EFFECTS OF MELATONIN ON CADMIUM ACCUMULATION AND HAEMATOLOGICAL PARAMETERS IN CADMIUM INTOXICATED PRUSSIAN CARP (*CARASSIUS GIBELIO* B.)*

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Abstract

The present study was performed to determine the effect of melatonin on cadmium accumulation and haematological parameters changes in females of Prussian carp (*Carassius gibelio* B.). The fish were exposed to a sublethal concentration of cadmium (0.4 and 4.0 mg/L) without or with melatonin implant (containing 18 mg melatonin hormone) for 1, 4, 7, 10 and 13 weeks. After the 7th week, Cd-exposed fish were divided into two groups. The first group of fish were subjected to depuration in clear water, while the second group remained exposed to the same concentrations of cadmium. After the 10th and 13th weeks of exposure the kidney, spleen, heart and blood were taken in order to determine the cadmium concentration and to analyse haematological parameters. The results of this study showed an increase in the concentration of cadmium in the tissues and blood in fish exposed to the highest dose (4.0 mg/L) of cadmium in water with a dependence on the exposure duration. Additionally, it was shown that melatonin administration reduced the cadmium concentration in the tissues and blood, and melatonin had a protective effect against cadmium accumulation. Moreover, the results showed a significant increase in blood parameters (RBC, Ht and Hb) in fish treated with the highest dose of cadmium. However, implantation of melatonin in cadmium-treated fish resulted in marked improvements in haematological parameters. Thus, melatonin can be a good implementation to alleviate, at least partially, the toxicity of cadmium.

Key words: cadmium, Prussian carp, haematological parameters, accumulation, depuration

Cadmium is a well-known, non-essential, highly toxic heavy metal and is recognised as one of the major aquatic pollutants and causes of toxic effects in living

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organisms (Ali et al., 2019). In polluted fresh water, the concentration of cadmium ranges from 40 to 120 µg/L, and in heavily polluted water, even up to 4000 µg/L (Witeska et al., 2010). Metals penetrate into fish organisms in different ways: mainly from water by means of the gills (respiratory tract) and skin (transcutaneous uptake), or with food through the gastrointestinal tract (Kondera et al., 2014). Cadmium is absorbed by fish organisms into the blood, and then taken up from the blood into the tissues. This metal is accumulated in fish tissues, especially in the liver, kidney and spleen, leading to their damage (Jasim, 2017; Sun et al., 2019). The kidney is an important organ in the body and the major function of the kidney, in addition to excretory functions, is to maintain both homeostasis and erythropoiesis (Jayakumar et al., 2016; Kaur et al., 2018). In the kidney, cadmium induces various pathological changes, such as damage and disorganisation to the tubules, and also glomerular oedema and necrosis (Jayakumar et al., 2016). The spleen is an organ which performs several haematologic functions. It is also the basal site for the elimination of aging blood cells. Furthermore, in a few fish species, such as goldfish (*Carassius auratus*), European perch (*Perca fluviatilis*) and common roach (*Rutilus rutilus*), this organ is an important erythropoietic site (Jasim, 2008). In the spleen cadmium causes necrosis of pancreatic cells and fatty changes in the pancreatic cells (Sun et al., 2019).

The toxic action of cadmium on fish includes tissue damage and dysfunction (such as kidney and spleen), as well as haematological disorders. Haematological changes are frequently used as an index to detect the physiological and health status of fish. This allows comparatively rapid estimation of the chronic toxicities of a compound such as cadmium (Al-Asgah et al., 2015). The disturbed blood profile of fish exposed to cadmium comprises changes in the haematological and biochemical parameters under the absorption of this toxicant (Witeska et al., 2010; Khan et al., 2015). Cadmium accumulates also in heart of fish. Some researchers have suggested that cardiac mitochondrial viability of fish was affected by Cd exposure and the heart is very sensitive to toxicity of this metal (Velasquez-Vottelerd et al., 2015; Jasim, 2017). Fish exposed to cadmium revealed disturbances in blood elements and differential blood count. Cadmium causes the destruction of erythrocytes, decreases or increases the haemoglobin concentration and haematocrit level (Khalesi et al., 2017; Rajeshwari and Sevarkodiyone, 2018).

Melatonin, which is an indolamine, is a naturally occurring hormone that plays an important role as a highly effective antioxidant and free radical scavenger in inactivating harmful free radicals produced by various stressors (Zhao et al., 2019). A number of studies suggest the protective effect of melatonin on the toxicity of cadmium (Chwelatiuk et al., 2006; El-Sokkary et al., 2010; Hussein et al., 2014). Chwelatiuk and Włostowski (2011) and Drąg-Kozak et al. (2018) demonstrated a decrease in cadmium content in the kidney, liver, ovary and brain of animals exposed to this metal and melatonin. This hormone is also known to influence haematological parameters and various immune parameters of fish (Esteban et al., 2013; Aripin et al., 2018). It is known that cadmium accumulates and exerts adverse effect on very important haemopoietic organs and/or organs connected with cardiovascular system in fish (Kondera et al., 2014; Velasquez-Vottelerd et al., 2015; Arantes

et al., 2016). Previously, we had found that exogenous melatonin could effectively reduce Cd accumulation in liver tissue of Prussian carp (Drag-Kozak et al., 2019). However, to the best of our knowledge, there is no information available on the combined effects of Cd²⁺ and melatonin on haematological parameters in female Prussian carp. Therefore, the present work was designed to study the efficiency of melatonin on haematological parameter disorders and Cd accumulation in the selected tissues (kidney, spleen, heart and blood) of female Prussian carp exposed to cadmium chloride (CdCl₂).

Material and methods

Experimental fish

The experiment was carried out on three hundred and forty-three Prussian carp (*Carassius gibelio* B.), which were three-year-old females (mean body weight 204.65 ± 12.58 g and body length 23.25 ± 0.49 cm) originating from the ponds of the Experimental Station of the Department of Animal Nutrition, Biotechnology and Fisheries of the University of Agriculture in Krakow. The fish were maintained in seven 700-L tanks (49 fish per tank), with constantly filtered and aerated water, at 18°C, dissolved oxygen concentration 9.0 mg/L and fed daily (3% of their body weight) with commercial dry pellets during the 3-month acclimation period and throughout the experiment. These pellets contained 37% crude protein, 12% crude fat and 32.5% carbohydrate.

After acclimation, the fish were randomly divided into seven groups. Control group fish were treated with neither cadmium nor melatonin. Group Mel was implanted intramuscularly with 18 mg of melatonin (Ceva Santa Animale, France). Many studies show that circulating melatonin levels measured after the implantation in fish demonstrate the increase from 4.0 pg/mL (control) to 1 ng/mL (melatonin implanted) during 5 months (Aarseth et. al., 2010; Sönmez et al., 2014). Group blank was sham-injected. Group 0.4 mgCd/L+Mel and 4.0 mgCd/L+Mel were exposed to cadmium 0.4 mg/L and 4.0 mg/L respectively in water and received intramuscular melatonin implants. Groups 0.4 mgCd/L and 4.0 mg/L were exposed to cadmium 0.4 mg/L and 4.0 mg/L respectively in water, and were not treated with melatonin. Cadmium concentrations were chosen considering Cd levels presented in surface waters (range from 1 to over 16 mg/L) (Witeska et al., 2010, Silva et al., 2017). Cadmium as (CdCl₂·2.5H₂O) was dissolved in distilled water. Every two days, the water was exchanged without disturbing the welfare of the fish. The procedure used for melatonin implants in the fish was according to Porter et al. (1998) and Mazurais et al. (2000).

The female Prussian carp were subjected to exposure to cadmium lasting 7 or 13 weeks. After the 7 weeks, the group of fish exposed to cadmium was divided into two groups. One of these was subjected to the same treatment (13 weeks of exposure), the second one, the residual metal-contaminated group, was kept in clean water for the following 3 or 6 weeks for the elimination experiments (Table 1).

Table 1. The configuration of treatment groups and cadmium doses in water during the 13 weeks exposure period and the following 6 weeks exposure or depuration period

Group	Control	Mel	blank	0.4 mgCd/L+Mel	0.4 mgCd/L	4.0 mgCd/L+Mel	0.4 mgCd/L
Cd dose in water (mg/L) during 1–7 weeks period	–	–	–	0.4	0.4	4.0	4.0
Number of fish at the beginning [n] (per tank)	49	49	49	49	49	49	49
Group during 7–10 weeks of experiment	control	Mel	blank	0.4 mgCd/ L+Mel	0.4 mgCd/ L+Mel-dep	4.0 mgCd/ L+Mel	4.0 mgCd/ L - dep
Cd dose in water (mg/L) during the 10–13 weeks period	–	–	–	0.4	0.4	–	–
Number of fish at the beginning of the 10th week of the exposure/depuration	14/14	14/14	14/14	14	14	14	14

The blood and tissue samples were collected immediately before metal exposure (0 weeks) and after 1, 4, 7, 10 and 13 weeks of exposure from 7 randomly caught fish from each group. The fish were anesthetized with Propiscin (0.3 ml/L; IRS, Żabinec, Poland) prior to all the manipulations (according to Kazuń and Siwicki, 2001).

The experiments were performed in accordance with the research protocols approved by the Local Animal Ethics Committee in Cracow, Poland (No. 97/V/2013).

Haematological analysis

After each experimental period, samples of blood (about 1 ml) were collected from the fish, which were anesthetized with Propiscin (0.3 ml/L) (Kazuń and Siwicki, 2001). Blood samples were obtained from the caudal vein by a sterile heparinized syringe. The haematological parameters such as red blood cell (RBC) count ($10^6/\text{mm}^3$), white blood cell (WBC) count ($10^6/\text{mm}^3$), haematocrit value (Ht, %) and haemoglobin concentration (Hb, g/dL) were estimated in fresh blood. RBC and WBC counts were counted according to the method described by Łuszczek-Trojnar et al. (2019). Haematocrit (Ht) was measured using a microhaematocrit centrifugation (at 12,000 rpm for 5 min). The results were expressed in percentages. Haemoglobin (Hb) was evaluated spectrophotometrically at 540 nm absorbance (cyanmethemoglobin method) (Drabkin, 1946). A number of blood parameters, such as mean corpuscular haemoglobin ($\text{MCH}=\text{Hb}\times\text{RBC}^{-1}$, pg), mean corpuscular haemoglobin concentration ($\text{MCHC}=\text{Hb}\times 100\times\text{Ht}^{-1}$, g/dL) and mean corpuscular volume ($\text{MCV}=\text{Ht}\times 10\times\text{RBC}^{-1}$, fL), were also calculated.

Cd determination

Samples (about 5 g) of the heart, kidney, spleen and blood were collected in order to determine cadmium concentrations after 1, 4, 7, 10 and 13 weeks of the experiment. The fish were euthanized by decapitation, and the heart, kidney, spleen tissues and blood were collected and stored at -20°C before the Cd concentration analysis was conducted. The preparation of fish tissues for heavy metal analysis and metal determination has been presented elsewhere (Drag-Kozak et al., 2018). Metal concentrations in the tissue samples and blood were measured by atomic absorption spectrometry (Unicam 929) (Agemian et al., 1980). The results were presented in milligrams of Cd per kilogram of wet tissue weight (ww).

Statistical analysis

The data received were analysed statistically by one-way analysis of variance (ANOVA). The differences between the means of the control and experimental groups, as well as between groups at successive weeks of exposure, were determined using the Mann-Whitney test. Furthermore, the relationship between the cadmium concentration in the heart, kidney, spleen and blood and the dose of exposure were determined using Spearman's correlation coefficients. The differences between the means were defined as significant for $P\leq 0.05$.

Table 2. Comparison of Cd concentration (mg/kg) in the kidney, spleen and heart of female Prussian carp during 13 weeks of fish exposure to different doses of cadmium and 6 weeks depuration. Spearman's correlation coefficients (*r*) for the relationship of Cd concentration tissues to cadmium doses during the exposure and depuration periods in different groups. The results of multiple comparisons Kruskal-Wallis test (*p*) for Cd concentration in tissues, value difference

Week	control	Exposure				Cd in kidney (mg/kg wet weight)				<i>r</i>	Statistic	Depuration				<i>r</i>	Statistic
		Mel	Blank	0.4 mgCd/ L+Mel	0.4 mgCd/ L	4.0 mgCd/ L+Mel	4.0 mgCd/ L	0.4 mgCd/ L+Mel- dep	0.4 mgCd/ L-dep			0.4 mgCd/ L+Mel-dep	0.4 mgCd/ L-dep	4.0 mgCd/ L+Mel-dep	4.0 mgCd/ L-dep		
0	0.23± 0.03 Aa	0.20± 0.02 Aa	0.26± 0.02 Aac	0.29± 0.03 Aa	0.25± 0.03 Aa	0.21± 0.02 Aa	0.29± 0.03 Aa	NT	NT	NS	NS	NT	NT	NT	NT	NT	NT
1	0.25± 0.01 Aac	0.29± 0.02 Ab	0.23± 0.02 Aa	0.83± 0.09 Bb	0.54± 0.09 Cb	2.33± 0.18 CDb	2.59± 0.12 Db	NT	NT	0.94***	p<0.0001	NT	NT	NT	NT	NT	NT
4	0.35± 0.03 Abc	0.34± 0.03 Ab	0.38± 0.02 Ab	5.09± 0.35 Bc	6.83± 0.85 Bc	35.22± 2.33 Cc	49.29± 1.63 Dc	NT	NT	0.94***	p<0.0001	NT	NT	NT	NT	NT	NT
7	0.61± 0.05 Ad	0.52± 0.06 Ac	0.49± 0.03 Acd	13.30± 64 Bd	16.10± 1.25 Bd	63.85± 3.56 Cd	112.5± 3.92 Dd	NT	NT	0.94***	p<0.0001	NT	NT	NT	NT	NT	NT
10	0.43± 0.03 Ab	0.97± 0.11 Bd	0.58± 0.04 Cd	19.07± 0.91 De	23.24± 1.10 Ee	139.9± 8.59 Fe	155.9± 9.87 Fe	15.30± 1.51 Dd	14.03± 1.21 Dd	0.94***	p<0.0001	108.6± 5.93 Ee	133.6± 8.57 Ed	105.0± 113.3±	113.3± 8.37 Fd	0.94***	p<0.0001
13	0.36± 0.04 Ac	0.53± 0.04 Bc	0.37± 0.04 Ceb	26.58± 1.65 Df	23.35± 1.41 De	116.8± 6.93 Ee	112.0± 9.39 Ed	23.77± 3.47 De	11.84± 1.19 Ed	0.94***	p<0.0001	105.0± 10.1 Fe	113.3± 8.37 Fd	113.3± 8.37 Fd	113.3± 8.37 Fd	0.94***	p<0.0001
Week	control	Exposure				Cd in kidney (mg/kg wet weight)				<i>r</i>	Statistic	Depuration				<i>r</i>	Statistic
		Mel	Blank	0.4 mgCd/ L+Mel	0.4 mgCd/ L	4.0 mgCd/ L+Mel	4.0 mgCd/ L	0.4 mgCd/ L+Mel- dep	0.4 mgCd/ L-dep			0.4 mgCd/ L+Mel-dep	0.4 mgCd/ L-dep	4.0 mgCd/ L+Mel-dep	4.0 mgCd/ L-dep		
0	0.09± 0.006 Aa	0.09± 0.004 Aa	0.09± 0.005 Aa	0.09± 0.004 Aa	0.09± 0.005 Aa	0.09± 0.005 Aa	0.09± 0.007 Aa	NT	NT	NS	NS	NT	NT	NT	NT	NT	NT
1	0.14± 0.01 Ab	0.14± 0.01 Ab	0.16± 0.01 Ab	0.33± 0.05 Bb	0.24± 0.03 Bb	0.58± 0.03 Cb	0.78± 0.06 Db	NT	NT	0.91***	p<0.0001	NT	NT	NT	NT	NT	NT
4	0.54± 0.01 Abc	0.11± 0.01 Bb	0.29± 0.02 Cc	0.74± 0.05 Dc	1.34± 1.00 Ec	2.74± 0.27 Fc	3.67± 1.18 Gc	NT	NT	0.94***	p<0.0001	NT	NT	NT	NT	NT	NT
7	0.17± 0.01 Ac	0.11± 0.01 Bb	0.14± 0.02 Abc	1.13± 0.18 Bcd	1.56± 2.23 Bc	5.26± 0.60 Cd	7.79± 0.53 Dd	NT	NT	0.94***	p<0.0001	NT	NT	NT	NT	NT	NT
10	0.16± 0.01 Abc	0.32± 0.02 Bc	0.16± 0.01 Ab	1.47± 0.13 Cd	1.64± 1.13 Cc	10.17± 0.37 De	18.06± 1.86 Ee	1.65± 0.12 Ce	1.05± 0.07 Dc	0.94***	p<0.0001	6.86± 0.38 Ec	11.61± 0.84 Fe	6.86± 0.38 Ec	11.61± 0.84 Fe	0.94***	p<0.0001
13	0.15± 0.01 Abc	0.24± 0.03 Bd	0.17± 0.02 Ab	2.01± 0.23 Ce	2.28± 0.17 Cd	15.02± 1.20 Df	30.91± 3.38 Ef	1.65± 0.15 Ccd	1.17± 0.14 Dc	0.94***	p<0.0001	11.25± 0.95 Ed	12.39± 1.00 Fe	11.25± 0.95 Ed	12.39± 1.00 Fe	0.94***	p<0.0001

	Cd in kidney (mg/kg wet weight)										Depuration			
	Exposure					NS					NT	NT	NT	NT
0	0.12± 0.01 Aa	0.12± 0.01 Aa	0.12± 0.02 Aa	0.12± 0.01 Aa	0.12± 0.01 Aa	0.12± 0.01 Aa	NS	NS	NS	NT	NT	NT	NT	NT
1	0.10± 0.02 Aa	0.17± 0.02 Bb	0.24± 0.03 Cb	0.22± 0.02 BCb	0.52± 0.03 Db	0.67± 0.04 Db	0.98± 0.07 Eb	0.84*** p<0.0001	NT	NT	NT	NT	NT	NT
4	0.72± 0.04 Cb	0.62± 0.03 Ac	0.67± 0.06 ACc	0.82± 0.05 Cc	1.57± 0.14 Bc	1.54± 0.10 Bc	2.72± 0.21 Dc	0.84*** p<0.0001	NT	NT	NT	NT	NT	NT
7	1.18± 0.06 ACc	1.17± 0.06 ACd	1.02± 0.06 Ac	1.57± 0.12 Bd	1.36± 0.08 CBc	4.11± 0.40 Dd	4.67± 0.28 Dd	0.86*** p<0.0001	NT	NT	NT	NT	NT	NT
10	1.31± 0.06 Ac	1.44± 0.10 Ad	1.37± 0.08 Ad	1.83± 0.08 Bd	2.29± 0.15 Cd	4.82± 0.30 Cd	6.59± 0.30 Be	0.91*** p<0.0001	0.38± 0.03 Bd	0.33± 0.05 Bd	2.51± 0.21 Ce	4.05± 0.29 Dc	0.36* p<0.0001	
13	1.20± 0.07 Abc	1.27± 0.10 Ad	1.20± 0.07 Acd	0.73± 0.08 Bc	0.80± 0.07 Be	4.55± 0.25 Cd	6.61± 0.45 De	0.40** p<0.0001	0.33± 0.03 Bd	0.12± 0.01 Ce	2.53± 0.14 CDe	2.73± 0.19 Dd	0.40** p<0.0001	

NT – not tested, NS – not significant.

Capital letters denote statistically significant differences ($P<0.05$) between the groups in different exposure weeks, while small letters indicate significant differences in the groups between successive weeks of the exposure. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Results

Behavioural activity

Some behavioural abnormalities, such as moving towards the surface, disturbed coordination in swimming activities and an increase in operculum movement were observed during the experiment only in the group which was exposed to the highest concentration of cadmium in water. No mortality was observed in either Prussian carp exposed to 0.4 mg/L and 4.0 mg/L Cd during the whole experimental period.

Accumulation of Cd

Table 2 and 3 show the Cd accumulation in the kidney, spleen, heart and blood of female Prussian carp during exposure to cadmium without melatonin and treated with melatonin.

The Cd accumulation increased significantly with the increased period of exposure to cadmium in all the tissues tested, especially in spleen and heart. The highest level of Cd accumulation was noted after the 10th week in Cd-exposed fish kidneys (155.9 ± 9.87 mg/kg w. w.). After 13 weeks of exposure, the cadmium accumulation was in the order kidney > spleen > heart > blood in the group of fish exposed to the highest concentration of Cd (4.0 mg Cd/L). The groups exposed to the highest dose of Cd showed a significantly higher accumulation level of Cd in the tissues and blood than the control group ($P < 0.05$), while in fish co-administered with cadmium and melatonin simultaneously, the cadmium concentration in all tissues and blood was low.

Kidney

The cadmium level in the kidney increased significantly ($P < 0.05$) in fish exposed to cadmium as soon as the first week of exposure and remained so until the end of the exposure period. The highest concentration of cadmium in the kidney was recorded in the 10th week of exposure. In the group exposed to the highest concentration of Cd (4.0 mgCd/L), an increase in the concentration of cadmium was observed in comparison to the control group and experimental groups during the whole experimental period. Administration of melatonin together with cadmium (4.0 mgCd/L+Mel) significantly ($P < 0.05$) reduced the accumulation of cadmium in the kidney. However, the level still remained higher than in the control and experimental groups. A positive correlation confirmed the relationship between cadmium concentration and Cd doses during the exposure (Table 2).

After 3 weeks of depuration, a statistically significant increase ($P < 0.05$) was observed in Cd levels in the kidneys of fish previously exposed to the highest doses of the metal.

During the depuration phase, the Spearman's correlation showed a significant positive association between the Cd kidney level and its previous exposure level (Table 2).

Spleen

In the fish spleen samples, the cadmium concentration was significantly higher in exposed groups than in the control ($P < 0.05$). The highest concentration of cadmium

in the spleen was noted after the 13th week of exposure. In the group of fish exposed to the highest Cd dose (4.0 mgCd/L), the Cd concentration in the spleen was significantly higher ($P<0.05$) than in the control and other groups during the whole experimental period. Administration of melatonin together with cadmium (4.0 mgCd/L+Mel) significantly decreased the accumulation of cadmium ($P<0.05$), which still continued to be significantly higher than in the control and experimental groups. A positive correlation confirmed the association between the cadmium concentration in the spleen and Cd doses during the exposure (Table 2).

After 3 and 6 weeks of depuration, a statistically significant increase ($P<0.05$) was observed in Cd levels in the spleen of females previously exposed to the highest doses of the metal. A positive correlation between Cd spleen level and cadmium doses was noticed after the 3rd and 6th week of the depuration period (Table 2).

Heart

A significant cadmium accumulation was noted in the heart of the exposed fish when compared to the control ($P<0.05$). The highest concentration of cadmium in the heart was noted after the 13th week of exposure. In the group of fish exposed to the highest Cd dose (4.0 mgCd/L), the concentration of cadmium in the heart increased significantly with the exposure period when compared to the control and other groups. The cadmium level decreased significantly ($P<0.05$) in the group with melatonin (4.0 mgCd/L+Mel) and still remained significantly higher than in the control and other experimental groups. Spearman's correlation analysis confirmed a positive relationship between the cadmium concentration in the heart and Cd doses during the exposure (Table 2).

After 3 and 6 weeks of depuration, there was a statistically significant decrease ($P<0.05$) in Cd levels in the hearts of fish previously exposed to the highest dose of the metal (Table 2).

Blood

In the blood, cadmium was accumulated in a high amount compared to the control ($P<0.05$). The highest concentration of cadmium in the blood was noted after the 7th week of exposure. In the group exposed to the highest concentration of Cd (4.0 mgCd/L), after 4, 7, 10 and 13 weeks, an increase in the concentration of cadmium was observed in comparison to the control group and experimental groups. A significant ($P<0.05$) decrease in the cadmium level in the blood was noted in the melatonin together with cadmium group (4.0 mgCd/L+Mel), but the level still remained higher than in the control ($P<0.05$). A positive correlation confirmed the association between the cadmium concentration in the blood and Cd doses during the exposure (Table 3).

A statistically significant decrease ($P<0.05$) in the Cd levels in the blood of fish previously exposed to the highest doses of the metal was observed after 6 weeks of depuration (Table 3).

Table 4. Effect of cadmium, melatonin and their combination on RBC ($10^3/\text{mm}^3$) and WBC ($10^3/\text{mm}^3$) in the blood of female Prussian carp during 13 weeks of fish exposure to different doses of cadmium and 6 weeks depuration. Spearman's correlation coefficients (r) for the relationship of RBC and WBC counts to cadmium concentration in blood during the exposure and depuration periods in different groups. The results of multiple comparisons Kruskal-Wallis test (p) for RBC and WBC level in blood, value difference

Week	Exposure					RBC (10 ⁶ /mm ³)					Depuration				
	control	Mel	Blank	0.4 mgCd/ L+Mel	0.4 mgCd/L	4.0 mgCd/ L+Mel	4.0 mgCd/L	r	Statistic	0.4 mgCd/ L+Mel-dep	0.4 mgCd/ L-dep	4.0 mgCd/ L+Mel-dep	4.0 mgCd/ L-dep	r	Statistic
0	0.85± 0.03 Aa	0.84± 0.02 Aa	0.83± 0.02 Aa	0.87± 0.02 Aa	0.83± 0.02 Aa	0.84± 0.03 Aa	0.84± 0.03 Aa	NS	NS	NT	NT	NT	NT	NT	NT
1	0.85± 0.03 Aa	1.01± 0.04 BD _b	0.94± 0.06	1.24± 0.07 C _b	0.96± 0.06 AB _{ac}	1.12± 0.07 CD _{bc}	1.12± 0.07 BC _b	0.46***	p<0.0024	NT	NT	NT	NT	NT	NT
4	1.06± 0.06 AB _b	1.06± 0.06 AB _b	1.05± 0.05 AB _b	1.21± 0.06 A _b	0.93± 0.06 B _{ac}	1.22± 0.05 AB _{bc}	1.15± 0.10 AB _b	NS	NS	NT	NT	NT	NT	NT	NT
7	1.12± 0.04 AC _b	1.27± 0.05 A _c	1.12± 0.06 AC _b	1.17± 0.05 AC _b	1.46± 0.05 B _b	1.11± 0.03 C _b	1.28± 0.06 AB _b	NS	p<0.0017	NT	NT	NT	NT	NT	NT
10	0.91± 0.06 AD _c	1.06± 0.05 AB _b	1.08± 0.04 AB _b	1.01± 0.1 A _b	0.97± 0.03 A _c	1.32± 0.09 C _c	1.18± 0.05 BC _b	0.40***	p<0.0006	1.19± 0.06 B _b	0.84± 0.05 D _c	1.01± 0.04 AB _b	1.05± 0.05 A _c	NS	p<0.0050
13	1.13± 0.03 AC _{bd}	1.07± 0.07 A _{bc}	1.13± 0.04 C _c	0.90± 0.05 B _a	1.13± 0.04 C _d	0.99± 0.05 C _d	1.27± 0.04 B _c	0.54***	p<0.0001	1.15± 0.04 A _b	1.31± 0.08 AB _b	1.30± 0.07 C _c	1.13± 0.04 B _c	-0.54***	p<0.0008
Depuration															
WBC (10 ³ /mm ³)															
Exposure															
0	50.62± 0.98 Aa	51.34± 0.91 Aa _{cd}	51.34± 1.25 Aa	52.06± 0.61 Aa	51.31± 1.32 Aa _c	52.06± 0.61 Aa	53.49± 1.22 Aa _b	NS	NS	NT	NT	NT	NT	NT	NT
1	50.63± 0.98 Aa	57.03± 1.67 B _{bcd}	50.95± 1.19 Aa	57.09± 1.49 Ba	50.56± 0.67 Aa	55.19± 1.01 Ba	50.31± 0.73 Aa	NS	p<0.0005	NT	NT	NT	NT	NT	NT
4	53.42± 0.75 ACa _b	62.42± 2.18 B _b	51.86± 2.65 ACa _b	65.43± 3.19 B _c	52.31± 0.59 Aa _c	68.11± 3.65 B _b	55.88± 1.01 Ca	-0.45***	p<0.0001	NT	NT	NT	NT	NT	NT

7	54.94± 0.88 ABb	58.19± 1.53 ABd	53.69± 0.91 Bab	56.19± 1.08 ABb	56.84± 1.29 ABb	53.89± 1.01 Ba	56.27± 1.37 ABa	NS	NS	NT	NT	NT	NT	NT	NT
10	53.10± 1.20 Aab	51.63± 1.67 Ac	55.46± 1.29 ACb	56.58± 1.77 Aabc	55.77± 1.57 Acb	54.24± 1.94 Aa	54.39± 1.86 Aa	NS	NS	73.75± 3.06 Bc	58.73± 1.11 Cb	57.79± 2.34 ADa	64.68± 1.37 Db	NS	NS
13	52.64± 1.16 Aab	54.25± 2.02 Acd	59.35± 2.10 Bb	53.41± 2.95 ABab	51.31± 2.21 Aac	51.32± 1.82 Aa	52.89± 0.90 Ab	NS	NS	55.32± 1.82 ABb	54.39± 1.50 ABc	54.63± 1.00 ABa	51.86± 1.10 Ac	NS	NS

NT – not tested, NS – not significant.

Capital letters denote statistically significant differences ($P < 0.05$) between the groups in different exposure weeks, while small letters indicate significant differences in the groups between successive weeks of exposure * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 5. Effect of cadmium, melatonin and their combination on level of MCV (fL), MCH (pg) and MCHC (g/dL) in the hepatopancreas of female Prussian carp during 13 weeks of fish exposure to different doses of cadmium and 6 weeks depuration. Spearman's correlation coefficients (*r*) for the relationship of MCV, MCH and MCHC to cadmium concentration in blood during the exposure and depuration periods in different groups. The results of multiple comparisons Kruskal-Wallis test (*p*) for MCV, MCH and MCHC level in hepatopancreas, value difference

Week	control	Exposure					MCV (fL)						Depuration				
		Mel	Blank	0.4 mgCd/L+Mel	0.4 mgCd/L	4.0 mgCd/L+Mel	4.0 mgCd/L	<i>r</i>	Statistic	0.4 mgCd/L+Mel-dep	0.4 mgCd/L-dep	4.0 mg Cd/L+ Mel-dep	4.0 mgCd/L-dep	<i>r</i>	Statistic		
0	380.9± 21.40 Aac	401.8± 35.23 Aa	390.2± 19.65 Aa	388.1± 27.80 Aa	391.5± 23.65 Aa	399.5± 29.9 Aa	388.0± 26.29 Aa	NS	NS	NT	NT	NT	NT	NT	NT		
1	385.8± 15.36 Aa	357.0± 26.93 ACab	361.4± 25.14 ACab	255.3± 22.37 Bb	338.6± 23.29 Aac	331.8± 13.24 Cab	345.4± 13.13 ACac	NS	p<0.0221	NT	NT	NT	NT	NT	NT		
4	330.2± 13.19 ACbc	317.4± 13.40 ABab	334.4± 14.42 AB-	285.7± 15.06 Bbc	353.7± 14.18 ACac	284.2± 25.36 ABbc	363.4± 12.67 Ca	NS	NS	NT	NT	NT	NT	NT	NT		
7	319.1± 12.57 Ab	293.9± 11.57 ACab	320.6± 20.64 Ab	307.5± 12.22 ACc	244.5± 10.59 Bb	260.8± 12.60 Cc	249.2± 15.80 CBb	-0.46***	p<0.0010	NT	NT	NT	NT	NT	NT		
10	360.6± 10.22 ACac	316.1± 18.57 ADab	324.2± 11.88 ADb	388.4± 17.85 Ca	361.9± 13.52 ACa	223.3± 6.98 Bc	312.4± 7.96 Dc	-0.37**	p<0.0008	316.5± 18.37 Ac	379.0± 32.65 Ac	329.9± 10.89 Ad	334.6± 10.06 Ac	NS	NS		
13	316.5± 10.45 Ab	379.0± 20.57 ABCab	329.9± 11.19 ABb	334.6± 19.98 Ba	300.1± 7.65 Ac	349.2± 10.22 Bab	261.4± 7.67 Cb	NS	p<0.0001	317.1± 13.53 Ac	306.6± 12.18 Ad	290.9± 14.79 Ad	285.5± 15.83 Ac	NS	NS		
0	155.9± 7.52 Aa	159.3± 6.44 Aa	161.4± 6.83 Aa	133.9± 4.25 Aa	161.3± 4.74 Aa	159.3± 6.63 A	159.4± 6.14 Aa	NS	NS	NT	NT	NT	NT	NT	NT		
1	148.1± 13.20 ACab	116.3± 6.91 ABb	123.8± 10.64 ABCb	98.75± 6.70 Bb	158.9± 11.27 Cab	100.6± 7.21 Bbc	146.2± 4.01 Cac	0.44**	p<0.0002	NT	NT	NT	NT	NT	NT		
4	118.1± 7.52 ACbc	110.9± 8.40 ABCbc	122.3± 6.47 ACb	93.87± 7.84 Bb	134.4± 11.42 ACab	97.83± 10.70 ABbc	126.2± 6.29 Cbc	NS	p<0.034	NT	NT	NT	NT	NT	NT		

	Exposure										Depuration									
	MCHC (g/dL)																			
7	95.25± 4.46 Ac	88.38± 7.96 ABc	96.31± 8.61 ABb	97.08± 6.73 Ab	70.85± 2.83 Cc	76.74± 3.43 Bb	84.99± 6.67 ABCd	-0.30*	p<0.0067	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
10	95.88± 4.66 ACc	111.9± 7.05 ABbc	108.2± 7.06 ABb	135.1± 15.37 Bab	127.9± 6.40 Bb	88.06± 4.61 Cb	109.1± 7.93 ABCbcd	NS	p<0.0055	61.48± 5.99 Dc	132.3± 11.28 Bd	130.8± 10.10 Bc	92.08± 7.98 Cd	NS	p<0.0001					
0	41.46± 2.60 Aac	41.12± 3.35 Aa	41.7± 1.90 Aa	41.24± 4.44 Aa	41.97± 2.54 Aac	41.97± 2.54 Aac	42.06± 2.84 Aac	NS	NS	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
1	38.39±2.90 ACDac	32.93± 1.11 ABabc	34.14± 1.63 ABCb	39.30± 1.97 Cab	47.3± 2.30 Da	30.11± 1.33 Bbd	42.56± 1.46 Da	0.032**	p<0.001	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
4	35.84± 1.65 ADa	35.01± 1.54 Bab	37.83± 3.61 ABab	33.08± 2.63 Aab	38.39± 1.91 Cc	34.27± 1.44 Bac	34.75± 1.30 DCc	NS	NS	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
7	30.04± 1.54 Ab	29.89± 1.80 ABabc	28.45± 0.82 Ac	31.67± 2.07 Ab	29.09± 0.94 Ab	29.52± 0.75 Acd	34.94± 1.09 Bc	NS	NS	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
10	27.31± 2.03 Ab	35.46± 1.44 Ba	33.28± 1.45 BDb	34.22± 1.42 Bab	35.42± 0.90 Bc	39.68± 2.32 Bab	34.81± 1.19 Bc													
13	40.45± 1.49 Ac	29.26± 1.36 Bc	31.88± 1.76 Bbc	32.60± 1.59 Bb	40.29± 2.57 Aac	29.68± 1.35 Bd	51.71± 2.06 Cb													

NT – not tested, NS – not significant.

Capital letters denote statistically significant differences ($P<0.05$) between the groups in different exposure weeks, while small letters indicate significant differences in the groups between successive weeks of exposure * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Haematological parameters

The results of haematological parameters analysis in the blood of fish exposed to cadmium and cadmium with melatonin are presented in Tables 3–5.

In the group of fish exposed to the highest tested concentration of Cd (4.0 mgCd/L), a significant ($P<0.05$) increase in the haemoglobin content was observed in comparison to the control group after the 1st, 10th and 13th week of exposure. The highest haemoglobin content was recorded after the 13th week of exposure to 4.0 mgCd/L. Simultaneously, co-administration of cadmium and melatonin (4.0 mgCd/L+Mel) significantly reduced the haemoglobin content in comparison to the 4.0 mgCd/L group (1st and 4th week) or both the 4.0 mgCd/L group and the control group (13th week). The Spearman's correlation for the relationship of the Hb level to the Cd concentration in the blood during the exposure was found after the 1st and 10th weeks of the exposure period (Table 3).

In the group of fish exposed to the highest concentration of Cd (4.0 mgCd/L), the haematocrit value significantly increased in the 1st, 4th and 10th week of exposure when compared to the control group. Administration of melatonin together with cadmium (4.0 mgCd/L+Mel) significantly ($P<0.05$) reduced the haematocrit value in comparison to single-exposed individuals (4.0 mgCd/L) (4th week) and to the control value (7th week). The Spearman's correlation did not confirm any association between haematocrit content and Cd concentration in the blood during the exposure, and a negative correlation was only noted after the 7th week (Table 3).

The fish exposed to Cd in water (4.0 mgCd/L) showed significantly higher RBC counts than in the control after the 1st, 10th and 13th week of exposure. However, the administration of melatonin in combination with cadmium (4.0 mgCd/L+Mel) significantly reduced ($P<0.05$) the RBC count compared to cadmium-treated fish (4.0 mgCd/L) in the 7th and 13th week. A statistically significant positive correlation between RBC and the cadmium concentration in the blood was observed after the 1st, 10th and 13th week of exposure (Table 4).

In fish from all groups implanted with melatonin, a significant ($P<0.05$) increase in WBC was noted after the 1st and 4th week of exposure in comparison to the control group.

The WBC was increased significantly ($P<0.05$) after 3 weeks of depuration in the fish group previously exposed to the highest doses of the metal, while after 6 weeks of depuration, the WBC count was similar in all groups tested (Table 4).

The MCV value exhibited a significant decrease in the group of fish exposed to the highest concentration of Cd (4.0 mgCd/L) when compared to the control group in the 7th, 10th and 13th week of exposure. A similar phenomenon occurred in fish from the 4.0 mgCd/L+Mel group after the 1st, 7th and 10th week. A significant negative relationship between MCV value and cadmium concentration in the blood during the exposure was found after the 7th and 10th week of exposure (Table 5).

The MCH value decreased significantly ($P<0.05$) in the group with melatonin (4.0 mgCd/L+Mel) after the 1st, 4th, and 13th week of exposure when compared to the group of fish exposed to Cd without melatonin (4.0 mgCd/L) (Table 5).

The MCHC value increased significantly ($P<0.5$) in fish exposed to Cd (4.0 mgCd/L) compared to the control group after the 7th, 10th and 13th week of

exposure. The MCHC value decreased significantly in group 4.0 mgCd/L+Mel after the 7th and 13th week of exposure when compared to animals not treated with melatonin (4.0 mgCd/L). A significant positive relationship between the MCHC value and cadmium concentration in the blood during the exposure was found after the 1th and 10th week. After 6 weeks of depuration, a statistically significant increase ($P<0.05$) was observed in the MCHC value in fish previously exposed to the highest doses of the metal (Table 5).

Discussion

Accumulation

In the present study, female Prussian carp exposed to cadmium demonstrated the increased Cd accumulation in the kidney, spleen and heart along with an increasing waterborne Cd concentration and the time of exposure. Furthermore, the results in female Prussian carp indicated that more than 3 and 17 times higher levels of Cd accumulated in the kidney than in the spleen and heart respectively (Table 2). Similarly, Sun et al. (2019), Ashraf (2005) and Ghiasi et al. (2011) also found an obvious higher cadmium accumulation in the kidney than in the spleen and heart. The increased concentration of Cd in the kidney could be due to the detoxification function of this organ and elimination of toxic substances. Moreover, proximal renal tubule cells are the main target for cadmium toxicity in the kidney (Prozialeck and Edwards, 2012).

In fish, the spleen and kidney have important functions in implementing immune defence and the maintenance of a stable internal environment (Kumar et al., 2018). In the kidney, both chronic and acute intoxication of cadmium have been indicated to cause nephropathies. As a result of the cadmium accumulation in the kidney, the tubular cells degenerate and the resorption processes are disturbed (Prozialeck and Edwards, 2012). Pollution such as heavy metals causes structural and functional changes in the spleen (Taheri et al., 2016). The increase in cadmium accumulation can lead to the destruction of the splenic white pulp, which may cause a reduction in lymphocytes, a reaction to pollutant stress, i.e., a drop in immunity (Arantes et al., 2016; Sun et al., 2019).

Melatonin is a naturally occurring antioxidant synthesized in the pineal gland that plays an important role in the protective effect against oxidative damage generated by a variety of heavy metals (including Cd), which was previously indicated by Drąg-Kozak et al. (2019). In this study, the melatonin implantation group showed a decrease in the cadmium concentration in tissues of fish exposed to the highest concentration of this metal in water. This decrease was observed after the 1st week of exposure and persisted to the end of the exposure period (Table 2). Although cadmium levels were still higher than in the control group, this finding can be accepted as a protective effect of melatonin against cadmium accumulation in tissues. According to Chwelatuiuk et al. (2006), Hussein et al. (2014) and Drąg-Kozak et al. (2018, 2019), after the administration of melatonin, the cadmium concentration decreases in animal tissues. Moreover, studies by Majumder et al. (2019) and Mitra et al. (2019) demonstrated that all tissue damage caused by cadmium was mitigated, wholly or

partially, by the administration of melatonin (the administration of melatonin prevented tissue damage).

The accumulation of cadmium in the kidney and spleen as the organs of the haematopoietic system may disturb haematopoietic processes (Taheri et al., 2016; Kumar et al., 2018). Kondera and Witeska (2013) and Sun et al. (2019) reported that cadmium reduced the haematopoietic potential in common carp (*Cyprinus carpio* L.) and yellow catfish (*Pelteobagrus fulvidraco*) in the head kidney and spleen, and caused histological lesions, antioxidative and inflammatory responses in both tissues.

The results of the present study showed that after the 1st week of the exposure of fish to the highest concentration of cadmium, its level in the blood increased and continued to increase until the end of the exposure (Table 3). These results were in line with those presented by Witeska (2003) and Kondera et al. (2014), who also found an obvious cadmium accumulation in the blood of common carp (*Cyprinus carpio*) exposed to this metal. About 50–60% of the cadmium in the bloodstream is found in erythrocytes in a form bound to a blood cell membrane or haemoglobin. The remaining part of this cadmium is transported in complex with albumin, cysteine and glutathione (Rubino, 2015).

In the present study, RBC and Hb were found to be significantly elevated after the 1st, 10th and 13th weeks of exposure to the highest concentration of cadmium. The increase in Ht was also significant after the 1st and 10th weeks of exposure (Table 3 and 4). Similar results have been observed by other authors. An increase in red blood parameters such as Ht, RBC and Hb after the exposure to cadmium was reported by Witeska et al. (2010) in common carp, *Cyprinus carpio* and by Lal Shah (2010) in tench, *Tinca tinca*. Moreover, the present study demonstrated a decrease in the MCV after the 7th, 10th and 13th week of exposure to the highest dose of cadmium, accompanied by an increase in the RBC count (Table 4 and 5). Similarly, Tort and Hernández-Pascual (1990) reported a decrease in the mean cell volume (MCV) and an accompanying increase in the number of red blood corpuscles after the sub-lethal cadmium exposure in dogfish (*Sarotherodon canicula*). An increase in MCHC values was observed after the 7th, 10th and 13th week of exposure in the group exposed to the highest dose of cadmium compared with the control group, which may suggest the stimulation of haemoglobin synthesis, which is consistent with the results of this study (Table 5). An increase in MCHC values has also been observed in fish exposed to copper and arsenic (Carvalho and Fernandes, 2006), as well as to cadmium (Dras-tichová et al., 2004).

The observed results of elevated levels of erythrocytes, haematocrit and haemoglobin in blood could be the response of organism to hypoxia, as the compensation of the oxygen deficiency (Lal Shah, 2010). Hypoxia may be caused by blood oxygen deficiency resulting from cadmium damage to the gills, which inhibits gas exchange and disrupts osmoregulation (Atli et al., 2016; Yancheva et al., 2016; Kayhan et al., 2017). The results of fish behaviour observation indicated an increase in opercular movement, a tendency of the fish to congregate next to the filter and erratic swimming in the groups of fish exposed to cadmium, which suggest that the fish had respiratory problems. Similarly, erratic swimming, increased breathing, accelerated ventilation with rapid arrhythmic opercular movement, frequent surfacing and loss

of equilibrium have been reported in guppy (*Poecilia reticulata*) and tench (*Tinca tinca*) after exposure to cadmium (Yilmaz et al., 2004; Lal Shah, 2010).

The abnormal oxygen intake (due to the impaired function of gills) may be compensated for by the release of erythrocytes from the spleen, where they are stored when the oxygen-carrying capacity must be increased to meet the increased oxygen demand (Parkash, 2016), which could be the reason of RBC enhancement observed in the study. On the other hand, an increase in the number of red blood cells may occur as a result of oxidative stress caused by cadmium, which accumulates in the spleen and, as demonstrated in the present study, may impair splenic function and cause an increase in the erythropoietic activity of the organ (Houston et al., 1996). Hypoxia may also occur when the arterial oxygen content is sufficient to meet the standard oxygen demand, and the oxygen deficiency is caused by the failure of the cardiovascular system, which is not able to ensure the normal blood flow. This may result from exposure to cadmium, which impairs the function of the oxygen transport system in the mitochondria (histotoxic hypoxia) or impairs tissues uptake of oxygen transported in the blood (methemoglobin) (Onukwufor et al., 2014). In the present study, an increase in the concentration of cadmium in the heart was already observed after one week of exposure and this increase persisted until the end of the exposure period (Table 2). Numerous studies on animals have shown that cadmium affects the function of the cardiovascular system through various mechanisms, for instance, by causing reactive oxygen species (ROS) production, which results in lipid peroxidation and changes in antioxidative enzymes, thus leading to oxidative stress (Alissa and Ferns, 2011; Mitra et al., 2019). Studies conducted by Soares et al. (2008) and Kumar and Singh (2010) on different species of fish have shown that cadmium accumulates in the blood and cardiac subcellular fractions and induces various responses in enzymatic antioxidative defence mechanisms. Studies on animals have also demonstrated that cadmium reduces myocardial contractility, which results in decreased stroke volume and cardiac output (Chen et al., 2015; Türkcan et al., 2015). Moreover, it affects the cardiac conduction system and impairs physiological and biochemical processes by blocking calcium channels (Limaye and Shaikh, 1999; Pawlak et al., 2013). Other *in vitro* and *in vivo* studies have also shown that cadmium has direct effects on the heart wall, which results in decreased coronary blood flow (Chen et al., 2015; Türkcan et al., 2015). The present study revealed that melatonin significantly reduced the accumulation of cadmium in the heart of the fish exposed to the highest dose of cadmium already after one week of exposure and that the reduction persisted until the end of the experiment (Table 2). Melatonin has a cardioprotective effect and prevents ischaemia-reperfusion injuries in different organs, including the heart. It may affect the cardiovascular system directly, through its receptors in the heart and arteries, or indirectly, by modulating the function of the nervous system or the immune system.

Melatonin, administered together with cadmium, prevented an excessive increase in accumulation and negative effects (Sun et al., 2016; Nduhirabandi and Maaman, 2018).

The mechanism by which melatonin reduces the concentration of heavy metals in tissues and affects blood parameters is not exactly clear. Different mechanisms are

probably involved in the prevention of the toxic effects of cadmium on blood parameters and cadmium accumulation in tissues by melatonin. Melatonin is likely to have a variety of action: it may induce cell antioxidative mechanisms, it is an effective metal chelating agent, it is very soluble in fats and can therefore pass freely through all cell barriers, thus facilitating the elimination of cadmium from tissues, and by repairing biological targets (Galano and Reiter, 2018; Mitra et al., 2019).

Changes in haematological parameters are also related to defence mechanisms (leukocyte counts). Leukocytes (white blood cells) are an important part of the organism defence system (Parkash, 2016). In the present study, no changes in the WBC count were observed in the groups exposed to cadmium. However, a significant increase in the WBC count was found in the melatonin-treated groups after the 1st and 4th week of exposure (Table 4). Those results corroborate those presented in previous studies, which demonstrated that the administration of melatonin increased the number of leukocytes in different animal species (Ahmed et al., 2011; Kurhaluk et al., 2017). The exact mechanism responsible for the melatonin-induced increase in the number of leukocytes is not known. However, in this case several mechanisms may be involved. One of them could be the direct effect of melatonin on the anterior/head kidney and spleen, which are lymphatic and haematopoietic tissues in fish, resulting in the acceleration of leukocytopoiesis (Esteban et al., 2013; Ngasainao and Lukram, 2016; Hoseini et al., 2019).

Depuration

Studies on the elimination of heavy metals from tissues and blood are important from the perspective of health. They make it possible to determine the ability of contaminated organisms to self-depurate and to assess the biological half-life of different metal contaminants (Kim et al., 2004). There are more metal elimination mechanisms than metal uptake mechanisms. However, metals accumulate at a faster rate than they are eliminated, which is probably due to the presence of metal-binding proteins. Metals are usually eliminated in fish through bile, urine, gills and mucus. Several factors influence the elimination of metals from fish tissues. Those factors include temperature, time, the age of the fish, their metabolic activity and the biological half-life of the metal concerned (Kalay and Canli, 2000; Kim et al., 2004). Information on the elimination of cadmium from organs and tissues such as the heart, spleen and blood is relatively limited in the available literature. In the present study, the order in which Cd was eliminated from the organs and tissues of the Prussian carp in the excretion period was as follows: heart > blood > kidney > spleen.

With all the concentrations analysed, the fastest cadmium elimination rate was observed in the heart, whereas a decrease in the concentration of Cd in the blood was observed after 6 weeks of detoxification. As for the spleen, an increase in the concentration of Cd was observed both after the 3rd and 6th week of the detoxification period in the group previously exposed to the highest Cd concentrations compared to the concentration observed after 7 weeks of exposure. In turn, the cadmium concentration in the kidney increased after the 3rd week of detoxification. After the 6th week, it decreased to the levels observed after 7 weeks of exposure in the groups

exposed to the highest Cd concentration in water. Relatively low metal elimination rates were observed in the kidney (Table 2). This was probably due to the movement of the metal from other tissues to the kidney for the purpose of detoxification. The highest concentration of the metal in the spleen (and kidney) observed after the exposure cessation may be due to continued redistribution between organs and tissues (Table 2). Moreover, the increase in cadmium concentration in the spleen during the detoxification period may be linked to the function of the spleen as a selective filter of the vascular system and an organ which performs haemocateresis, whose aim is to destroy old red blood cells that may contain Cd (Sales et al., 2017).

An increase in the cadmium concentration in the kidney and spleen during the excretion phase was also observed in the group of melatonin-treated fish which were previously exposed to the highest Cd concentration. However, the increase was smaller than the one observed in the group which did not receive melatonin. In turn, a decrease in cadmium concentration in the heart was observed in the melatonin-treated group. The decrease was significantly lower than that observed in the groups which did not receive a melatonin implant, which confirms the protective role of that indoloamine (Table 2).

Conclusion

In conclusion, the present study shows that the administration of melatonin significantly reduced the accumulation of Cd in all the analysed tissues and blood of Prussian carp and prevented the negative effects of cadmium on blood parameters. Melatonin as a natural antioxidant and a potent scavenger of free radicals may play an important role in the protection of cells from oxidative damage caused by cadmium. Thus, melatonin may be an effective agent able to prevent and mitigate, at least partially, the toxicity of that metal. Based on the results presented in this paper, as well as the reports of other authors, we believe that the administration of melatonin may be an appropriate method for treating acute or chronic Cd toxicity, especially considering its safety margin and low cost.

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