Histological Aspects Regarding the Antioxidant and Chelating Potential of Chlorella in Experimental Pb Contamination of Carassius gibelio Bloch species

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Abstract

The purpose of this study was to highlight the histological aspect of some tissue of Prussian carp's specimens, subjected to sub lethal Pb intoxication with and without chlorella dietary supplementation.

90 Prussian carps, with weighing between 10 and 12 g were divided according to the following treatments for 21 days: C (without treatment), E1 (75 ppm Pb into water), E2 (75 ppm Pb into water+2% lyophilized chlorella in feed). Samples of skin, muscle and gonads were removed at the end of experimental period and analyzed in light microscopy and a specific QuickPHOTO Micro 2.2 software has been used for the histological study.

Mentioned epithelium suffered evident Pb-induced histomorphological alterations while the active principles of chlorella powder has attenuated the destructive action of lead to a greater or lesser extent, showing its chelating and antioxidant potential.

Keywords: chlorella, experimental lead intoxication, fish, histological lesions

1. Introduction

Heavy metals are chemical elements that are naturally present in ecological systems [1], but with exploitation they have become pollutants. All these exploitations have led to the appearance in the aquatic environment of the dangers from anthropogenic sources which have started to be much higher compared to the inputs from natural sources [2].

A main problem that is associated with the persistence of metals is related to their bioaccumulation and bio amplification ability, phenomena that may cause an increase of the metal level in the ecosystem [3], leading to longer-term risks in ecological systems.

Heavy metal contamination of ecosystems is a problem of great importance because they enter the food chains and have effects on the functioning of the biocenosis [4].

Lead and its compounds, if present in aquatic environments in sufficient amounts, can cause acute or chronic toxicity to organisms [5].

The role of various antioxidants in protection against heavy metal poisoning has been documented in many studies. Mourente et al., (2002) [6] and Senug et al., (2007) [7] reported that antioxidants contained in various natural sources significantly counteracted the effects of heavy metal toxicity on fish.

Chlorella, a single-cell green freshwater algae with the ability to bind heavy metals (in animal models) [8] has also been used to detoxify wastewater from metal contaminants [9].

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Chlorella's strong antioxidant capacity is associated with its phenolic compounds as salicylic, trans cinnamic, synaptic, chlorogenic, and caffeic acids found in the methanolic extract [10].

Fish have been at the center of many eco toxicological studies because they are at the top of the aquatic food chain, being considered ideal indicators of heavy metal contamination in aquatic ecosystems [11].

In view of the above-mentioned considerations, we set out to highlight the histopathological changes caused by lead experimental intoxication in some tissue of *Carassius gibelio* Bloch specimens and antioxidant and chelating potential of Chlorella against lead toxicity.

2. Materials and methods

Materials and methods are the same with those presented in a previous paper [12] except: the heavy metal investigated, in this case-Pb in dose of 75 ppm as $Pb(NO_3)_2$ x ½H_2O_2 and the tissue sampled – muscle, gonads and skin.

3. Results and discussion

Pb addition in water in a dose of 75 ppm, for 3 weeks resulted in its retention in the organs of intoxicated animals, as evidenced by histological alterations of the examined tissues.

The muscle is at the same time an edible part of the fish, but it is also the tissue that comes in close contact with the water-dissolved pollutants [13, 14]. According to Saad et al., (2012) [15], if fish from polluted waters show epithelial lesions of muscle tissue, it is very likely that they will be invaded by microorganisms that could cause severe epidermal pathology, resulting in the degeneration of the muscle connections.

Permanent histological slides performed on muscle tissue highlights the structural integrity of the fish organ in the control group (Figure 1 and Figure 2).

Figure 1. Muscle-LC
striated muscle tissue-longitudinal section overview; striated muscle fibers; endomysium
(H-E stain, 10x)

Figure 2. Muscle-LC
striated muscle tissue-cross section
(H-E stain, 10x)

In contrast, muscle fibers become thickened and inter fibrillary edema is present in individuals intoxicated with Pb (Figure 3). In addition, the muscle bundles degenerate and install focal necrotic processes (Figure 4).

The results of the present study are corroborated with the findings of Abbas and Ali (2007) [16], who observed identical histological lesions, such as the destruction and vacuolation of the muscle cells of the Oreochromis species, after exposure to chromium. Also, Patnaik et al. (2011) [17] studied *C. carpio* histology exposed to sub-lethal and lead concentrations. The authors reported the thickening and separation of the muscle fibers with intracellular edema.

Similarly, Fatama (2008) [18], has reported the degeneration of muscle connections together with the aggregation of inflammatory cells between them, focal areas of necrosis, vacuolar degeneration in muscle fibers and atrophy of muscle connections in fish exposed to different pollutants.

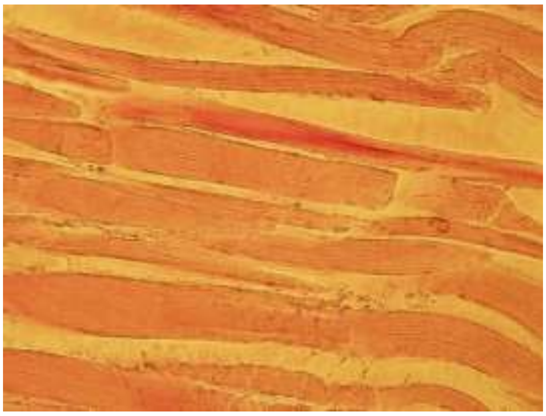

Figure 3. Muscle-LE1
thickened muscle fibers; interfibrillary edema
(H-E stain, 10x)

Figure 4. Muscle-LE1
degeneration of muscle bundles;
focal necrotic processes (H-E stain, 10x)

The microscopic observations reported in the LE2 specimens suggest the exercise of the protective effect of the active principles of chlorella in the muscle tissue. Thus, the muscle fibers appear only slightly thickened, the fibrillary lesions, and the interfibrillary edema, although persistent, is less severe (Figure 5 and Figure 6).

Figure 5. Muscle-LE2
slightly thickened muscle fibers
(H-E stain, 10x)

Figure 6. Muscle-LE2
fibrillar lesions;
mild interfibrillary edema (H-Estain, 10x)

Heterogeneous oocytes are present in the structure of the female gonad as development: the primary stage with oocytes with basophilic cytoplasm; the cortical alveolar stage, with the enlarged oocyte, the cytoplasm around the nucleus slightly opaque and the nucleoli are arranged on the inner face of the nuclear envelope. A very large number of oocytes are in the vitellogenic stage: the oocyte is increased in volume and the cytoplasm is occupied by vitellus granules (Figure 7 and Figure 8).

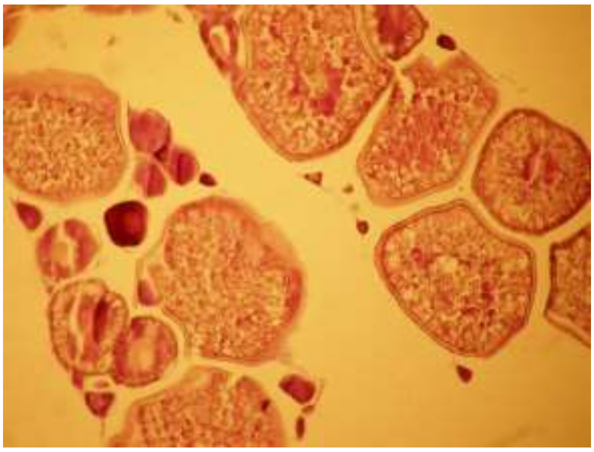

Figure 7. Ovary-LC
oocytes in varying degrees of development
(H-E stain, 10x)

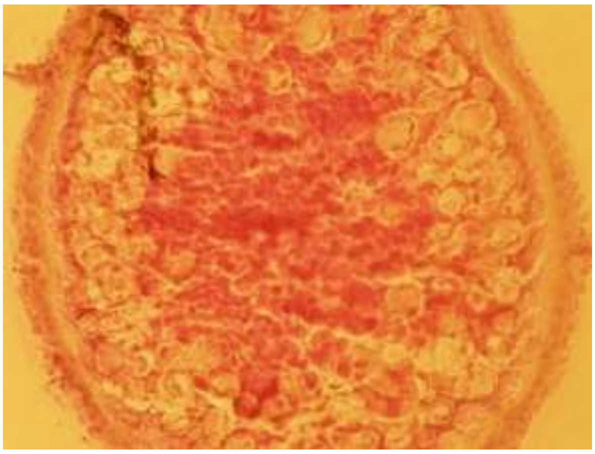

Figure 8. Ovary-LC-detail
(H-E stain, 40x)

Microscopic analysis of the histological slides obtained on the ovary reveals follicular atresia manifested both in the primary follicles, by processes of oocyte degeneration, but also in the follicles in the vitellogenic stage in intoxicated individuals. In this case, the atresia is manifested by processes of resorption of yolk granules and fusion in the central area (Figure 9 and Figure 10). Increasing the number of atretic follicles was also found by Doaa and Hanan (2013) [19] in *Oreochromis niloticus* specimens exposed to higher concentrations of lead acetate, and Fayhaa Salim (2015) [20] reported degenerative ova in Common carp under heavy metals pollution.

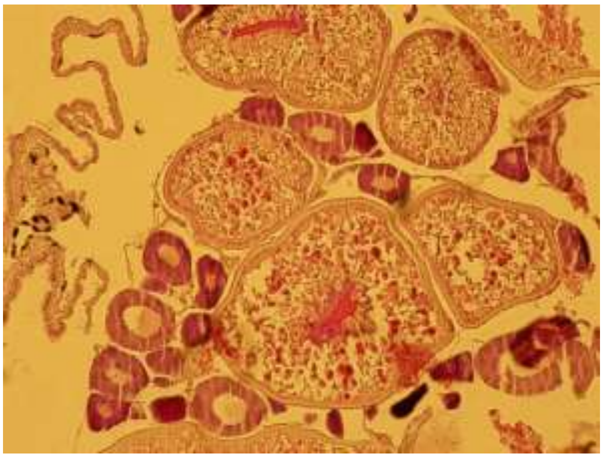

Figure 9. Ovary-LE1
follicular atresia (H-E stain, 10x)

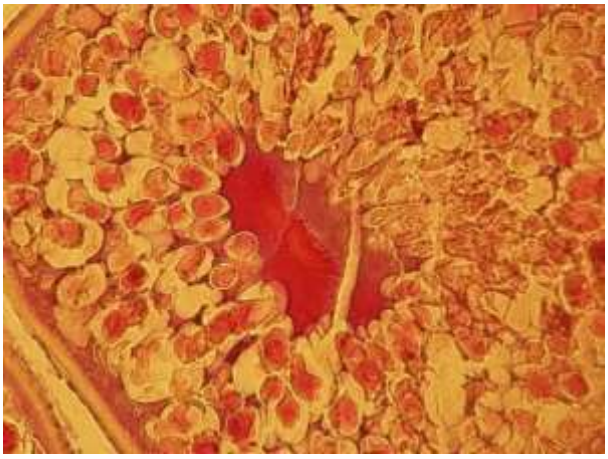

Figure 10. Ovary-LE1- detail
(H-E stain, 40x)

In the LE2 group, which received additionally chlorella in feed, the frequency of follicular atresia is reduced (Figure 11 and Figure 12), which means that, the protective effect of the active compounds is manifested on this organ.

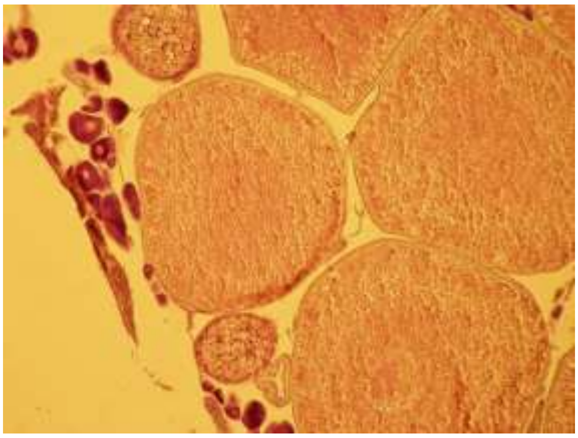

Figure 11. Ovary-LE2-
follicular atresia (H-E stain, 10x)

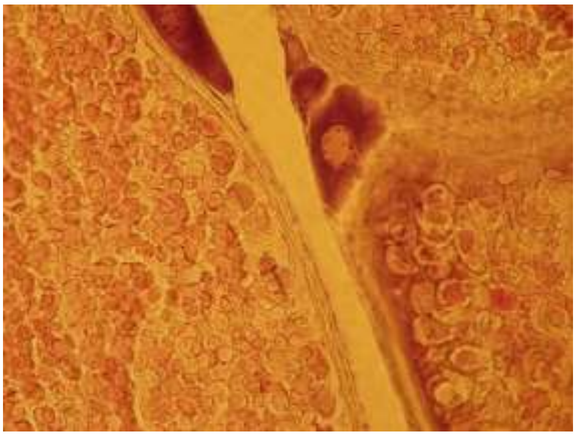

Figure 12. Ovary-LE2- detail
follicular atresia (H-E stain, 10x)

Microscopic analysis of the histological slides of testis highlights the structural integrity of the organ in the control individuals (Figure 13 and Figure 14). Thus, normal-looking seminiferous tubules are lined with intact seminiferous epithelium, and blood capillaries are visible in the interlobular interstitium.

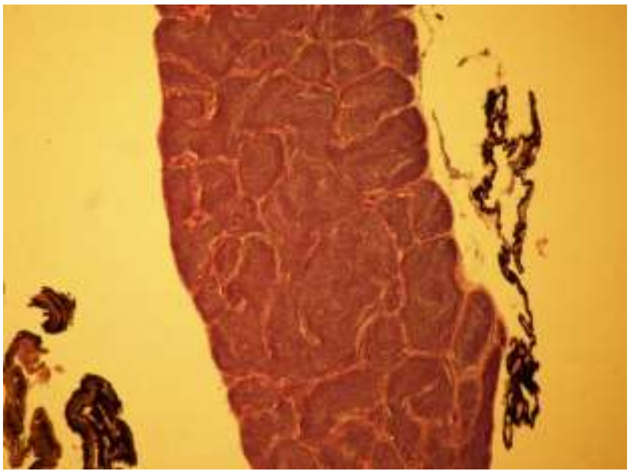

Figure 13. Testis-LM
overview (H-E stain, 10x)

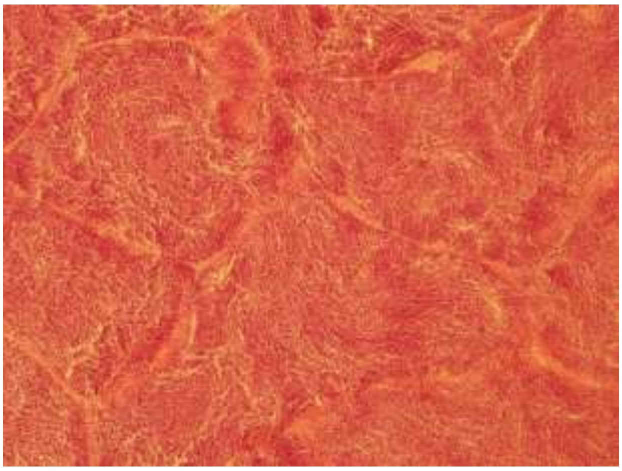

Figure 14. Testis-LM-detail
seminiferous tubules with normal appearance;
intact seminiferous epithelium;
interlobular interstitium with blood capillaries
(H-E stain, 40x)

Instead, clear histopathological changes are observed in the testicular parenchyma of the specimens from the group exposed to sub lethal doses of Pb: disorganized aspect of the testicular architecture; rupture of the wall of the seminiferous tubules, epithelial lesions with thinning of the seminal epithelium; diffuse interstitial hyperplasia, with Leydig cell damage, hypertrophies and vascular lesions (Figure 15 and Figure 16). Histopathological lesions in the testicular parenchyma become less severe in the LE2 group: it has a somewhat orderly appearance; the seminiferous tubules have an intact wall; only in restricted territories appear tubular lesions and disordered appearance of the parenchyma (Figure 17 and Figure 18).

As a result, lead has adverse effects of on the hypothalamic-pituitary-gonadal axis [20] suggesting a direct action on the gonads.

Figure 17. Testis-LE1- overview
rupture of the wall of the seminiferous tubules; epithelial lesions; diffuse interstitial hyperplasia, hypertrophies and vascular lesions (H-E stain, 10x)

Figure 18. Testis-LE1-detail
(H-E stain, 40x)

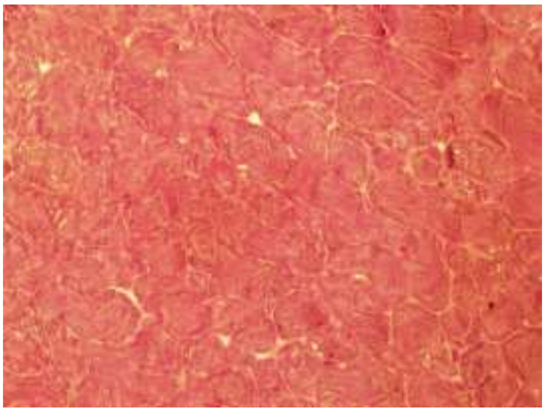

Figure 19. Testis-LE2-overview
testicular parenchyma;
seminiferous tubes with intact wall;
restricted tubular lesions
(H-E stain, 10x)

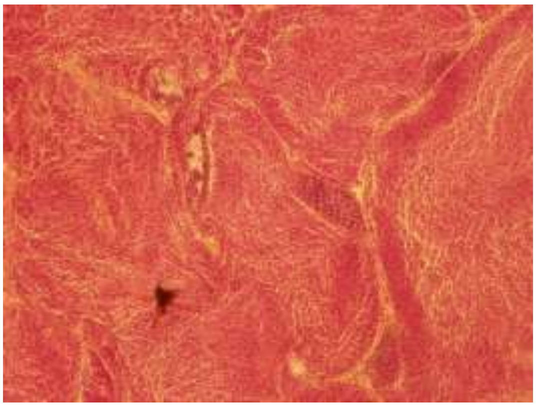

Figure 20. Testis-LE2-detail
disordered appearance of seminiferous tubules on restricted territories; mild vascular hypertrophy; mild hyperplasia in Leydig interstitial cells
(H-E stain, 40x)

Compared to the normal appearance of the sections obtained from the skin tissue of the control individuals (Figure 21 and Figure 22), in those intoxicated with Pb, lesions with exfoliation of the cell layers in the epidermis are observed; edema in the hypodermis and hyperchromatic territories in the epidermis (Figure 23 and Figure 24), which are also found in specimens that have received chlorella supplement in feed; in the latter, the exfoliation of the cell layers is absent (Figure 25 and Figure 26).

Alterations as inflammatory cells infiltration or sub cutaneous adipose tissue partly with fibrosis were noticed by Fayhaa Salim (2015) [20] in Common carp from heavy metals polluted freshwater. Our finds suggest that beside gills and digestive tract, fish can accumulate heavy metals from water by diffusion via skin.

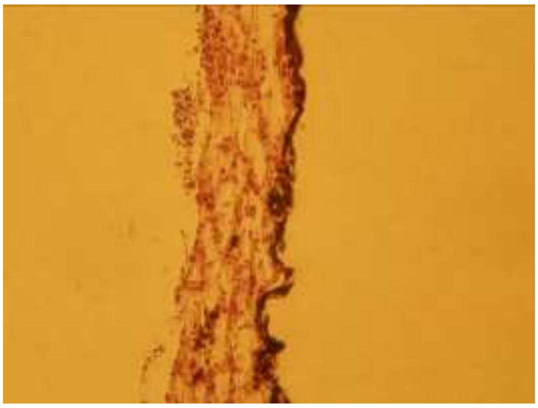

Figure 21. Skin-LC-Epidermis
(H-E stain, 10x)

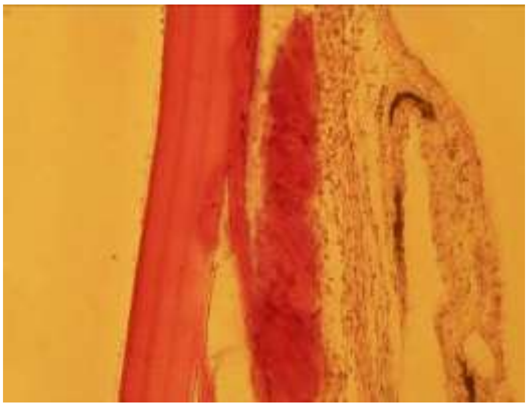

Figure 22. Skin-LC
Skin section-normal appearance (H-E stain, 10x)

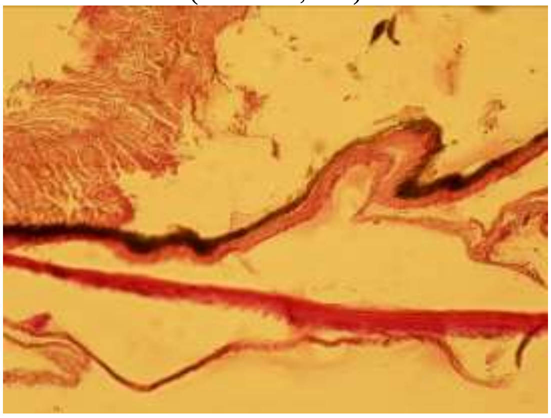

Figure 23. Skin-LE1
exfoliation of cell layers in the epidermis;
edema in the hypodermis (H-E stain, 10x)

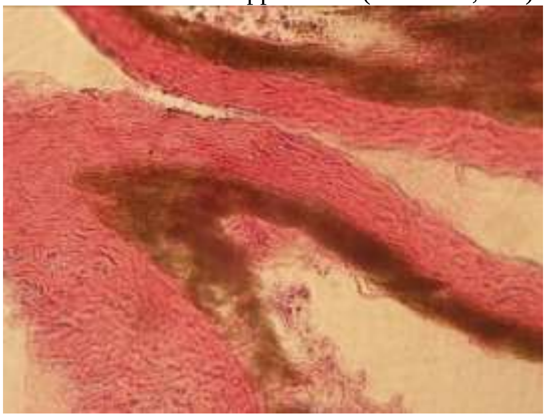

Figure 24. Skin-LE1
hyperchromatic territories in epidermis
(H-E stain, 10x)

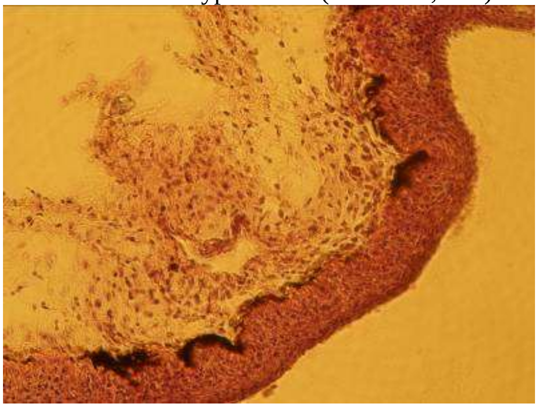

Figure 25. Skin-LE2
edema in the hypodermis (H-E stain, 10x)

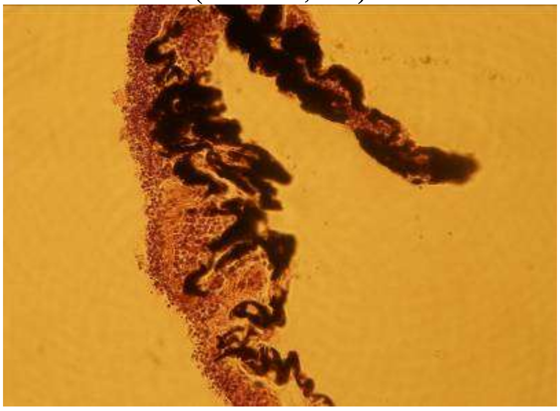

Figure 26. Skin-LE2
hyperchromatic territories (H-E stain, 10x)

4. Conclusions

The results obtained and presented in this paper, allow us to state the following conclusions:
- Pb has induced histopathological lesions and cellular alterations in muscle, gonads and skin belong to specimens exposed to sub lethal lead poisoning;
- tissue damage is less common and less severe in histological slides from individuals who have received chlorella in feed, which means that the active compounds in chlorella lyophilisate show chelating and antioxidant potential for Pb.

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