Assessment of gentamicin effect on oxidoreductive balance and microstructure of trunk kidney in Prussian carp 
(*Carassius gibelio*)

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Abstract: Assessment of gentamicin effect on oxidoreductive balance and microstructure of trunk kidney in Prussian carp (*Carassius gibelio*). The aim of this study was to investigate the potential toxic effects of gentamicin on the Prussian carp (*Carassius gibelio*) by determining its effect on oxidant-antioxidant balance and by histological image analysis of trunk kidney. The fishes were injected with single standard therapeutic dose of gentamicin of 5 mg·kg⁻¹. The samples of trunk kidney were collected in 3 days post antibiotic administration. Concentration of reduced glutathione (GSH) and activity of enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were examined. The concentration of GSH and the activity of studied enzymes did not show statistically significant differences between control and gentamicin-exposed group. The pathological changes were not observed in the trunk kidney structure. Renal tubules as well as renal corpuscles had correct structure. The obtained results indicate that a single injection of gentamycin at a dose of 5 mg·kg⁻¹ does not lead to disturbance of oxidant-antioxidant balance or histopathological changes in the trunk kidney of Prussian carp. Gentamicin administration did not change oxidoreductive balance and trunk kidney microstructure in Prussian carp (*Carassius gibelio*).

Key words: antibiotic, oxidative stress, histopathology, toxicity

INTRODUCTION

Gentamicin is an aminoglycoside antibiotic. It is a product of *Micromonospora* actinomycetes and exhibits activity against aerobic bacteria, in particular Gram negative bacilli. Gentamicin mechanism of action is typical for aminoglycosides – inhibiting bacterial protein synthesis (Bakker 1992). Gentamicin is used to treat respiratory infections in animals (Lappin et al. 2017), inflammation of hair follicles (Hillier et al. 2014), chronic foreign body associated sternal osteomyelitis (Wainberg et al. 2015). Nephrotoxicity is a primary negative effect of gentamicin on animals. The application of gentamicin causes reduction in kidney blood flow...
and glomerular filtration with increase of vascular resistance. Such effects may occur without permanent morphological changes in the glomerulus and are independent from tubular damage. Main mechanism of gentamicin nephrotoxic action is tubular cytotoxicity. Tubular damage is expressed through necrosis of tubular epithelial cells and modification of function of main cellular components involved in transport of water and solutes (Randjelovic et al. 2017). Therefore, the treatment of animals with gentamicin is associated with necrosis (Edwards et al. 2007) as well as apoptosis of tubular epithelial cells (Li et al. 2009). High doses of gentamicin cause mild glomerular enlargement and changes in circular shape and density with neutrophil infiltration (Stojiljkovic et al. 2008). Despite the relatively widespread use of gentamicin in veterinary medicine, there are few studies in the scientific literature on the toxic effects of this antibiotic on animal organisms. However, Spangler et al. (1980) showed the neurotoxic effect of gentamicin in dogs. Similar effect was also reported in the case of rats (Pramila Padmini and Vijay Kumar 2012). The nephrotoxicity of gentamicin was found in fish such as Nile tilapia (*Oreochromis niloticus*) (Augusto et al. 1996) and oyster toadfish (*Opsanus tau*) (Reimschuessel et al. 1996). It was shown that in response to gentamicin, larval zebrafish (*Danio rerio*) develop renal failure with the typical features observed in higher organisms (Hentschel et al. 2005). Gentamicin is also ototoxic to hair cells in the lateral line system of Mexican blind cave fish (*Astyanax fasciatus*) and zebrafish (*Danio rerio*) (Van Trump et al. 2010). The use of this antibiotic in aquaculture is one of the pathways responsible for releasing it into the aquatic environment (Iliev et al. 2015). Numerous organic substances polluting the aquatic environment can cause chemical stress in fish, which is expressed, among others, as disturbance of the oxidation-reduction balance (Dorval et al. 2003, Isik and Celik 2008, Slaninova et al. 2009, Asifa and Chitra 2017). A similar effect was showed in the case of many pharmacological agents, such as ibuprofen (Bartoskova et al. 2013), captopril (Cortes-Diaz et al. 2017) and antibiotic oxytetracycline (Yonar et al. 2011). There are few studies in the literature, on the influence of pharmaceuticals on the histological structure of internal organs of fish. Reda et al. (2013) showed histopathological changes in the kidneys and liver tissue of Nile tilapia (*Oreochromis niloticus*) caused by the antibiotics: oxytetracycline and florfenicol. Studies on the effect of oxytetracycline on carp (*Cyprinus carpio*) have shown that this substance leads to histopathological changes in spleen and kidney tissues (Svobodová et al. 2006). Literature data on the toxic effects of gentamicin on the fish organism are insufficient. A better understanding of its impact on fish is important in terms of the safety of using this antibiotic in veterinary practice, as well as in terms of learning the effects of contamination of the aquatic environment with pharmaceuticals. The aim of this study was to determine the effects of gentamicin used at standard therapeutic dose on oxidative stress parameters and trunk kidney microstructure in Prussian carp (*Carassius gibelio*).
MATERIAL AND METHODS

Animals and experimental design

The study was approved by the II Local Ethic Commission in Krakow (permission No. 129/2018). The Prussian carp (Carassius gibelio) obtained from Experimental Fishery Station of University of Agriculture in Krakow (Poland) were used in this study. The fish (52 individuals) of body length 18–22 cm were divided into 2 equinumerous groups (experimental and control) and kept in 700 l plastic tanks. The water parameters were: pH 7.23 ±0.08; NH₃ 0.01 ±0.01 mg·l⁻¹; NO₂ 0.02 ±0.01 mg·l⁻¹ and total hardness 17.5 ±0.84 °n. After 1 week of acclimation to the laboratory conditions fish were injected with gentamicin (experimental group) or sterile deionized water (control group). Gentamicin (Sigma Aldrich) was administrated at a standard therapeutic dose of 5 mg·kg⁻¹ as a sterile solution in deionized water. Scraps of trunk kidney were collected after 3 days post administration for biochemical and histological analyses.

Biochemical analyses

Scraps of trunk kidney were taken from 20 experimental and 20 control fish for analyses of reduced glutathione (GSH) concentration and activity of enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

Concentration GSH was determined according to Ellman (1959) method. The homogenates were prepared in an ice-cold solution containing 1.15% KCl and 0.01 M sodium phosphate buffer (pH = 7.4). The supernatants were prepared by centrifugation at 14,000 rpm for 5 min at 4°C. The GSH measurements were performed at λ = 412 nm using Tecan Sunrise microplate reader in deproteinized supernatant, which was due to the reaction between the thiol groups of GSH and 5,50-dithiobis-(2-nitrobenzoic acid) (DTNB).

For the analyses of enzyme activity, the tissues were homogenized in ice-cold potassium phosphate buffer (100 mM, pH = 7.0). The activities of GPx and SOD were determined using the MARCEL 330 spectrophotometer. GPx activity was determined using the procedure described by Lück (1962). The absorbance was measured at λ = 340 nm. The measurements were based on the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), with the concomitant oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH).

Activity of SOD was analysed according to the method described by Kono (1978). The assay is based on the inhibiting influence of SOD on the reduction of cytochrome C by the superoxide anion, which is generated by xanthinexanthine oxidase.

Activity of CAT measurements were conducted with an electrochemical method using Clark’s oxygen electrode at pH = 7.0 and 25°C according to the method described by Formicki and Stawarz (2006). The total protein concentrations were determined with Bradford’s method (1976) using a microplate reader at λ = 595 nm.

Histological analyses

Scraps of trunk kidney were taken from 6 experimental and 6 control fish for histological analyses. The collected tissue was embedded in paraffin wax. Paraffin blocks of trunk kidney were cut to 6 μm
thick sections and stretched. Microtome sections were deparaffinized in xylene and hydrated by passing through graded alcohols. After deparaffinization, sections were stained with Haematoxylin–Eosine and observed under light microscopy. The microstructures of the tissue were examined in the randomly selected 10 sections from each fish. The analyses were conducted using Nikon Eclipse Ci microscope.

Statistical analyses
The Shapiro-Wilk’s test was used to test the normality of distribution. The statistical verification of differences in GSH concentration as well as differences in activity of tested enzymes between control and experimental groups were performed by the Student’s t-test for unpaired data. Statistical analyses were carried out at the significance level of 0.05. The analyses of basic statistics were also performed. The results were statistically analysed using procedures of the PQStat software.

RESULTS
Biochemical analyses
Statistical analysis indicated the normal distribution of results in case of all measured parameters, both in the experimental and the control groups. The statistical verification of GSH concentration did not show significant differences between experimental and control group. The mean values were similar in both groups (Table). Also the results of all measured enzymes activities did not show statistically significant differences between the control and the experimental groups (Table).

Histological analyses
Histopathological changes were not observed in the trunk kidney of both control and experimental fish. The structural details of the kidney, glomeruli and renal tubules, showed normal appearance in each group of fish (Fig.).

DISCUSSION
The discovery of antibiotics changed the treatment of infectious diseases, leading to a significant reduction in morbidity and mortality of humans and animals. The effective medication to which pathogenic microorganisms are sensitive is required for treatment of infectious diseases in fish. The widespread use of pharmaceuticals in aquaculture poses a risk of contamination of the aquatic

<table>
<thead>
<tr>
<th>Tested parameter</th>
<th>Control group (mean ±SD)</th>
<th>Experimental group (mean ±SD)</th>
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<tr>
<td>GSH [μmol·mg protein⁻¹]</td>
<td>2.32 (±1.18)</td>
<td>1.74 (±0.74)</td>
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<tr>
<td>SOD [U·mg⁻¹]</td>
<td>8.06 (±2.75)</td>
<td>9.57 (±3.01)</td>
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<tr>
<td>GPx [U·mg⁻¹]</td>
<td>0.38 (±0.23)</td>
<td>0.35 (±0.23)</td>
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<tr>
<td>CAT [U·mg⁻¹]</td>
<td>12.17 (±1.31)</td>
<td>11.98 (±1.27)</td>
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environment. The high load of antibiotics has been found in sediments. The detected concentrations were enough to inhibit the growth of bacteria (Küm-merer 2008). The research conducted by Wollenberger et al. (2000) showed that antibiotics often have a low biodegradability. Another important issue is the subject of the toxicity of antibiotics to target and non-target organisms. Antibiotics can have a wide variety of damaging effects on animals including direct toxicity, adverse interactions with other drugs, tissue necrosis, impairment of the animal immune or defence mechanisms and damage to foetal or neonatal tissues (Guardabassi and Kruse 2008). In case of fish, antibiotics may have direct effects on their organisms, including toxicity, immune suppression, alterations in normal bacterial flora of digestive tract, and on growth (Reimschuessel et al. 1996, Gelsleichter et al. 1998, Tafalla et al. 1999, Navarrete et al. 2008). One of the significant indicators of the toxicity of chemical compounds is their effect on the histological state of internal organs, including kidney. Kidney of fish receives the largest proportion of postbranchial blood, and therefore renal lesions might be a good indicator of environmental pollution (Cengiz 2006). In the present

FIGURE. Normal structure of control (A) and gentamicin-treated (B) Prussian carp (Carassius gibelio) renal tubules; normal glomerulus of gentamicin-treated (B) fish; RT – renal tubule, G – glomerulus (HE staining, 400×).
study, histopathological changes in *Carassius gibelio* trunk kidney were not found. This may indicate lack of toxicity of a single administration of gentamycin at the dose of 5 mg·kg$^{-1}$ to Prussian carp. However, according to Augusto et al. (1996) exposure of Nile tilapia *Oreochromis niloticus* to gentamicin induced acute tubular necrosis that peaked in severity at 2 days following injection of 25 mg·kg$^{-1}$ and at 4 to 7 days following injection of 5 mg·kg$^{-1}$. Necrosis following higher dose exposures was more severe than that following a low dose of tested antibiotics. Reimschuessel et al. (1996) evaluated the nephro-toxic effects of gentamicin in kidneys of toadfish (*Opsanus tau*). Gentamicin was administered at doses of 2.5, 3.5, 5, 15, and 50 mg·kg$^{-1}$. The researchers noticed extensive necrosis in the proximal tubules in each fish injected with gentamicin. In 28 days after antibiotic injection, sections of kidney that were examined were essentially devoid of proximal tubules. Research conducted by Hentschel et al. (2005) demonstrated a decline in glomerular filtration rate after exposure of larval zebrafish (*Danio rerio*) to gentamicin. Gentamicin was injected into the cardiac venous sinus of zebrafish embryos and histological analysis at 96 h post fertilization showed lysosomal phospholipidosis, flattening of the brush border, accumulation of debris in the tubular lumen, as well as tubular and glomerular distention (Hentschel et al. 2005). Chemical stress may be also expressed by disturbing the oxidoreductive balance of the body. In the studies of oxidative stress in animals, the enzymatic indices, such as GPx, SOD and CAT activity, and non-enzymatic ones, especially the concentration of GSH, are usually evaluated. CAT and GPx catalyze the transformation of hydrogen peroxide into neutral substances: water and oxygen (Fantel 1996, Andreoli 2002). SOD is a first-line antioxidant and loss of its activity undoubtedly potentiates tissue injury through increased formation of reactive oxygen species (Comhair et al. 2000). The most important non-enzymatic antioxidant is reduced glutathione (GSH), which cooperates with enzymatic antioxidants to neutralize hydrogen peroxide and protects the thiol groups of active enzyme sites (Meister 1998). It is considered that oxidative stress has a central role in gentamicin induced nephrotoxicity. However, there is no available data on whether gentamicin can cause oxidative stress in fish. Our results indicate that a single administration of gentamicin at the dose of 5 mg·kg$^{-1}$ did not lead to oxidation-reduction imbalance in Prussian carp (*Carassius gibelio*) trunk kidney. However, gentamicin can change the oxidation-reduction balance in other animals. In treated rats renal hydrogen peroxide generation was increased (Guidet and Shah 1989) and reduced glutathione concentrations were decreased (Ali 2002, Sener et al. 2002). The results of biochemical and histological analysis conducted by us did not show toxic effects of gentamicin in Prussian carp. Nevertheless, the results obtained by other authors do not allow to make unambiguous conclusions on the toxicity of this antibiotic to fish. Further research that will take into account the influence of factors such as fish species and the amount of antibiotic dose, which probably have an influence on possible toxic effects, is needed.
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Streszczenie. Ocena wpływu gentamycyny na równowagę oxydoredukcyjną i mikrostrukturę nerki tułowiowej karasia srebrzystego (Carassius gibelio). Celem pracy było zabranie potencjalnego toksycznego oddziaływania gentamycyny na organizm karasia srebrzystego (Carassius gibelio) poprzez określenie jej wpływu na równowagę oksydoredukcyjną oraz analizę histologiczną nerki tułowiowej. Do badań użyto karasi srebrzych, którym podano jednorazowo standardową dawkę terapeutyczną gentamycyny (5 mg·kg⁻¹). Skrawki nerki tułowiowej pobrano po 3 dniach od iniekcji antybiotyku. Następnie określono stężenie glutattonu zredukowanego (GSH) i aktywność enzymów: dysmutazy ponadtlenkowej (SOD), peroksydazy glutattonowej (GPx) i katalazy (CAT) oraz wykonano preparaty histologiczne. Stężenie GSH i aktywność badanych enzymów nie różniły się istotnie pomiędzy grupą kontrolną a grupą ekspонowaną na gentamycynę. Nie zaobserwowano również zmian patologicznych w nercie tułowiowej. Kanaliki nerkowe oraz ciałka nerkowe odznaczały się prawidłową strukturą. Uzyskane wyniki pokazują, że jednorazowa iniekcja gentamycyny w dawce 5 mg·kg⁻¹ nie prowadzi do zaburzeń równowagi oksydoredukcyjnej ani powstania zmian histopatologicznych w nercie tułowiowej karasia srebrzystego (Carassius gibelio).

Słowa kluczowe: antybiotyk, stres oksydacyjny, histopatologia, toksyczność

MS received 23.01.2019
MS accepted 8.04.2019

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