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Effects of acute exposure to deltamethrin and recovery time on common carp (*Cyprinus carpio* L.)

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Abstract

OBJECTIVES: The aim of this study is to evaluate effects of the insecticide Decis Mega (DM; active substance deltamethrin 50 g.L^{-1}) on common carp on the basis of haematological profile, oxidative stress, antioxidant enzymes and histopathology.
DESIGN: Fish were exposed two concentrations of DM 6.56 µg.L^{-1} (1DM) and 65.6 µg.L^{-1} (2DM) for 96 h. Then the remaining fish were transferred into DM-free water for depuration for another period of 96 h.
RESULTS: Exposure to 1DM and 2DM proved effect on enzymatic activity of superoxide dismutase, catalase, glutathione reductase and on oxidative damage of cells in gills, liver and kidney (p<0.05, p<0.01). Exposure to 1DM showed differences (p<0.05, p<0.01) in hematocrit and hemoglobin in blood. Histopathological changes were observed after acute exposure to DM as well as to DM-free water in gills, liver and kidney.
CONCLUSION: This study concludes that deltamethrin has influence on the haematological parameters, activity of antioxidant enzymes and caused oxidative damage, and histopathological changes in the fish. However, antioxidant balance in the body was restored after placing the fish in clean water for 4 days, however, this time was not sufficient complete regeneration.

Abbreviations

ANOVA	- analysis of variance
CAT	- catalase
EC50	- median effective concentration
Er	- erythrocyte count
GPx	- glutathione peroxidase
GR	- glutathione reductase
GSH	- reduced glutathione
GSSG	- oxidized glutathione
GST	- glutathione S-transferase
Hb	- hemoglobin concentration
LC50	- median lethal concentration
Leuko	- leukocyte count
LPO	- lipid peroxidation
MCH	- erythrocyte hemoglobin
MCHC	- mean corpuscular hemoglobin concentration
MCV	- mean erythrocyte volume
NADPH	- nicotinamide adenine dinucleotide phosphate
NBT	- nitroblue tetrazolium
PCV	- hematocrit
ROS	- reactive oxygen species
SOD	- superoxide dismutase
TBARS	- thiobarbituric acid reactive substances

ORIGINAL ARTICLE

INTRODUCTION

Deltamethrin ((S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylate) is a synthetic broad-spectrum pyrethroid insecticide, whose insecticidal effect is to interact with ion channels on the axons of the target species (FAO 2000). Deltamethrin is used for direct application to a wide variety of food, feed crops, stored grains, and in food and feed handling establishments. In addition, it is used indoors and outdoors in residences, on pets, and in paint additive products (US EPA 2010).

Deltamethrin, as with the whole group of pyrethroids has a low toxicity to mammals and birds. On the other hand, these substances are very toxic to bees and aquatic organisms (Glickman & Lech 1982; Koprucu & Aydin 2004). The widespread use of these pesticides, mainly in agriculture, leads to exposures and toxic effects on non-target organisms, mainly in aquatic ecosystems (Koprucu & Aydin 2004). Levels of deltamethrin in surface water has been reported from 0.057 $\mu g.L^{-1}$ in the Ebro River, Spain (Feo *et al.* 2010), 0.45 $\mu g.L^{-1}$ in the Ravi River, Pakistan (Mahboob *et al.* 2015) and to 1.43 $\mu g.L^{-1}$ in the Lorens River, Holland (Dabrowski *et al.* 2002). Toxicity of deltamethrin on non-target aquatic organisms varies on different species, for example glass shrimp (*Paratya australiensis*) (60hEC50) is from 0.038 to 0.181 $\mu g.L^{-1}$ (Thomas *et al.* 2010), water flea (*Daphnia magna*) (48hLC50) is 0.037 $\mu g.L^{-1}$, rainbow trout (*Oncorhynchus mykiss*) is 2.3 $\mu g.L^{-1}$ (96hLC50) (Dabrowski *et al.* 2002), common carp 48hLC50 for embryo 0.213 $\mu g.L^{-1}$, larvae 0.074 $\mu g.L^{-1}$ (Koprucu & Aydin 2004) and 96hLC50 for juvenile 1.45 $\mu g.L^{-1}$ (Svobodova *et al.* 2003).

Many studies have been conducted on acute toxicity of pyrethroids to fishes and to other non-target aquatic organisms. Although, deltamethrin is highly toxic to fish, it very rapidly degrades in water with a half-life of 8 to 48 hours, depending on the mode of introduction into water, pH and temperature (Erstfeld 1999). The present paper is focused on both the acute toxicity of deltamethrin on carp and their recovery, when they remained in free water after acute exposure to deltamethrin for another period of 96 hours. The assessment of toxicological risks to deltamethrin was done by using biomarkers of antioxidant enzymes, oxidative stress, haematological and histopathological examination.

MATERIALS AND METHODS

Chemicals

The pyrethroids insecticide Decis Mega (DM) containing active substance of 50 $g.L^{-1}$ deltamethrin.

Experimental protocol

One and one half year old common carp (*Cyprinus carpio* L.) were obtained from a local commercial hatchery (Vodnany, Czech Republic) with a mean total length 22.6±2.3 cm and weight 172.5±46.1 g.

At first, sixty fish were randomly selected and 10 each were placed into six 200-L aquaria with continuous aeration. Dissolved oxygen saturation was maintained at 97.5±46.1%, with a temperature of 19.7±0.5 °C, pH of 8.2–8.7 and light cycle of 12 h light:12 h dark was set. Basic physical and chemical indices of the diluting water were for acid neutralisation capacity – $ANC_{4.5}$ 1.12 $mmol.L^{-1}$, sum of Ca and Mg 12 $mg.L^{-1}$, total ammonia 0.03 $mg.L^{-1}$, $N0_3^-$ 1.13 $mg.L^{-1}$, NO_2^- 0.004 $mg.L^{-1}$, PO_4^{3-} 0.02 $mg.L^{-1}$ and chemical oxygen demand – COD_{Mn} 1.4 $mg.L^{-1}$.

Fish were acclimatized for 10 days before the beginning of the experiment and the exposure to two concentrations of DM 6.56 $\mu g.L^{-1}$ (10% 96hLC50) and 65.6 $\mu g.L^{-1}$ (96hLC50) and control fish were monitored at the same time. Each experimental group was performed in duplicate. The test was performed semi-statically with the bath exchange every 12 h. The tested substances were maintained at above 80% of the nominal concentration throughout the experimental period. DM concentrations in water were analyzed using gas chromatography with electron capture detection (Mekebri *et al.* 2008). After acute exposure to DM, 6 fish were randomly collected from each group for the haematological examination of blood and were killed so the tissues samples could be taken for the determination of oxidative stress and antioxidants enzymes activity (brain, gill, intestine, liver, muscle and kidney) and for the histopathological examination (liver, gill, and kidney). Then the remaining fish were held in DM-free water during the next 96 h. After the recovery period 5 fish were randomly selected from each group for the same analysis as after acute exposure to DM. Carps were not fed during the experiment. Acute toxicity testing was performed according to OECD guideline No. 203 (OECD 1992).

Haematological examination

Blood was sampled from the *v. caudalis* using heparinised syringe (Heparin inj., Leciva, Czech Republic) at a concentration of heparin sodium salt of 0.01 mL per 1 mL blood. The indices measured for evaluation of the haematological profile included erythrocyte count (Er), hemoglobin concentration (Hb), hematocrit (PCV), mean erythrocyte volume (MCV), mean erythrocyte hemoglobin (MCH), mean colour concentration (MCHC) and leukocyte count (Leuko). The procedures were based on standard methods for haematological examination of fish by Svobodova *et al.* (1991).

Antioxidant enzymes and lipid peroxidation

Enzymatic activities and level reactive oxygen substance were measured in liver, gill, kidney, intestine, muscle and brain. The tissues were immediately frozen after removal and stored at –80 °C until processing. Frozen tissue samples were weighed and homogenized using an Ultra Turrax homogenizer (Ika, Germany) with 50 $mmol.L^{-1}$ potassium phosphate buffer (1:10, w/v), pH 7.0, containing 0.5 $mmol.L^{-1}$ EDTA. The homoge-

nate was divided and used for analyzes. Total superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by the method of Marklund & Marklund (1974). Superoxide dismutase activity was assessed spectrophotometrically at 420 nm and expressed as the amount of enzyme per milligram of protein. The catalase (CAT; EC 1.11.1.6) activity assay, using the spectrophotometric measurement of H_2O_2 breakdown at 240 nm, was performed following the method of Beers & Sizer (1952). Glutathione reductase (GR) activity was determined spectrophotometrically, measuring nicotinamide adenine dinucleotide phosphate (NADPH) oxidation at 340 nm (Carlberg & Mannervik 1975). The thiobarbituric acid reactive substances (TBARS) method described by Lushchak *et al.* (2005) was used to evaluate lipid peroxidation (LPO). Protein levels were estimated spectrophotometrically by the method of Bradford (1976) using bovine serum albumin as a standard.

Histopathology

Gills, liver and kidney from each fish were fixed in 10% neutral buffered formalin. The fixed tissues 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 and observed under light microscope.

Statistical analysis

The statistical software program STATISTICA (version 8.0 for Windows, StatSoft) was used to compare differences the test groups with control group. Prior to analysis, all measured variables were checked for normality (Kolmogorov–Smirnov test) and homoscedasticity of variance (Bartlett's test). If these conditions were met, a one-way analysis of variance (ANOVA) was employed to determine differences in measured variables among the experimental groups. When a significant difference was detected ($p<0.05$, $p<0.01$), Dunnett's multiple range test was applied. In case the conditions for ANOVA were not met, a non-parametric test (Kruskal-Wallis) was used.

RESULTS

Fish behaviour

The behavioural and survival carps observed in the present study were typical of pyrethroid poisoning, which correspond to tested concentrations of DM. Fish showed differences in sheltering, escaping, and in rate of movement among the treatment groups during the trial. Mortality rate was 10% in concentration of 6.56 μg.L^{-1} 1DM and 45% in second concentration of 65.6 μg.L^{-1} 2DM during the acute exposure for 96 h. The remaining fish in both concentrations to DM after acute exposures had the normal behavioral symptoms as well as the control group during the recovery time of 96 h and mortality was not observed then.

Haematological examination

Fish exposed to a concentration of 6.56 μg.L^{-1} 1DM showed a significantly decreased PCV ($p<0.01$) and Hb ($p<0.05$) after acute exposure 96 h (Table 1). Significant increase ($p<0.01$) was observed in MCHC in fish exposed to 2DM (65.6 μg.L^{-1}) compared to the carp in the control after recovery time.

Tab. 1. Haematological parameters in common carp affected by 96 h acute exposure to DM and 96 h recovery time.

Index	Exposure time	Control	1DM (6.56 μg.L^{-1})	2DM (65.6 μg.L^{-1})
PCV (L.L^{-1})	acute 96h	0.26±0.03	0.18±0.04**	0.25±0.02
	recovery 96h	0.21±0.07	0.24±0.08	0.25±0.05
Hb (g.L^{-1})	acute 96h	61.71±9.37	45.95±10.85*	62.6±7.27
	recovery 96h	40.61±15.03	47.81±16.56	60.80±14.18
Er (T.L^{-1})	acute 96h	1.31±0.25	1.03±0.33	1.20±0.22
	recovery 96h	0.74±0.40	1.10±0.39	1.35±0.35
MCV (fL)	acute 96h	203.74±23.67	192.11±60.07	218.71±44.63
	recovery 96h	349.24±157.14	214.88±19.90	194.16±29.06
MCH (pg)	acute 96h	47.50±4.59	49.10±19.68	53.39±6.94
	recovery 96h	64.46±30.67	43.51±4.21	46.11±6.51
MCHC (L.L^{-1})	acute 96h	0.23±0.01	0.25±0.03	0.25±0.03
	recovery 96h	0.19±0.02	0.20±0.02	0.24±0.01**
Leuko (G.L^{-1})	acute 96h	75.33±18.87	59.33±33.19	55.67±25.72
	recovery 96h	54.20±20.10	56.00±17.36	65.00±27.56

Data are means ± standard deviation. Experimental groups significantly different (*$p<0.05$, **$p<0.01$) from the control group.

Antioxidant enzymes and lipid peroxidation

A decrease ($p<0.01$) in SOD activity in liver, gill, intestine and brain, and increased ($p<0.01$) activity SOD were in muscle and kidney were found in fish exposed to 1DM concentration. Fish exposed to 2DM showed a reduced ($p<0.01$) activity of SOD in liver, gill and intestine compared with control fish after a 96-h acute exposure, but there were no significant differences in the activity of SOD between test groups after recovery time (Table 2).

GR activity was lower ($p<0.01$) in gill and intestine in both concentrations after acute exposure for 96 h. After recovery time, we observed only higher activity ($p<0.01$) of GR in gill in concentration 2DM compared with control (Table 3).

Exposure to DM, in both tested concentrations, showed increased ($p<0.01$) in activity of CAT in liver, gill, intestine, brain and kidney, only in the muscle was reduced CAT activity. CAT activity remained increased ($p<0.01$) in intestine in fish exposed to 2DM compared with control fish after recovery time 96 h (Table 4).

Exposure fish to both concentrations DM led to increasing ($p<0.01$) in lipid peroxidation in liver, gill and brain, compared with control fish after acute exposure 96 h. In lipid peroxidation were no observed changes after recovery times (Table 5).

Histopathology

Gills tissue had pathological alternations of secondary and primary lamellae including haemorrhagiae,

Tab. 2. Activity of SOD (nmol NBT.min^{-1}.mg^{-1} protein) in common carp after 96 h exposure to DM and after 96 h recovery time.

Tissue	Exposure time	Control	1DM (6.56 µg.L^{-1})	2DM (65.6 µg.L^{-1})
Liver	acute 96h	0.205±0.05	0.092±0.05**	0.122±0.11**
	recovery 96h	0.112±0.04	0.144±0.04	0.177±0.04
Gill	acute 96h	0.249±0.07	0.046±0.03**	0.028±0.11**
	recovery 96h	0.113±0.03	0.146±0.02	0.112±0.02
Muscle	acute 96h	0.037±0.02	0.203±0.06**	0.093±0.01
	recovery 96h	0.014±0.01	0.021±0.02	0.137±0.11
Intestine	acute 96h	0.390±0.07	0.077±0.03**	0.065±0.09**
	recovery 96h	0.090±0.05	0.131±0.04	0.142±0.02
Brain	acute 96h	0.696±0.09	0.267±0.31**	0.770±0.09
	recovery 96h	0.091±0.03	0.106±0.02	0.083±0.03
Kidney	acute 96h	0.068±0.03	0.204±0.05**	0.122±0.13
	recovery 96h	0.132±0.01	0.123±0.02	0.093±0.02

Data are means ± standard deviation. Experimental groups significantly different (**$p<0.01$) from the control group.

Tab. 3. Activity of GR (nmol NADPH.min^{-1}.mg^{-1} protein) in common carp after 96 h exposure to DM and after 96 h recovery.

Tissue	Exposure time	Control	1DM (6.56 µg.L^{-1})	2DM (65.6 µg.L^{-1})
Liver	acute 96h	0.177±0.06	0.160±0.07	0.113±0.04
	recovery 96h	0.102±0.04	0.143±0.05	0.100±0.06
Gill	acute 96h	0.898±0.23	0.218±0.08**	0.258±0.12**
	recovery 96h	0.185±0.02	0.214±0.01	0.334±0.03**
Muscle	acute 96h	0.027±0.02	0.014±0.01	0.041±0.02
	recovery 96h	0.017±0.01	0.020±0.01	0.018±0.01
Intestine	acute 96h	0.304±0.10	0.131±0.03**	0.160±0.03**
	recovery 96h	0.121±0.07	0.116±0.03	0.176±0.04
Brain	acute 96h	0.085±0.03	0.145±0.09	0.124±0.06
	recovery 96h	0.151±0.10	0.157±0.04	0.096±0.01
Kidney	acute 96h	0.185±0.10	0.346±0.13	0.337±0.16
	recovery 96h	0.171±0.09	0.310±0.11	0.190±0.10

Data are means ± standard deviation. Experimental groups significantly different (**$p<0.01$) from the control group.

Tab. 4. Activity of CAT (μmol H_2O_2.min^{-1}.mg^{-1} protein) in common carp after 96 h exposure to DM and after 96 h recovery.

Tissue	Exposure time	Control	1DM (6.56 μg.L^{-1})	2DM (65.6 μg.L^{-1})
Liver	acute 96h	0.520±0.14	1.164±0.20**	1.185±0.36**
	recovery 96h	0.751±0.10	0.578±0.16	0.568±0.16
Gill	acute 96h	0.077±0.04	0.261±0.07**	0.246±0.04**
	recovery 96h	0.354±0.04	0.472±0.05	0.437±0.05
Muscle	acute 96h	0.134±0.05	0.060±0.04**	0.042±0.02**
	recovery 96h	0.052±0.01	0.096±0.04	0.083±0.03
Intestine	acute 96h	0.119±0.06	0.433±0.08**	0.556±0.20**
	recovery 96h	0.867±0.17	0.624±0.03	0.956±0.13**
Brain	acute 96h	0.030±0.00	0.133±0.03**	0.154±0.03**
	recovery 96h	0.268±0.06	0.208±0.03	0.290±0.07
Kidney	acute 96h	0.521±0.14	0.741±0.07**	0.996±0.16**
	recovery 96h	0.955±0.05	0.993±0.12	0.885±0.08

Data are means ± standard deviation. Experimental groups significantly different (**p<0.01) from the control group.

Tab. 5. Content of TBARS (nmol.mg^{-1} protein) in common carp after 96 h exposure to DM and after 96 h recovery.

Tissue	Exposure time	Control	1DM (6.56 μg.L^{-1})	2DM (65.6 μg.L^{-1})
Liver	acute 96h	0.497±0.03	0.613±0.04**	0.646±0.05**
	recovery 96h	0.664±0.05	0.574±0.05	0.588±0.06
Gill	acute 96h	0.193±0.06	0.390±0.04**	0.388±0.05**
	recovery 96h	0.436±0.02	0.453±0.03	0.468±0.03
Muscle	acute 96h	0.310±0.08	0.351±0.10	0.355±0.07
	recovery 96h	0.389±0.02	0.481±0.09	0.548±0.17
Intestine	acute 96h	0.307±0.09	0.466±0.05	0.417±0.07
	recovery 96h	0.561±0.10	0.549±0.09	0.515±0.04
Brain	acute 96h	0.331±0.05	0.441±0.08**	0.483±0.02**
	recovery 96h	0.519±0.05	0.503±0.11	0.590±0.05
Kidney	acute 96h	0.474±0.10	0.439±0.03	0.470±0.02
	recovery 96h	0.648±0.05	0.612±0.02	0.588±0.08

Data are means ± standard deviation. Experimental groups significantly different (**p<0.01) from the control group.

fusions, hyperplasia, lifting of epithelial cells and focal degeneration of secondary lamellae occurring in all examined groups. The liver of exposed fish (Figure 1) was characterized by lipid degeneration of hepatocyte cytoplasm and lodes of perivascular oedema. Most pronounced changes included strong swelling of the hepatocytes and nuclear degenerations in group 2DM. The apparent changes characteristic for the processes of tissue reparation (fibrosis and the presence of polyploid cells) were noticed in group 2DM after the 96 h regeneration period.

Histopathological changes in the kidney tissue (Figure 2) were characterized by the presence of occlusive hyperemia in 1DM. More pronounced changes were observed in higher concentration of deltamethrin (2DM), where extensive locations of altered tubules with the presence of macrophages were present. In both exposed groups, these lesions were less pronounced after a 96 h recovery period.

DISCUSSION

In the course of 96 h toxicity test of deltamethrin-based pyrethroid preparation Decis Mega on common carp, mortality occurred in groups exposed to DM. Fish mortality corresponded to the toxicity of test concentrations. During exposure to DM changes were also observed in the carp behavior. Alterations of fish

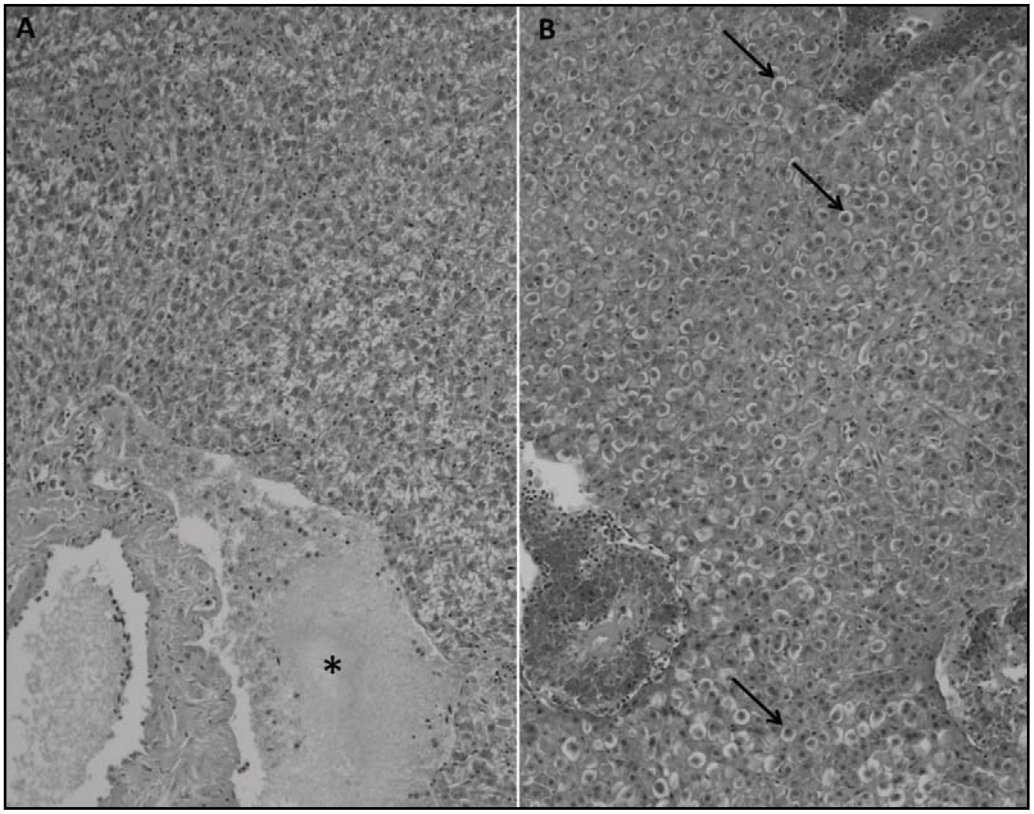

Fig. 1. Liver of common carp after 96 h exposure to deltamethrin (haematoxylin and eosin, 200×). A – 1DM (6.56 µg.L^{-1}), the asterisk indicates perivascular oedema; B – 2DM (65.6 µg.L^{-1}), the arrows mark noticeable swelling of the hepatocytes.

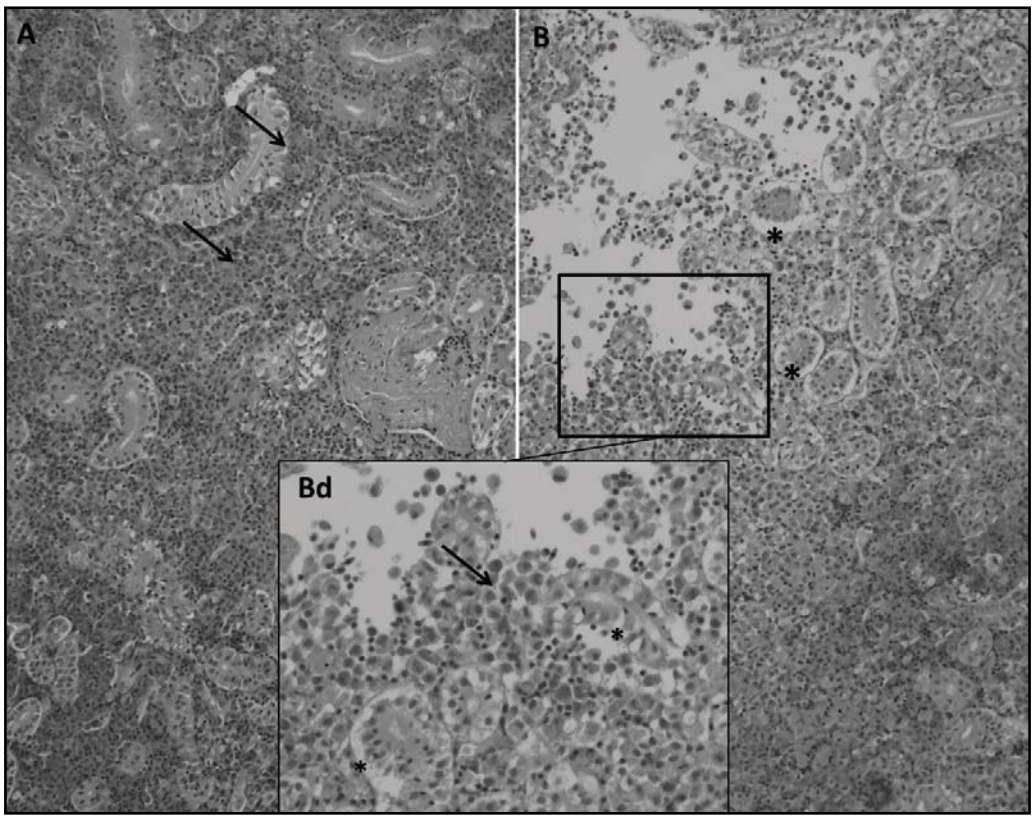

Fig. 2. Caudal kidney of common carp after 96 h exposure to deltamethrin (haematoxylin and eosin, 200×). A – group 1DM (6.56 µg.L^{-1}), the arrows indicate blood congestion places; B – 2DM (65.6 µg.L^{-1}), see disintegrated tissue with destructed tubular epithelium in upper-left side. The asterisks mark hypertrophy of epithelial cells of renal tubules. Bd – selected detail of part B, the arrow indicates macrophage aggregates.

behavioral responses are sensitive and are first indicators to identify accidental pollution (Huang *et al.* 2014). The response to DM observed in this study are identical to those reported in other studies. The first response is hyperactivity, change in swimming speed, erratic swimming, rapid gill and opercular movements and also swimming at the water surface. Subsequently, fish become lethargic, oriented vertically in the water or laid

on one side and loss of movement coordination and jerky movements. Seizures alternating with lethargy phase, there is a gradual slowing of breathing and death of fish (Velisek *et al.* 2009; Stara *et al.* 2013; Huang *et al.* 2014). However, fish inserted into DM-free water after exposure to DM was gradually recovering and there was restoration of coordinated movements and normal respiration rates.

In this study we observed only a few haematological changes in blood. It is concluded that DM1 induced anemia in carp, when there were lower PCV and also hemoglobin concentration. Similarly, Svobodova *et al.* (2003) measured lower values Hb, PCV and Er in carp blood after acute exposure 96hLC50 to deltamethrin at concentration 1.45 $\mu g.L^{-1}$. Vani *et al.* (2011) exposed of catla (*Catla catla*) to deltamethrin at concentration 1.61 $\mu g.L^{-1}$ for 45 days. The study result indicated a significant decrease in Er, Hb and Leuko. Whereas, in the study of Hedayati & Tarkhani (2014) with iridescent shark-catfish (*Pangasius hypophthalmus*) were observed significantly increased values Er, Hb, PVC and MCV after 7 days exposure to deltamethrin at concentrations 15 and 20 $\mu g.L^{-1}$. In our study carp were also kept in DM-free water after acute exposure to DM was found to increase MCHC in the group 2DM with compared other groups. However, these values of 0.24 $L.L^{-1}$ correspond to the physiological interface for carp 0.20–0.26 $L.L^{-1}$ (Svobodova *et al.* 1991). Haematological parameters can be used as fish health indicators and also for the habitat conditions, but there are many factors that limit its interpretation, for example the variability of fish species, differences in the methodology used and interpretation of cell types (Pimpao *et al.* 2007).

Acute exposure fish to DM led to increased lipid peroxidation production and also antioxidant enzymes changes were found. Reactive oxygen species (ROS) are naturally in the cells, but overproduction of ROS may cause cell or organism damages or death. This imbalance is referred to as oxidative stress (Davies 1995; Dinu *et al.* 2010). Dinu *et al.* (2010) assessed the deltamethrin at concentration 2 $\mu g.L^{-1}$ on goldfish (*Carassius auratus*). Lipid peroxidation increased in the liver already after 3 days and in the intestine LPO increased after 7 days. The changes in the activity of SOD, CAT and GR had been observed already after the first day of exposure fish to deltamethrin. SOD and CAT are the first defense enzymes that break overproduction of ROS into less toxic substances (Slaninova *et al.* 2009; Stara *et al.* 2012). Another no less important enzymes are GR, glutathione S-transferase (GST), reduced glutathion (GSH) and glutathione peroxidase (GPx) (Zivna *et al.* 2013; Plhalova *et al.* 2014). Ensibi *et al.* (2013) exposed carp to concentration of deltamethrin 0.4 $\mu g.L^{-1}$, had increased enzymatic activity CAT and GR in liver after 4 day of exposure, ROS growth occurred until 14 days exposure. Increased ROS production may, thus, be associated with the metabolism of deltamethrin leading to the peroxidation of membrane lipids. Reduction of enzymatic activity SOD, GPx, GSH and the increased levels ROS were in liver, kidney and gills in Nile tilapia (*Oreochromis niloticus*) exposure to deltamethrin in a concentration 1.46 $\mu g.L^{-1}$ after 28 days (Abdel-Daim *et al.* 2015). In study Amin & Hashem (2012), LPO content was increased and CAT activity in liver, kidney and gill of catfish (*Clarias gariepinus*) exposed to deltamethrin in a concentration of 0.75 $\mu g.L^{-1}$ for 48 h was reduced. The manifestations of enzymatic activities and oxidative stress vary in terms of fish species, organs, dose toxicant and time to exposure (Slaninova *et al.* 2009; Stara *et al.* 2013). However, keeping the fish in clean water for few days, after exposure to deltamethrin, apparently allowed some depuration and led to rebalance of the formation of ROS and antioxidant biomarkers.

Histological studies of the gills, hepatic and kidney tissues confirmed damage induced by deltamethrin. In this study, histological examination of control groups did not present significant differences among exposure periods, indicating good experimental conditions. The histopathological changes of fish tissues after acute exposure of 96 h to deltamethrin were similar to those described by various authors after deltamethrin (Al-Ghanbousi *et al.* 2012; Al-Ghanbousi *et al.* 2014) or organophosphate exposition (Nandan & Nimila 2012). The appearance of the liver suggests reversible cell damage, where cellular swelling and fatty change of cells dominated among other changes. These two commonly encountered specific cell changes are usually associated with toxic exposures (Kumar *et al.* 2012). Caudal kidney exhibited a number of changes which were more pronounced with increasing deltamethrin concentration. The tissues examined 96 h after remaining in DM-free water showed comparable changes with previous examination suggested persistent damage of examined organs, however, beginning reparation processes representing by fibrosis and presence of polynuclear cells were observed.

CONCLUSION

Acute exposure to deltamethrin demonstrated significant differences in biochemical parameters and led to the histopathological changes in carp. In fish held to DM-free water after acute exposure recovered normal behaviour and there was a balance of ROS formation after 4 days. For the full recovery of fish it was necessary to allow a longer period in clear water. This study summarizes the findings about the acute effect of deltamethrin and suggests that there is little potential for bioaccumulation in fish. Moreover biomarkers of antioxidant enzymes and oxidative stress used in this study they seem to be helpful in assessing the risk of environmental contaminants, as well as haematological and histopathological studies.

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