Spirulina - a "Green" Chelating Agent in Lead-Intoxicated Freshwater Fish

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Abstract

Our work mainly focused on highlight the histological alterations induced by experimental lead poisoning on *Carassius gibelio* Bloch species and lead-detoxifying ability of spirulina powder added 2% in fish diet. 90 1 year old Prussian carps, were divided in 3 groups as follow: C (control group), E1 group (75 ppm Pb into water as $Pb(NO_3)_2$ x ½H_2O), E2 group (75 ppm Pb into water+2% spirulina powder in feed), according to the experimental protocol. Fish tissue (gills, intestine, liver, kidney and gonads) were sampled and histologically processed at the end of a 21-day trial period. Histological sections analyzed with an Olympus microscope have revealed the lead-harmful effects to the fish tissues, while spirulina powder has showed an ameliorating effect on the tissue structural changes caused by lead.

Keywords: freshwater fish, histological alterations, lead intoxication, spirulina powder

1. Introduction

Heavy metals are the most toxic inorganic pollutants impacting aquatic ecosystems [1, 2]. The sources of heavy metals in water are natural (volcanic eruptions and rock disaggregation) [3] and anthropogenic (fossil fuels combustion, mining, agriculture, nuclear power plants, industry) [4-7]. They are non-biodegradable pollutants that persist in the environment [8]. Heavy metals pose a particular threat to the health of aquatic animals, as they can easily enter their bodies via the skin, respiratory and gastrointestinal tract [9] and bioaccumulate [10].

Removing heavy metals from the body can be done through a medical procedure that involves the administration of chelating agents. They have an affinity for a certain heavy metal, with which they form complexes that are then easily eliminated from the body. But synthetic chelating agents exhibit not only therapeutic benefits, but a number of side effects, mainly associate to essential metals homeostasis disorders and their transport and the formation of depot complexes in certain organs [11-15].

On the other hand, there are also bioactive compounds from natural sources that can act as oxygen free radical scavengers or as metal chelators, which allows them to be used as natural antagonists of heavy metals [13, 14, 16, 17].

Spirulina is a cyanobacterium belonging to the blue-green algae group. It has been proven to exhibit antioxidant and chelating activity in many previous studies [17-19].

Lead is one of the most feared heavy metal [20], because nowadays, as a result of human activity, its level in the aquatic environment and industrialized societies is 3 times higher than in the pre-industrial ones.

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2. Materials and methods

Biological material

Test organisms in the experiment were 90 1 year old Prussian carps coming from a local fish farm located 46.42209N, 21.94159E in the west of Romania.

Animal housing

The fish were housed in 3 aquariums with a capacity of 60 l (stocking density of 30 fish/aquarium) equipped with individual air pumps where they were acclimatized to laboratory conditions for 2 weeks. Water quality indicators were daily monitored: water temperature and dissolved oxygen were measured using the Hanna Hi 9145 Water Resistant Processor Oxygenmeter; pH, NO^{-2}, NO^{-3}, water hardness were determined with TERMATEST kits.

Experimental Design

Fish were divided in 3 groups for a 21 days experimental period as follow: C (control group), E1 group (75 ppm Pb into water as $Pb(NO_3)_2$ x $½H_2O$), E2 group (75 ppm Pb into water+2% spirulina powder in feed). Once a week, the water from each aquarium was refilled, adding the appropriate concentration of Pb to the replaced amount.

Tested product and toxic metal

Freeze-dried spirulina (*Arthrospira platensis*) administered to the group E2 was acquired from Bucharest Medica Laboratories and incorporated into the feed in a proportion of 2%, going through the following stages: bringing the feed granules to very fine particle sizes; incorporation of freeze-dried material; homogenization and moistening of the obtained mixture; regranulation; drying the obtained granules.

The investigated metal - Pb as $Pb(NO_3)_2$ x $½H_2O_2$, was purchased from S. C. Corozin S. R. L. Timişoara and administered in water in a concentration of 75 ppm. The dose used was established based on the average lethal concentration LC50 for an interval of 3 weeks [26].

Animal feeding

Fish were fed 2 times/day with a granulated commercial product during the acclimatization and experimental period.

Sampling

Fish tissue (gills, intestine, liver, kidney) were sampled and histologically processed at the end of a 21-day trial period. Histological sections have been examined and photographed under Olympus light microscope equipped with a digital camera.

3. Results and discussion

Normal and histopathological tissue sections of C group Pb- intoxicated group (E1) are those of our previous report [27-29].

Gill

The morphological aspects observed in optical microscopy on the gill tissue shows an unaffected respiratory barrier in the case of individuals belonging to the control group (Figure 1). Instead, obvious histological alterations in the Pb-intoxicated group appear: disorganized gill lamellae architecture-hypertrophic lamellar epithelium cells with vacuolated cytoplasm; alteration of the connective structure in the lamellar axis; the fusion of the gill lamellae (Figure 2), massive epithelial and connective degenerations (Figure 3), with the shortening to the point of destruction of the secondary gill lamellae; vascular congestion.

In E2 group, the secondary gill lamellae, although thin, maintain the uniform appearance; slight vascular hypertrophies appear at the top of the primary (Figure 4) and secondary lamellae (Figure 5); only punctual hyperplastic territories are also present (Figure 6), suggesting a slightly affected respiratory barrier.

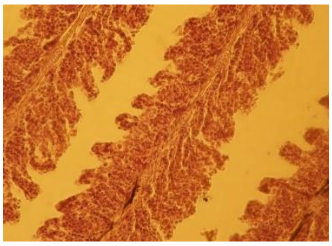

Figure 1. Gill-C
normal aspect

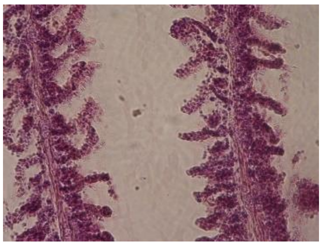

Figure 2. Gill–-E1
hypertrophic lamellar epithelium,
vacuolated cytoplasm
(H-E stain, 100x)

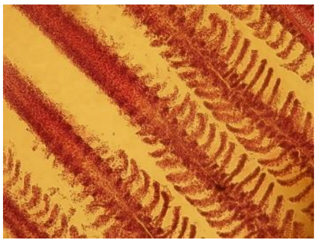

Figure 3. Gill-E1
massive epithelial and connective degenerations; secondary gill lamellae shortening; vascular congestion
(H-E stain, 100x)

In E2 group, the secondary gill lamellae, although thin, maintain the uniform appearance; slight vascular hypertrophies appear at the top of the primary (Figure 4) and secondary lamellae (Figure 5); only punctual hyperplastic territories are also present (Figure 6), suggesting a slightly affected respiratory barrier.

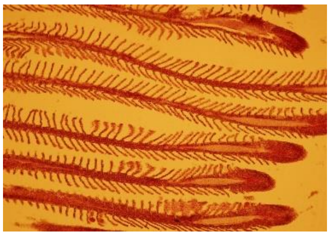

Figure 4. Gill-E2
punctual epithelial hypertrophy
(H-E stain E, 100x)

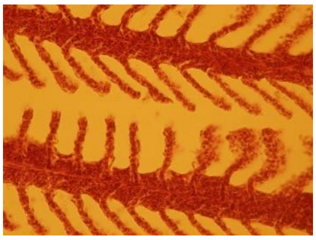

Figure 5. Gill E2
secondary lamellae epithelial hypertrophies (H-E stain, 100x)

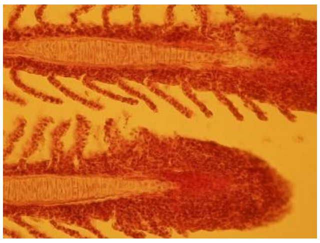

Figure 6. Gill-E2
cellular hyperplasia processes;
(H-E stain, 100x)

Intestine

The histological structure of the intestine of the control group is adapted for the function of: - completion of chemical digestion - secretion - absorption (Figure 7). Thus, the intestinal mucosa has dense villi, with a rectangular appearance. The intestinal epithelium is intact; it consists of absorptive cells (enterocytes) and goblet cells; the villous chorion that includes blood capillaries has a normal appearance (Figure 8). The intestinal mucosa of Pb-intoxicated fish has villi with a wide appearance and reduced in height (Figure 9).

Figure 7. Intestine-C
cross section
(H-E stain, 100x)

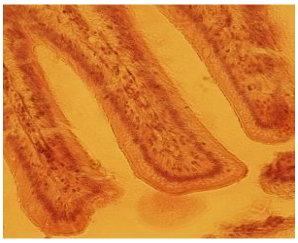

Figure 8. Intestine-C
enterocytes and goblet cells
blood capillaries with normal appearance (H-E stain, 100x)

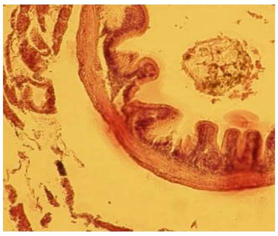

Figure 9. Intestine-E1
villi with wide appearance, reduced in height (H-E stain, 100x)

Villous territories with evident altered epithelium up to the disappearance of cells and microvilli; leukocyte infiltrative cells are present in the chorion (Figure 10). In group E2, the intestinal mucosa shows high villi, with a rectangular and triangular appearance. Only slight epithelial detachments are observed in the apical portion of the villi (Figure 11).

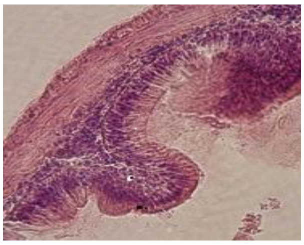

Figure 10. Intestine-E1
cross section - altered epithelium;
disappearance of cells and microvilli;
leukocyte infiltrative cells (H-E stain, 100x)

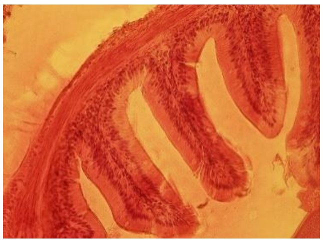

Figure 11. Intestine-E2
cross section; apical epithelial
detachments (H-E stain, 100x)

Liver

Liver parenchyma of control group specimens, consists of liver cells arranged in hepatocyte cords with an ordered appearance. The polygonal liver cells have spherical nucleus with an obvious nucleolus and heterochromatin on the inner face of the nuclear envelope (Figure 12 and Figure 13). Sinusoidal capillaries, with a normal appearance, are arranged between the hepatocyte cords. The liver of individuals subjected to chronic lead poisoning loses its normal architecture; hepatocyte cords have a disorganized appearance, hepatocytes are hypertrophic, with clear, vacuolated cytoplasm and pyknotic nucleus; frequent pericentrilobular necrosis with hypertrophies of the sinusoidal capillaries can be seen in the figure 14. In figure 15, the disorganized appearance of the hepatocyte cords and hypertrophy of the centrilobular vein is evident, and in figure 16, pericentrilobular hepatocyte necrosis with sinusoidal hypertrophies.

No structural and architectural changes were found in the fish liver that additionally received lyophilized spirulina in the daily diet. Sinusoidal capillaries with a normal appearance, are arranged among the ordered hepatocyte cords of these individuals (Figure 17).

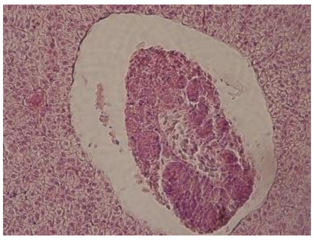

Figure 15. Liver-–E1
hypertrophic centrilobular vein
(H-E stain, 100x)

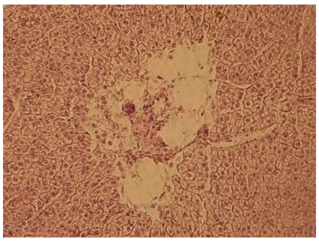

Figure 16. Liver-E1
pericentrilobular hepatocyte necrosis
sinusoidal hypertrophies
(H-E stain, 100x)

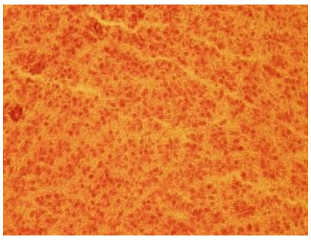

Figure 17. Liver-E2
ordered hepatocyte cords;
(H-E stain, 400x)

Kidney

Kidney histological sections of control group reveal homogeneous renal corpuscles in size and appearance and urinary tubules lined with cubic or prismatic nephrotic epithelium, provided at the apical pole with a brush border (Figure 18). Serious histological alterations of the renal parenchyma and stroma in fish experimentally contaminated with Pb can be observed: hypertrophic renal corpuscle with glomerular capillaries lesions and enlarged capsular space; peritubular edema (Figure 19), nephrocyte and interstitial necrosis (Figure 20 and Figure 21). On the histological slides obtained from the kidney of the fish that received spirulina in diet, the renal corpuscles are homogeneous in size and appearance.

Urinary tubes are lined with cubic or prismatic nephrotic epithelium, provided at the apical pole with a brush border. Only mild and infrequent peritubular edemas (Figure 22) were found.

The present study reveals that the fish exposed to Pb, suffered tissue structural alterations as a result of its accumulation in their organism and oxidative damages.

Thus, gills ensure the interface between the external and the internal environment, their structure (thinnest epithelium among all the organs) [30], facilitating the retention of toxic substances and their access to the body. Severe histopathological changes on the gill tissue of the heavy metal poisoned fish suggests damage to the respiratory barrier, as previous authors reported [27, 28, 31, 32]. Our founding on gills such as lamellar fusion, edema, hyperplasia, vacuolation and epithelial necrosis, congestion of blood vessel, were generally due to the toxic effects of lead, [31, 33]. Similar observation have been reported by Mustafa et al. 2020 [34] and Mustafa 2020 [35] in gills tissues of *Cyprinus carpio*, *Carasobarbus luteus* and *Luciobarbus xanthopterus* or Kaoud et al. (2012) [36] in *Oreochromis niloticus* exposed to heavy metal intoxication. The gastrointestinal tract is another major route of entry of wide varieties of toxic substances coming from the feed or the aquatic environment in which they live, into the fish body. Disrupting effect of Pb on intestine is marked by the histological alterations on its internal structure. Wall rupture, wider and shortening of villi height in Prussian carp from current study, are also found in juvenile Nile Tilapia *Oreochromis niloticus*, under experimental Pb poisoning [37] or in Asian sea bass *Lates calcarifer* (Bloch) exposed to copper [38].

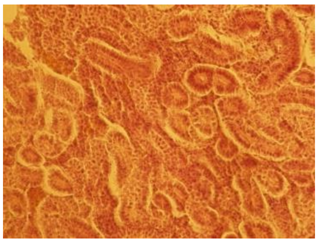

Figure 18. Kidney-C
normal structure of the renal parenchyma
(H-E stain, 100x)

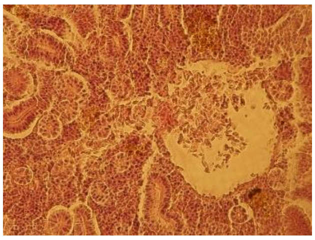

Figure 19. Kidney-E1
hypertrophic renal corpuscle;
glomerular capillaries lesions;
enlarged capsular space;
peritubular edema
(H-E stain, 100x)

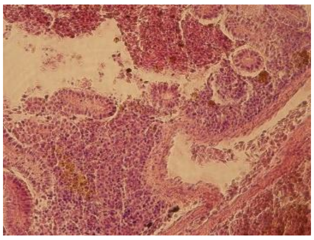

Figure 20. Kidney-E1
peritubular edema,
vascular hypertrophies,
nephrocyte and interstitial necrosis
(H-E stain, 100x)

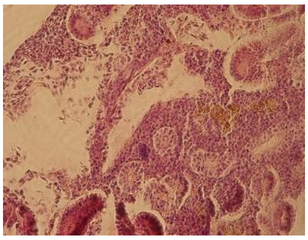

Figure 21. Kidney-E1
nephrocyte degenerative processes;
cytoplasm vacuolation (H-E stain, 10x)

Figure 22. Kidney-E2
mild peritubular edema (H-E stain, 10x)

Liver is one of the organs most exposed to harmful factors despite the many functions it performs in the body. It is also one of the most targeted organs by the polluting factors in the water, and their increased level in water and fish feed leads to exceeding liver detoxification and biotransformation capacity. Identical hepatic lesions caused by lead in our study, can be found in reports of many authors in freshwater fish exposed to heavy metal pollutants [34, 39]. Hepatocyte vacuolation and necrosis seems to be a common response to exposure of fish to a variety of toxic agents [40-43].

While the gills largely ensure the entry of heavy metals into the fish's body, the kidney is responsible for their "exit" control, which is why we observed severe changes at this level. Kidney are considered more susceptible to sever histopathological lesion than gill or liver tissue due to their intense blood perfusion [44]. Nephrotic and interstitial necrosis, peritubular edema, vascular hypertrophies, damaged glomerulus were have been found in Nile tilapia *Oreochromis niloticus* exposed to Pb, Ni and Cu [39] or in major carp *Catla catla* exposed to Cd and Cu [45].

4. Conclusions

We can conclude that: Pb induce histomorphological alterations in vital organs tissue structure; active principles from the spirulina powder show obvious chelating power; spirulina inclusion of in fish diet seems to be a beneficial approach aimed at reducing the oxidative stress induced by exposure to toxic agents.

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