, Stepanova S, Siroka Z, Sevcikova M, *et al.* (2013). Oxidative stress parameters in fish after subchronic exposure to acetylsalicylic acid. Neuroendocrinol Lett. **34**: 116–122.