Garlic, Cilantro and Chlorella's Effect on Intestine Histoarchitecture Changes in Cd-Intoxicated Prussian Carp *(Carassius gibelio)*

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Abstract

Bioactive compounds from natural sources can act as oxygen free radical scavengers or metal chelators, which enables them to be used as natural antagonists to heavy metals toxicity. So the present study was carried out to compare histologically the aspect of intestine tissue of Prussian carp specimens, subjected to chronic Cd intoxication with and without garlic, cilantro and chlorella dietary supplementation.150 Prussian carps, with weight of 10-12 g were divided according to the following treatments for 21 days: C (without treatment), E1 (10 ppm Cd into water), E2 (10 ppm Cd into water+2% lyophilized garlic in feed), E3 (10 ppm Cd into water+2% lyophilized cilantro in feed), E4 (10 ppm Cd into water+2% lyophilized chlorella in feed). Cadmium toxicity and the potential protective effect of the three lyophilized products against the impact of cadmium toxicity were histopathologically assessed. For this purpose, fragments of intestine were removed and routinely processed at the end of experimental period and analyzed in light microscopy. A specific QuickPHOTO Micro 2.2 software has been used for the histological study. Tissue alterations were assessed using the histopathological score ranging from – to +++ depending on the degree and extend of lesions: (-) none, (+) mild occurrence, (++) moderate occurrence, (+++) severe occurrence. Our research findings show that Cd induces a significant increase in histopathological changes like vascular network hypertrophies and reach infiltrating leukocyte cells. In the same time, chlorella powder added to the fish diet, expressed the most effectiveness on the intestinal recovery of the cadmium-intoxicated fish followed by while cilantro and garlic powder.

Keywords: cadmium toxicity, fish, intestine histopathology, lyophilization, natural chelators.

1. Introduction

Cadmium is a non-essential metal which alters various physiological processes in the blood and tissue of fish [1, 2].

The common tool to reduce heavy metal poisoning is chelation therapy [3]. Natural chelating agents from natural sources [4-12] have heavy metal detox ability without causing any harm to other essential minerals of the body as synthetic chelating agents do [13-15.]

Beside its antibacterial [16, 17], anticarcinogenic [18, 19], hypolipidemic [20], hypoglycemic, [21, 22], antifungal [23, 24], cardio protective, chemotherapeutic, antidiabetic and hepatoprotective [25, 26], antiulcerogenic, [27, 28], anti-platelet aggregation, antibiotic, antioxidant properties [29, 30], garlic (*Allium sativum* L.) is antidote for heavy metal poisoning [31, 32], modulating the detoxification and defending against free radicals damage.

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So garlic's protective property against Cd and Pb toxicity can be attributed to (1) its antioxidative ability, provided by organo-sulphur compounds such as diallyl tetrasulfide; (2) its chelation ability, provided by sulphur-containing amino acids and compounds with free carboxyl and amino groups, which in turn promotes the excretion of Pb or Cd from the body; and (3) the prevention of Cd and Pb intestinal absorption, by its sulphur-containing amino acids such as S-allyl cysteine and S-allyl mercaptocysteine [3].

Cilantro (*Coriandrum sativum*) by its principal constituent – linalool - can bind and immobilize cadmium chloride from *liver and kidney* [33] and decrease toxic tissues cadmium accumulation in cultured rainbow trout *Oncorhynchus mykiss* [34]. Coriander seeds can promote the hepatic antioxidant system [35].

Chlorella, a unicellular green algae with the ability to bind cadmium (in animal models) [36, 37], has been used to detoxify wastewater of metal contaminants as well [38-41].

Histopathological biomarkers have been primarily used in fish to identify and evaluate the toxic effects of exposure to contaminants [42]. As such we have propose to investigate chelating and antioxidant property of chlorella, coriander and garlic in Prussian carps' cadmium-induced intoxication by comparative evaluation of the histopathological aspects of intestine tissue.

2. Materials and methods

Materials and methods are the same with those presented in a previous paper [43], except the tissue which was investigated.

3. Results and discussion

Histological study of intestine belong to the control group

Intestinal mucosa of control specimens shows dense villi with rectangular appearance. Epithelium lining mucosa is prismatic monolayer predominantly formed of enterocytes provided with microvilli at the apical pole (Photo 1), among which goblet cells are arranged (Photo 2).

Mucosal chorion consists of lax connective tissue composed of thin collagen fibers, fibroblasts and incorporates a vascular smooth network and a low leukocyte infiltration.

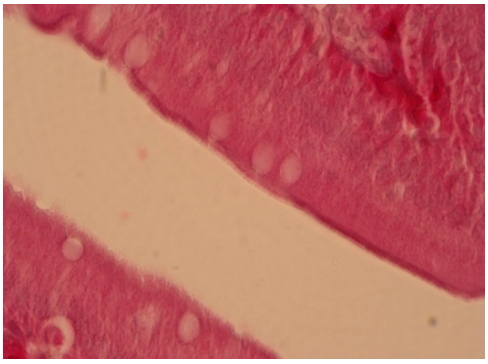

Photo 1. Intestine –C
prismatic monolayer epithelium;
enterocytes provided with microvilli at the apical pole
(trichrome Mallory staining, 400x)

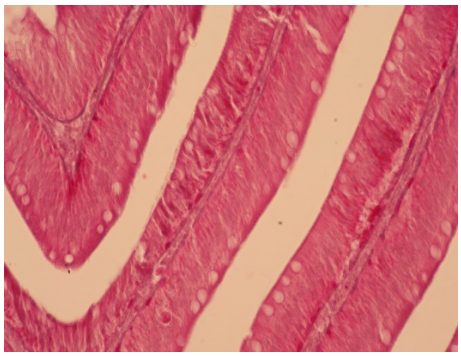

Photo 2. Intestine–C
goblet cells arranged between enterocytes
(trichrome Mallory staining, 1000x)

Histopathological study of intestine belong to E1 group

In the Cd-intoxicated group, villi of intestinal mucosa are rarer and more heterogeneous than those of the control one. Thus, the mucosa shows rectangular villi alternating with triangular extremely small villi. Mucosal epithelium seems slightly disorganized and rarefied, crossed by infiltrating leukocyte cells (Photo 3 and Photo 4).

Vascular network is hypertrophic in the lax connective tissue of the chorion villi structure, emphasized aspect to the top of the villi (Photo 5). Also, a noted aspect throughout of the connective tissue is the presence of leukocyte infiltrative cells in migrating to the surface of the mucosa.

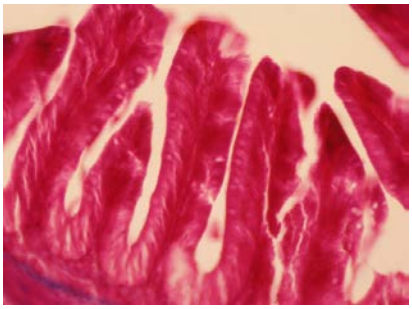

Photo 3. Intestine-E1
disorganized and rarefied epithelium;
infiltrating leukocyte cells
(trichrome Mallory staining, 400x)

Photo 4. Intestine-E1
disorganized and rarefied epithelium;
infiltrating leukocyte cells
(trichrome Mallory staining, 100x)

Table 1. List of severity of intestine lesions in Prussian carps specimens

Intestine's lesions	Control group	E1 10 ppm $CdCl_2$	E2 10 ppm $CdCl_2$ +2% garlic	E3 10 ppm $CdCl_2$ +2% cilantro	E4 10 ppm $CdCl_2$ +2% chlorella
hypertrophic vascular network	-	+++	+	-	+
leukocyte infiltrative cells	+	+++	++	++	+
disorganized and rarefied epithelium	-	+	-	-	-
goblet cells	+	+++	+	+	+

(−) none, (+) mild, (++) moderate, (+++) severe occurrence

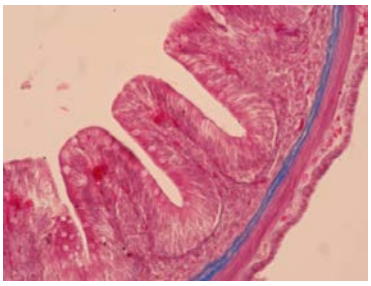

Photo 5. Intestine-E1
hypertrophic vascular network;
leukocyte infiltrative cells
(trichrome Mallory staining, 400x)

Histological appearance of these intestinal samples proves that digestive tract generally allow the heavy metal absorption due to consumption of feed, water or contaminated sediments.

Histopathological study of intestine belong to E2 group (Cd+garlic powder)

Intestinal mucosa has rectangular villi and numerous goblet cells are present among the absorptive cells of the epithelial structure. Subepithelial basement membrane is rich in collagen fibers and is evident. Villous and basal chorion contents of lax connective tissue, it shows a slightly hypertrophic vascular network and numerous infiltrating leukocyte in migration toward mucosal surface (Photo 6, Photo 7).

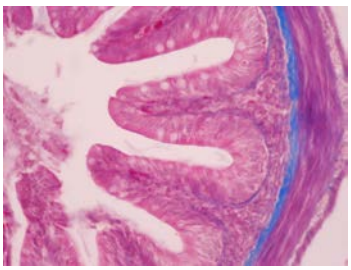

Photo 6. Intestine-E2
slightly hypertrophic vascular network;
infiltrating leukocyte
(trichrome Mallory staining, 400x)

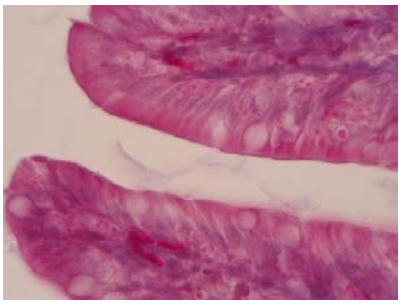

Photo 7. Intestine-E2
slightly hypertrophic vascular network;
infiltrating leukocyte
(trichrome Mallory staining, 1000x)

Histopathological study of intestine belong to E3 group (Cd+cilantro powder)

Intestinal villi of this group have a rectangular shape, and a homogenous development. Epithelium lining mucosa consists predominantly of enterocytes, provided with striated plateau to the apical pole, between which are numerous goblet cells. Subepithelial basement membrane and villi capillaries are obvious. Lax connective tissue, of mucosa chorion contains numerous infiltrative leukocyte cells (Photo 8 and Photo 9).

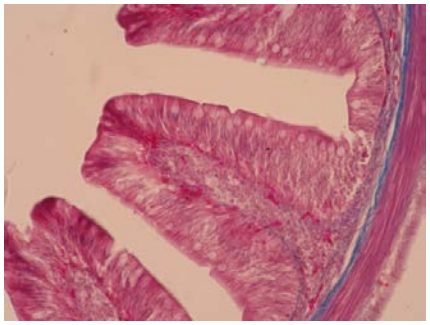

Photo 8. Intestine-E3
numerous infiltrative leukocyte
(trichrome Mallory staining, 400x)

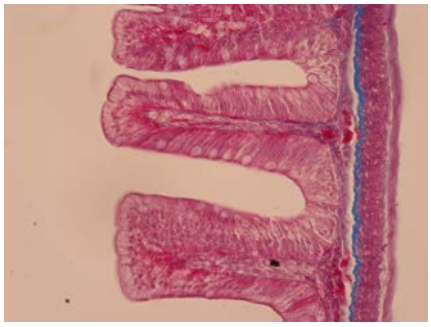

Photo 9. Intestine-E3
numerous infiltrative leukocytes
(trichrome Mallory staining, 400x)

Histopathological study of intestine belong to E4 group (Cd+chlorella powder)

Mucosa villi have a rectangular appearance and uniform size. Epithelium lining mucosa consists predominantly of enterocytes lining exhibiting a striated well developed plateau to their apical pole (Photo 10), and goblet cells are arranged between these cells.

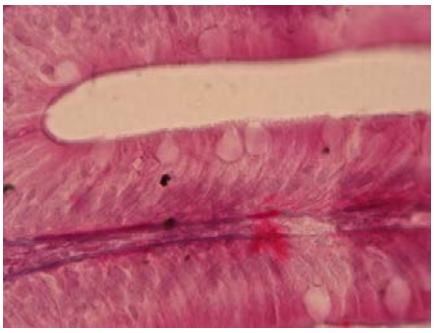

Photo 10. Intestine-E4
enterocytes; goblet cells
(trichrome Mallory staining, 1000x)

Histological damages detected in all intoxicated groups suggest that toxic metal enter the digestive tract of fish via the food, water or sediments that they consume, inducing an alteration of structures and functions in the fish's intestine.

Mucous-secreting goblet cells have proliferated and multiplied in all treated groups, indicating a defense mechanism against the severe pathological changes induced by $CdCl_2$ contamination as Sayed et al., 2013 [44] have noticed on intestine of Nile Tilapia, *Oreochromis niloticus*, exposed to sublethal concentrations of cadmium.

The similar pathologies but in different occurrence (Table 1) in the four Cd-intoxicated groups with and without garlic, cilantro or chlorella powder are in agreement with those observed by many investigators, studying the effects of metals on fish intestine [45, 46].

4. Conclusions

The present study has revealed severe damages to the intestinal tissues of the Prussian carps upon contamination of the fish environment with $CdCl_2$. Garlic, cilantro and chlorella powder have minimized the histopathological changes induced in intestine by sublethal exposure to $CdCl_2$.

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