ORIGINAL ARTICLE

Evaluation of the toxic effect of peracetic acid on grass carp (*Ctenopharyngodon idella*) juveniles

Latifeh Chupani, Alzbeta Stara, Josef Velisek, Eliska Zuskova

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic

Correspondence to: MVDr. Eliška Zusková, PhD.
Faculty of Fischeries and Protection of Waters
University of South Bohemia in České Budějovice
Laboratory of Aquatic Toxicology and Ichthyopathology
Zatisi 728/II, 389 25 Vodnany, Czech Republic.
TEL: +420 387 774 623; E-MAIL: zuskova@frov.jcu.cz

Submitted: 2014-09-23 *Accepted: 2014-11-08* *Published online: 2014-11-30*

Key words: **antioxidant system; histopathology; haematological parameters; biochemical parameters; Persteril; micronucleus frequency; fish**

Neuroendocrinol Lett 2014; **35**(Suppl. 2):86–92 **PMID:** 25638371 NEL351014A09 © 2014 Neuroendocrinology Letters • **www.nel.edu**

Abstract

OBJECTIVES: The aim of present study was to evaluate the effect of peracetic acid (PAA) on haematotological and biochemical indices, antioxidant status, micronucleus induction and histopathological alterations of liver and gill in grass carp.
METHODS: Grass carp (*Ctenopharyngodon idella*) juveniles were exposed to therapeutic concentrations (1, and 3 $mg.l^{-1}$) of PAA for a period of 10 days. Selected haematotological indices – the erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and leukocyte count (WBC), and biochemical indices – glucose (Glu), total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), and lactate dehydrogenase (LDH) were evaluated in plasma. Activity of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR), as well as levels of thiobarbituric acid reactive substances (TBARS) were assessed in gill and liver. Micronucleus frequency in peripheral erythrocytes was counted in control and experimental fish. Histological examinations of gill and liver were performed.
RESULTS: No significant differences were found in haematological parameters measured. Statistically significant ($p<0.05$) alterations in the activities of AST, CK and LDH were found in treated fish compared to control groups. Fish exposed to 1 $mg.l^{-1}$ of PAA showed significantly lower ($p<0.05$) SOD activity in liver and gill while catalase activity indicated a significant decrease ($p<0.05$) only in gill tissue. Other significant changes were observed in GR activity in gill in both PAA exposed groups, while GR activity in liver remained unchanged. There was no significant difference in the count of micronuclei between control and exposed fish. Haemorrhage, fusion of primary lamellae, degeneration of secondary lamellae, some clubbing on primary and secondary lamellae tips, and lifting of epithelial cells were found in gill tissues in both control and treated fish.
CONCLUSION: The results show that PAA could induce alterations in biochemical parameters in blood plasma, antioxidant enzymes response and histopathological changes in gill; however, it seems that these changes are reversible. Subsequently, lower concentration (1 $mg.l^{-1}$) is useable as a treatment concentration for grass carp.

Abbreviations:

ALT - alanine aminotransferase
AST - aspartate aminotransferase
BCA - bicinchoninic acid
CAT - catalase
CK - creatine kinase
Glu - glucose
GPx - glutathione peroxidase
GR - glutathione reductase
GSH - glutathione
GSSG - oxidized glutathione
LDH - lactate dehydrogenase
MN - micronucleus
NBT - nitro blue tetrazolium
PAA - peracetic acid
PMS - phenazine methosulfate
SOD - superoxide dismutase
TBA - thiobarbituric acid
TBARSs - thiobarbituric acid reactive substances
TP - total protein

INTRODUCTION

Peracetic acid (PAA) is a strong oxidant agent which has been known for its germicidal properties for a long time (Duong 2005). It has been widely used as a disinfectant in laboratories, the food, beverage, medical and pharmaceutical industries as well as for the treatment of municipal waste water to inactivate many pathogenic and indicator microbes (Kitis 2004). PAA have been recently approved for the use in aquaculture as a sanitizer (Straus *et al.* 2012a; Kouba *et al.* 2012) which has drawn a high attention as an alternative biocide for malachite green and formaldehyde in aquaculture. The use of malachite green for food fish has been forbidden in Europe since 2000 by the reason of its accumulation in exposed fish (Sudova *et al.* 2007). The application of formaldehyde has been recommended to be limited as regards workers' safety and possible harmful effects on water body (Pedersen *et al.* 2013). The most desirable attributes for PAA include its wide spectrum of antimicrobial activity, relative stability in environments containing low organic matter, harmless by-products, neutral residuals, easy-to-use in water bodies (Pedersen *et al.* 2009). PAA products are commercially available in the form a quaternary equilibrium aqueous solution containing PAA, hydrogen peroxide (HP), acetic acid, and water (Falsanisi *et al.* 2006). Although there is no comprehensive data about the distinction activity of PAA, two feasible mechanisms are hypothesized. First one is the production of active oxygen (Lefevre *et al.* 1992; Liberti *et al.* 1999) and hydroxyl radicals (-OH) (Lubello *et al.* 2002) which would lead to disruption of sulphydril (-SH) and sulphur (-S-S) bonds in the cell membrane proteins (Reichert & Young 1997) and the second one is the disruption of the chemiosmotic function of lipoprotein cytoplasmic membrane, denaturation of microbial macromolecules and metabolites (Gómez-López 2012).

In recent years, some data has been published about PAA treatment against fish ectoparasite *Ichthyophthirius multifiliis* (Straus & Meinelt 2009; Meinelt *et al.* 2009; Sudová *et al.* 2010; Meinelt *et al.* 2007), its use in the control of fungal infections of channel fish (Straus *et al.* 2012a) and of crayfish plague (Jussila *et al.* 2011). The results of these studies have shown promising perspectives of PAA as a disinfectant useful in fish cultures. However, there is a paucity of information on the toxic impacts potential in terms of PAA application on fish (Straus *et al.* 2012b). It identifies the necessity of toxicity data on different fish species for safe use of PAA in aquaculture.

Haematological and blood biochemical parameters have been considered as valuable indicators for the assessment of physiological and pathological alterations in fish health statue through toxicological research (Saravanan *et al.* 2011). Furthermore, fish antioxidant system factors, either enzymatic or non-enzymatic component with histopathological alteration have been approved as sensitive indices for the evaluation of fish health condition (Li *et al.* 2010). Erythrocyte micronucleus frequency, which was originally adapted for mammalian species, has been proposed as a useful tool for estimating of cytogenetic damages in fish under laboratory and field conditions (Udroiu 2006).

Given the narrow available information on consequences of PAA treatment in fish, we designed the recent study to assess possible changes in health status of grass carp juveniles (*Ctenopharyngodon idella)* exposed to PAA through the analysis of the alterations in haematological and biochemical parameters as well as the alterations in antioxidant system, micronuclei induction in peripheral erythrocytes and histopathological changes.

MATERIAL & METHODS

Fish and water parameters

Grass carp (mean weight ± SD, 72.3±14.0 g) were obtained from a local fish hatchery and maintained for 2 weeks in aquaria with dechlorinated tap water. Fish were fed a commercial diet (BioMar, 47%protein, 26% fat) every day, at 2.5% of total body weight. Water quality parameters were: temperature 16.3–18.2 °C; dissolved oxygen >69%; pH 7.2–7.9; ANC4.5 (acid neutralisation capacity) 1.2 mmol.l^{-1}; COD_{Mn} (chemical oxygen demand) 1.5 mg.l^{-1}; total ammonia 0.04 mg.l^{-1}; sum of Ca^{2+}+Mg^{2+} 14 mg.l^{-1}; Cl^{-} 11 mg.l^{-1}; PO_4^{3-} 0.01 mg.l^{-1}.

Experimental protocol

The 10-day test was carried out using duplicate groups of 7 fish held in six 150-l tanks. Grass carp juveniles were subjected to 0 (C, control group), 1 mg.l^{-1} (E1, experimental group) and 3 mg.l^{-1} (E2, experimental group) of PAA for ten days. PAA concentrations (1 and 3 mg.l^{-1}) were obtained by addition of Persteril 36 (Eurosarm) two-times a day (at 8:30 a.m. and 4:30

p.m.) to the waterThe desired concentration of PAA was first carefully diluted with enough water from the target aquarium and then was applied into the rest of water volume with fish to ensure even distribution of the active ingredient. The test baths were constantly aerated and renewed daily before the first application of PAA. The range of examination consisted of haematological, biochemical and, histopathological analyses, the evaluation of antioxidant status and counting of micronuclei in erythrocytes.

Fish sampling and haematological examination

The blood samples were taken by puncturing the caudal vessel with heparinised syringes and the blood was stabilised by 40 IU of sodium heparin (Heparin inj., Leciva, Czech Republic) per 1 ml blood. Immediately after sampling, erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and leukocyte count (WBC) were determined in the blood samples according to *Svobodova et al. (1991).*

Biochemical examination

Blood plasma was separated by centrifugation (10 min at 12 000 × *g*) at 4 °C and then glucose (Glu), total proteins (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatine kinase (CK) were determined using VETTEST 8008 Analyser (IDEXX Laboratories, Westbrook, ME, USA). The analyser works on the basis of dry chemistry and colorimetric analysis. The analyses were carried out using testing slides (Multi-layer film slides, Kodak).

Biomarkers of oxidative stress and antioxidant parameters

Livers and gill samples were taken, quickly frozen and stored in −80 °C until analysis. For the determination of catalase (CAT) and superoxide dismutase (SOD) activities, frozen samples were homogenized in 50 mM potassium phosphate buffer (KH_2PO_4 with 1 mM EDTA, pH 7.4), centrifuged at 30 000 × *g* for 30 minutes, and supernatants were taken. Total SOD activity was assessed according to the method based on the inhibition of nitro blue tetrazolium (NBT) and production of superoxide anions by NADH and phenazine methosulfate (PMS). SOD activity was measured spectrophotometrically at 560 nm and reported as the nmol of NBT per min per mg protein (Ewing & Janero 1995). CAT activity was determined regarding to the decomposition rate of hydrogen peroxide by spectrophotometric method at 240 nm (Abei 1984). Glutathione reductase (GR) activity was evaluated spectrophotometrically by the rate of NADPH oxidation at 340 nm (Carlberg & Mannervik 1975). In order to determine GR and lipid peroxidation rate, the tissues were homogenized in phosphate saline buffer at pH 7.2. The level of TBARS as an index of lipid peroxidation was calculated spectrophotometrically at 532 nm according to the method of TBA (thiobarbituric acid) assay with some slight modifications (Uchiyama & Mihara 1978).

Total protein concentration in various samples was calculated by bicinchoninic acid (BCA) method using bovine serum albumin as a standard (Bradford 1976).

Micronucleus test

Peripheral blood samples were taken from caudal vein by heparinized syringe and smeared on microscope glasses. The blood smears were fixed in absolute ethanol for 20 minutes, air-dried and stained by 10% Giemsa solution for 25 minutes. Two slides were prepared for each fish and 1000 erythrocyte cells per slides were analyzed under light microscope (1 000×). Micronuclei were identified according to Al-Sabti and Metcalfe (1995) as non-reflective, small ovoid or circular chromatin bodies, displaying the same staining properties and with a diameter 1/3–1/20 of the nucleus.

Histopathology

Liver and gill from each fish were fixed in 10% neutral buffered formalin. The fixed tissue samples were dehydrated through a series of graded ethanol, cleared in xylene, and embedded in paraffin. Sections were cut using a rotary microtome at 3–4 μm, stained with haematoxylin and eosin (H&E) and observed under light microscope.

Statistical analysis

The statistical analysis was performed using the STATISTICA (version 8.1 for Windows, StatSoft). Data was expressed as means ± SD and *p*-value less than 0.05 was considered as statistically significant. Kolmogorov-Smirnov and Bartlett's tests were conducted to evaluate normality and homoscedasticity of variance respectively. One-way ANOVA was applied in the case of data normal distribution. If the data showed a "non-normal" distribution, a non-parametric test (Kruskal-Wallis) was used.

RESULTS

Mortality

No mortality occurred in control and E1 groups. In contrast, high mortality rate (71.5%) was observed in fish exposed to 3 $mg.l^{-1}$ PAA (E2).

Haematological and biochemical indices

Haematological and biochemical values are shown in Table 1 and 2. There are no significant differences in haematological parameters between PAA exposed groups (E1 and E2) and control. On the other hand, the results of biochemical values show a significant increase in AST, CK and LDH activities in E1 group compared with control.

Copyright © 2014 Neuroendocrinology Letters ISSN 0172–780X • www.nel.edu

Biomarkers of oxidative stress and antioxidant parameters

No significant differences were observed in the levels of TBARS in liver and gill tissues between control and exposed groups. SOD activity in liver and gill tissue was significantly lower ($p<0.05$) in treated fish (E1, E2). There was a significant decrease in CAT activity of gill in both groups of E1 and E2 compared with control, whereas in liver significant decrease was just observed in E group. On the other side, GR activity significantly increased in gill tissues of E1 and E2 groups compared with control (Tables 3 and 4).

Tab. 1. Haematological parameters.

Parameters	C n=14; mean±SD	E1 n=14; mean±SD	E2 n=4; mean±SD
RBC ($T.l^{-1}$)	2.20±0.13	2.32±0.51	1.95±0.08
WBC ($G.l^{-1}$)	42.56±7.45	37.94±7.15	29.25±4.75
PCV ($l.l^{-1}$)	0.31±0.03	0.32±0.02	0.30±0.01
Hb ($g.l^{-1}$)	66.99±9.18	65.56±7.22	63.02±8.24
MCV (fl)	142.02±13.41	142.10±30.32	155.34±5.09
MCH (pg)	29.63±4.34	28.84±7.55	32.20±2.90
MCHC ($l.l^{-1}$)	0.22±0.03	0.21±0.02	0.21±0.03

Tab. 2. Biochemical parameters.

Parameters	C n=14; mean±SD	E1 n=14; mean±SD	E2*
GLU ($mmol.l^{-1}$)	3.21±0.83[a]	2.87±0.42[a]	–
TP ($g.l^{-1}$)	29.00±4.50[a]	28.30±2.70[a]	–
AST ($U.l^{-1}$)	93.00±24.00[a]	158.30±30.27[b]	–
ALT ($U.l^{-1}$)	18.80±6.80[a]	23.50±8.00[a]	–
CK ($U.l^{-1}$)	576.30±101.70[a]	1 160.00±232.70[b]	–
LDH ($U.l^{-1}$)	1 780.00±821.00[a]	2 837.50±366.80[b]	–

* all samples missed for this group
Values with the different alphabets within each row are significantly different ($p<0.05$)

Micronucleus frequency

Micronucleus assay revealed no significant differences in E1 and E2 groups compared with control.

Histopathology

The liver of exposed fish (E1 and E2) showed normal physiological structure, while gill tissue revealed some pathological alterations in secondary and primary lamellae including haemorrhagiae, fusions, focal degeneration of secondary lamellae, clubbing of primary lamellae tips and lifting of epithelial cells (Figure 1). Furthermore, as side findings, there were observed undifferenced protozoan parasites in histological section of gill of control group.

DISCUSSION

Peracetic acid is a disinfectant, which was relatively recently introduced into aquaculture for fish treatment (Elia *et al.* 2006). Regarding the growing interest in the application of PAA as therapeutic agent, toxicity data on different fish species is needed for the safe application security.

Haematological and biochemical indices in peripheral blood provide a reliable index of health status in various organisms including fish (De Pedro *et al.* 2005). The activity of enzymes can be affected as a result of environmental stress, diseases and tissue damage (Kori-Siakpere *et al.* 2011). The present study indicated higher

Tab. 3. Lipid peroxidation and antioxidant enzymes activities in liver of grass carp.

Group	SOD (nmol NBT/min/mg protein) (mean±SD)	CAT (H_2O_2/min/mg protein) (mean±SD)	GR (nmol NADH/min/mgprotein) (mean±SD)	TBARS (nmol/mg protein) (mean±SD)
C (n=14)	0.1017±0.0332[a]	2.0956±0.0683[a]	0.2274±0.0927[a]	0.1808±0.0249[a]
E1 (n=14)	0.0706±0.0267[b]	2.1505±0.2668[a]	0.2292±0.0833[a]	0.2034±0.0333[a]
E2 (n=4)	0.0683±0.0054[b]	1.3102±0.1425[b]	0.2708±0.1177[a]	0.1670±0.0018[a]

Values with the different alphabets within each column are significantly different ($p<0.05$).

Tab. 4. Lipid peroxidation and antioxidant enzymes activities in gill of grass carp

Group	SOD (nmol NBT/min/mg protein) (mean±SD)	CAT (H_2O_2/min/mg protein) (mean±SD)	GR (nmol NADH/min/mgprotein) (mean±SD)	TBARS (nmol/mg protein) (mean±SD)
C (n=14)	0.1809±0.0896[a]	0.0585±0.0119[a]	0.2399±0.0683[a]	0.1140±0.0093[a]
E1 (n=14)	0.0080±0.0038[b]	0.0287±0.0072[b]	0.3535±0.0435[b]	0.1510±0.0218[a]
E2 (n=4)	0.0110±0.0060[b]	0.0392±0.0124[b]	0.2889±0.0292[b]	0.1145±0.0048[a]

Values with the different alphabets within each column are significantly different ($p<0.05$).

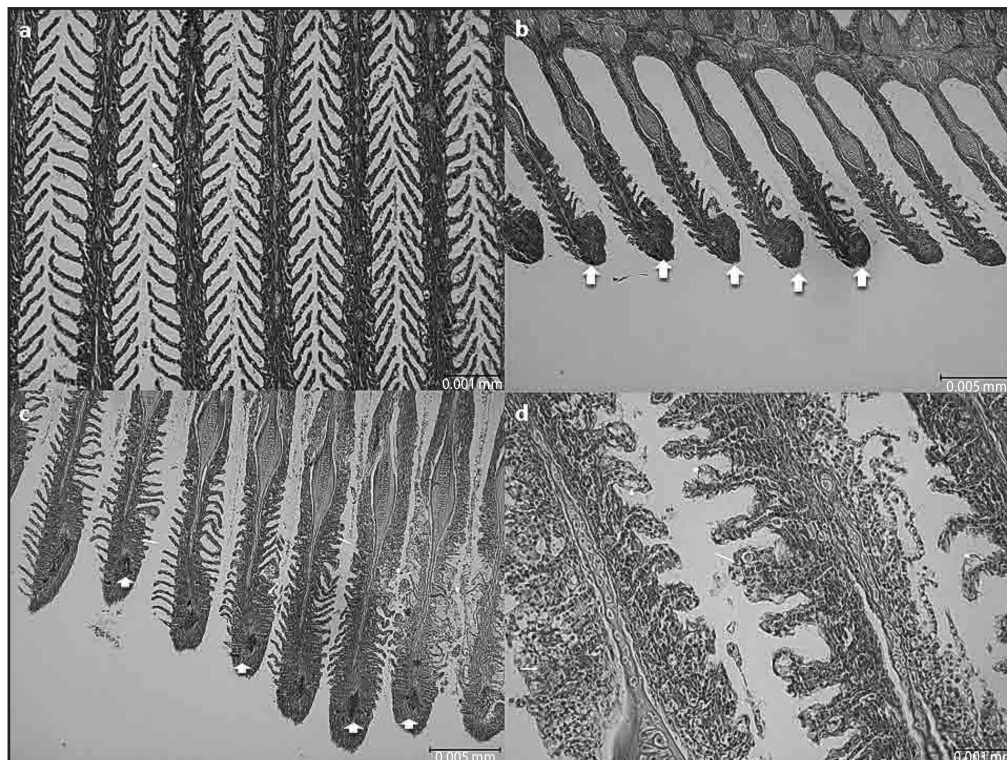

Fig. 1. Histological sections of gill of grass carp (H&E). a) Normal structure of gill; b–d) gill with histopathological alterations; b) club deformation of primary lamellae tips c) aneurysm (head arrows); fusion of some secondary lamellae (arrows); sever degeneration of the secondary lamellae (asterisks); d) hyperplasia in primary lamellae (arrows); lifting of epithelial cells (head arrows).

AST, LDH, and CK activities in fish exposed to PAA. The increase in the activities of the relevant enzymes is well-known as an indicator of physiological disorder due to stress (Velisek *et al.* 2006) reported under different contamination exposure and stress condition in fish (Banaee *et al.* 2014a; Agrahari *et al.* 2007; Almeida *et al.* 2002). Furthermore, AST is one of important enzymes involved in nitrogen metabolism, amino acid oxidation and liver gluconeogenesis. Increased levels of the aminotransferases can have a vital role in supplying energy the under the stress condition (Banaee *et al.* 2014b). LDH activity is generally associated with the reduction of pyruvate, the final product of glycolytic pathway, which allows keeping glycogenesis. This could be vital in the conditions that additional amount of energy required such as stress situation related to chemical exposure (Diamantino *et al.* 2001). The elevation of LDH may attribute to a shift from aerobic metabolism to anaerobic pathway in treated fish. CK, engaged in the regeneration of ATP by reversible transfer of phosphate from the phosphoryl group of phosphocreatine to ADP, play an essential role in providing required energy in the process of environmental adaptation (Kori-Siakpere *et al.* 2011). The reduction in SOD and CAT activities was observed in gill tissues of treated fish, whereas, GR activity was increased. PAA can produce free radicals (Booth & Lester 1995). GR is one of important enzymes engaged in the detoxification of reactive oxygen species through the reduction of oxidized glutathione (GSSG) to reduced form (GSH) (Chang *et al.* 1978). In general, high activity of GR is proposed as an indices of oxidative stress in living cells (Stageman *et al.* 1992). Increase in the activity of GR in treated fish could indicate an increment in the consumption of glutathione used by glutathione peroxidase (GPx), one of crucial enzymes involved in antioxidant system, to protect cells against oxidative stress. Weakening SOD and CAT activities observed in our study might be attributed to enhanced ROS production. The ROS overproduction could overwhelm the oxidative detoxification, resulting in decrease of antioxidant enzymes activities or inactivation of enzymes (Kono & Fridovich 1982). Furthermore, PAA causes the inactivation of catalase (Block 1983).

The data obtained for MN assay indicated that micronuclei frequency was not elevated by fish exposure to PAA. Our results are in line with the finding of exposure of common carp with PAA (Sapone *et al.* 2007) and could be a confirmation for non genotoxic effect of PAA as reported in other studies with other organisms (Maffei *et al.* 2005; Bolognesi *et al.* 2004).

The histopathological analysis of liver tissue in both control and treated fish did not show any alterations. Our data from histopathology corresponded to the constant level of ALT activity in plasma as already confirmed that ALT activity is more specific enzyme for the evaluation of liver damage than AST (Singh *et al.* 2011). Normal structure with slight variations in antioxidant enzyme activities may point out that liver is not a target organ for PAA exposure in fish.

As fish gills have an extensive surface area exposed to the external environment, they could be affected by

Copyright © 2014 Neuroendocrinology Letters ISSN 0172–780X • www.nel.edu

multiple factors in water, such as physical and chemical changes (Flores-Lopes & Thomaz 2011). There is rare published data on gill histopathological alterations due to PAA exposure of fish. The histopathological alterations including fusion, club-shape of primary lamellae tips, and lifting of epithelial cells observed in gill tissues during present study have been reported in several studies which have investigated the effects of hydrogen peroxide on fish (Tort *et al.* 2002; Rach *et al.* 1997). It is reported that the recovery of minor damage gill can begin relatively fast, but intensive damage caused by hydrogen peroxide can be recovered within weeks (Henriksen *et al.* 2014). The mechanism of action of PAA is thought to be similar to the mechanism of hydrogen peroxide (Finnegan *et al.* 2010) and since the main structure of gill revealed unchanged in most cases in our study, histological changes reported in our study may be repairable. The lesions observed in gill tissues in control group could be caused by protozoan parasites. Protozoan parasites constitute the most common parasites group encountered in fish (Omeji *et al.* 2011). Gill filament fusion, aneurism, filament hyperplasia, epithelial lifting were reported in fish infected by protozoan parasites (Khorramshahr 2012; Baticados *et al.* 1984; Singh & Kaur 2013). No parasites were found in gill of treated fish which might be due to biocide impact of PAA on protozoan parasites.

Although PAA sub-acute exposure (1 $mg.l^{-1}$) of grass carp can affect some enzymes of antioxidant system and induces histopathological changes in gill, it seems that these alterations are slight and can be recovered. The present results may propose that the concentration of 1 $mg.l^{-1}$ of PAA can be applied as a therapeutic concentration in grass carp.

ACKNOWLEDGMENTS

The study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic – projects „CENAKVA"(No. CZ.1.05/2.1.00/01.0024), "CENAKVA II" (No. LO1205 under the NPU I program), and by the Grant Agency of the University of South Bohemia in Ceske Budejovice (No. 018/2014/Z).

REFERENCES

1 Abei H (1984). Catalase in vitro. Method Enzymol **105**: 121–126.
2 Agrahari S, Pandey KC, Gopal K (2007). Biochemical alteration induced by monocrotophos in the blood plasma of fish (*Channa punctatus*) (Bloch). Pestic Biochem Phys **88**: 268–272.
3 Almeida J, Diniz Y, Marques S, Faine L, Ribas B, Burneiko R, Novelli E (2002). The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. Environ Int **27**: 673–679.
4 Al-Sabti K, Metcalfe CD (1995). Fish micronuclei for assessing genotoxicity in water. MUTAT RES-GEN TOX EN **343**: 121–135.
5 Banaee M, Haghi BN, Ibrahim ATA (2014a). Sub-lethal toxicity of chlorpyrifos on common carp, *Cyprinus carpio* (Linnaeus, 1758): Biochemical response. Intl J Aquat Biol **1**: 281–288.
6 Banaee M, Sureda A, Zohiery F, Hagi BN, Garanzini DS (2014b). Alterations in biochemical parameters of the freshwater fish, *Alburnus mossulensis*, exposed to sub-lethal concentrations of fenpropathrin. Intl J Aquat Biol **2:** 58–68.
7 Baticados MCL, Quinitio GF, Bulnheim H (1984). Occurrence and pathology of an Amyloodinium-like protozoan parasite on gills of grey mullet, *Mugil cephalus*. Helgol Meeresunters **37**: 595–601.
8 Block SS (2002). Peroxygen compounds. Chapter 9 in Disinfection, sterilization, and preservation. 5th Ed. Philadelphia: Lippincott Williams & Wilkins. p.185–204.
9 Bolognesi C, Buschini A, Branchi E, Carboni P, Furlini M, Martino A, Monteverde M, Poli P, *et al.* (2004). Comet and micronucleus assays in zebra mussel cells for genotoxicity assessment of surface drinking water treated with three different disinfectants. Sci Total Environ **333**: 127–136.
10 Booth R, Lester J (1995). The potential formation of halogenated by-products during peracetic acid treatment of final sewage effluent. Water Res **29**: 1793–1801.
11 Bradford MM (1976). Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein dye binding. Anal Biochem **72**: 248–254.
12 Carlberg I, Mannervik B (1975). Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem **250**: 5475–5480.
13 Chang J, Van Der Hoeven L, Haddox C (1978). Glutathione reductase in the red blood cells. Ann Clin Lab Sci **8**: 23–29.
14 De Pedro N, Guijarro AI, López-Patiño MA, Martínez-Álvarez R, Delgado MJ (2005). Daily and seasonal variations in haematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. Aquac Res **36**: 1185–1196.
15 Diamantino TC, Almeida E, Soares AM, Guilhermino L (2001). Lactate dehydrogenase activity as an effect criterion in toxicity tests with *Daphnia magna* Straus. Chemosphere **45**: 553–560.
16 Duong NTH (2005).The sanitizing efficiency of different disinfectants used in the fish industry. UNU – Fisheries Training Programme.
17 Elia AC, Anastasi V, Dörr AJM (2006). Hepatic antioxidant enzymes and total glutathione of *Cyprinus carpio* exposed to three disinfectants, chlorine dioxide, sodium hypochlorite and peracetic acid, for superficial water potabilization. Chemosphere **64**: 1633–1641.
18 Ewing JF, Janero DR (1995). Microplate superoxide dismutase assay employing a nonenzymatic superoxide generator. Anal Biochem **232**: 243–248.
19 Falsanisi D, Gehr R, Santoro D, Dell'erba A, Notarnicola M, Liberti L (2006). Kinetics of PAA demand and its implications on disinfection of wastewaters. Water Qual Res J Can **41**: 398–409.
20 Finnegan M, Linley E, Denyer SP, Mcdonnell G, Simons C, Maillard J-Y (2010). Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms. J Antimicrob Chemother **65**: 2108–2115.
21 Flores-Lopes F, Thomaz A (2011). Histopathologic alterations observed in fish gills as a tool in environmental monitoring. Braz J Biol **71**: 179–188.
22 Gómez-López VM editor, (2012). Decontamination of fresh and minimally processed produce. Ames, IA: Wiley-Blackwell.
23 Henriksen M, Kania P, Buchmann K, Dalsgaard I (2014). Effect of hydrogen peroxide and/or *Flavobacterium psychrophilum* on the gills of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis (in press)
24 Jussila J, Makkonen J, Kokko H (2011). Peracetic acid (PAA) treatment is an effective disinfectant against crayfish plague (*Aphanomyces astaci*) spores in aquaculture. Aquaculture **320**: 37–42.
25 Khorramshahr I (2012). Histopathological study of parasitic infestation of skin and gill on oscar (*Astronotus ocellatus*) and discus (*Symphysodon discus*). AACL Bioflux **5**: 88.
26 Kitis M (2004). Disinfection of wastewater with peracetic acid: a review. Environ Int **30**: 47–55.
27 Kono Y, Fridovich I (1982). Superoxide radical inhibits catalase. J Biol Chem **257**: 5751–5754.

28 Kori-Siakpere O, Ogbe M, Ikomi R (2011). Variations in lactate dehydrogenase and creatine kinase activities in the plasma of the african catfish: *Clarias gariepinus* (Burchell, 1822) exposed to sublethal concentrations of potassium permanganate. Ann Biol Res **2:** 19–25.
29 Kouba A, Kuklina I, Niksirat H, Machova J, Kozak P (2012). Tolerance of signal crayfish (*Pacifastacus leniusculus*) to Persteril 36 supports use of peracetic acid in astaciculture. Aquaculture **350**: 71–74.
30 Kristan J, Stara A, Turek J, Policar T, Velisek J (2012). Comparison of the effects of four anaesthetics on haematological and blood biochemical profiles in pikeperch (*Sander lucioperca* L.). Neuro Endocrinol. Lett. **33** (Supp.3): 66–71.
31 Lefevre F, Audic J, Ferrand F (1992). Peracetic acid disinfection of secondary effluents discharged off coastal seawater. Water Sci Technol **25**: 155–164.
32 Li Z-H, Velisek J, Zlabek V, Grabic R, Machova J, Kolarova J, Randak T (2010). Hepatic antioxidant status and hematological parameters in rainbow trout, *Oncorhynchus mykiss*, after chronic exposure to carbamazepine. Chem-Biol Interact **183**: 98–104.
33 Liberti L, Lopez A, Notarnicola M (1999). Disinfection with peracetic acid for domestic sewage re-use in agriculture. Water Environ J **13**: 262–269.
34 Lubello C, Caretti C, Gori R (2002). Comparison between PAA/UV and H2O2/UV disinfection for wastewater reuse. Water Sci Technol **2**: 205–212.
35 Maffei F, Buschini A, Rossi C, Poli P, Forti GC, Hrelia P (2005). Use of the comet test and micronucleus assay on human white blood cells for in vitro assessment of genotoxicity induced by different drinking water disinfection protocols. Environ Mol Mutagen **46**: 116–125.
36 Meinelt T, Matzke S, Stüber A, Pietrock M, Wienke A, Mitchell A, Straus D (2009). Toxicity of peracetic acid (PAA) to tomonts of *Ichthyophthirius multifiliis*. Dis Aquat Organ **86**: 51–56.
37 Meinelt T, Richert I, Stüber A, Bräunig I (2007). Application of peracetic acid to the parasite *Ichthyophthirius-multifiliis* in Sander (*Sander lucioperca*) breeding. Deut Tierarztl Woch **114**: 244–251.
38 Omeji S, Solomon S, Idoga E (2011). A comparative study of the common protozoan parasites of clarias gariepinus from the wild and cultured environments in Benue State, Nigeria. J Parasitol Res **2011:** 8.
39 Pedersen L-F, Meinelt T, Straus DL (2013). Peracetic acid degradation in freshwater aquaculture systems and possible practical implications. Aquacult Eng **53**: 65–71.
40 Pedersen L-F, Pedersen PB, Nielsen JL, Nielsen PH (2009). Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems. Aquaculture **296**: 246–254.
41 Rach J, Schreier T, Howe G, Redman S (1997). Effect of species, life stage, and water temperature on the toxicity of hydrogen peroxide to fish. Prog Fish-Cult **59**: 41–46.
42 Reichert M, Young JH (1997). Sterilization technology for the health care facility. 2nd ed. Jones and Bartlett Learning. p. 307.
43 Sapone A, Gustavino B, Monfrinotti M, Canistro D, Broccoli M, Pozzetti L, Affatato A, Valgimigli L*, et al.* (2007). Perturbation of cytochrome P450, generation of oxidative stress and induction of DNA damage in *Cyprinus carpio* exposed *in situ* to potable surface water. Mutat Res Genet Toxicol Environ Mutagen **626**: 143–154.
44 Saravanan M, Prabhu Kumar K, Ramesh M (2011). Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. Pestic Biochem Phys **100**: 206–211.
45 Singh A, Bhat T, Sharma O (2011). Clinical biochemistry of hepatotoxicity. J Clinic Toxicol S **4**: 2161–0495.
46 Singh S, Kaur P (2014). Histology of gills of Labeo rohita and *Hypophthalmichthys molitrix* infested by monogenean and copepod parasites. Int J Fish Aqua Stud **1**: 1–6.
47 Stegeman JJ, Brouwer M, DiGiulio RT, Forlin L, Fowler BA, Sanders BM, Van Veld PA (1992). Molecular response to environmental contamination: Enzyme and protein systems as indicators of chemical exposure and effect. In: Huggett RJ, Kimerle RA, Mehrle PM, Bergman HL (eds.). Biomarkers: Biochemical, physiological, and histological markers of anthropogenic stress. Lewis Publishers. pp. 235–335.
48 Stara A, Steinbach Ch, Wlasow T, Gomulka P, Ziemok E, Machova J, Velisek J (2013). Effect of zeta-cypermethrin on common carp (*Cyprinus carpio* L.). Neuroendocrin Lett **34** (Suppl. 2): 37–42.
49 Straus D, Meinelt T, Farmer B, Mitchell A (2012a). Peracetic acid is effective for controlling fungus on channel catfish eggs. J Fish Dis **35**: 505–511.
50 Straus DL, Meinelt T (2009). Acute toxicity of peracetic acid (PAA) formulations to *Ichthyophthirius multifiliis* theronts. Parasitol Res **104**: 1237–1241.
51 Straus DL, Meinelt T, Farmer BD, Beck BH (2012b). Acute toxicity and histopathology of channel catfish fry exposed to peracetic acid. Aquaculture **342**: 134–138.
52 Sudova E, Machova J, Svobodova Z, Vesely T (2007). Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: a review. Vet Med-Czech **52**: 527.
53 Sudova E, Straus DL, Wienke A, Meinelt T (2010). Evaluation of continuous 4-day exposure to peracetic acid as a treatment for *Ichthyophthirius multifiliis*. Parasitol Res **106**: 539–542.
54 Svobodova Z, Pravda D, Palackova, J (1991). Unified methods of haematological examination of fish.Research Institute of Fish Culture and Hydrobiology, Vodnany, 31 p
55 Tort M, Jennings-Bashore C, Wilson D, Wooster G, Bowser P (2002). Assessing the effects of increasing hydrogen peroxide dosage on rainbow trout gills utilizing a digitized scoring methodology. J Aquat Anim Health **14**: 95–103.
56 Uchiyama M, Mihara M (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem **86**: 271–278.
57 Udroiu I (2006). The micronucleus test in piscine erythrocytes. Aquat Toxicol **79**: 201–204.
58 Velisek J, Wlasow T, Gomulka P, Svobodova Z, Dobsikova R, Novotny L, Dudzik M (2006). Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). Vet Med-Czech **51**: 469.

 *Copyright © 2014 Neuroendocrinology Letters ISSN 0172–780X • **www.nel.edu***