EFFECTS OF PROLONGED EXPOSURE TO CADMIUM ON THE HEMATOPOIETIC ORGANS IN GRASS CARP (*CTENOPHARYNGODON IDELLA*, CYPRINIDAE)

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ABSTRACT

Destruction of spleen and kidney components in *C. idella* started on the 17th day in specimens subjected to 20 $\mu g \cdot L^{-1}$ Cd . The spleen was actually destructed after 73 days of exposure to 60 $\mu g \cdot L^{-1}$ Cd , where the two pulps can not be distinguished clearly, as the splenic blood vessels contained less blood cells . The kidney showed a complete destruction and the hematopoietic tissue lacked a distinguishable border whereas the necrosis covered large area after 60 days at 60 $\mu g \cdot L^{-1}$Cd .

The degree of destruction was lineary proportional to concentration of toxicant and period of exposure although the period was somewhat more effective .

It was concluded that the histological alterations in spleen and kidney could be used as an indicator for water pollution with cadmium and for fish health as well .

INTRODUCTION

Fishes inhabiting natural aquatic systems can be exposed to toxic metals, such as cadmium. Cadmium is a non-nutrient metal and toxic to fish even at low concentration, for example it is toxic to rainbow trout (*Oncorhynchus mykiss*, Salmonidae) when concentration exceeds 3 $\mu g \cdot L^{-1}$ for 30 days of exposure{1}.Likewise, it is toxic to steelhead *Salmo gairdneri* and coho salmon *Oncorhynchus kisutch* at 5.2 and 3.7 $\mu g \cdot L^{-1}$ respectively after more than a week of exposure {2}. The fluctuations in cadmium levels in fish seem to be not correlate with

seasonal variation in its concentration in water or diet rather than with pH, as observed in Arctic char (*Salvelinus aplinus,* Salmonidae) from an oligotrophic Alpine lake {3}.

Microscopic examination for the presence of lesions, can provide early indication of organ influence in relation to chemical exposure. Renal interstitial tissue, in addition to head kidney and spleen are major hemopoietic sites in the fish {4}. The kidney is a main target for metals {3}; also, the long-term storage can occur in kidney {1}. The spleen, in turn, is a main site for antibody production in fishes and basically wherever lymphocytes congregate {5}. The dysfunction of these two hematopoietic organs can affect the health, and so the growth of fish.

Grass carp *C. idella* is one of most cultivated fishes in Iraq at farms and some shallow aquatic systems for human consumption because of its conversion of vegetable matter into animal protein. Some aquatic systems may be polluted by cadmium as a result of car batteries thrown in them, batteries of sunk boats and some industrial discharges. The objective of this study was to investigate the effect of cadmium on the spleen and kidney in this species

MATERIALS AND METHODS

A total of 200 specimens of grass carp *C. idella* measuring 78-113mm in total length were employed in the investigation. The tests were conducted in 45 liter round plastic tanks with a water volume of 35 liter. Twenty five specimens were introduced in each tank. The fishes were acclimated for 21 days, where they fed a fresh grass and pellets of commercial diet. Fluorescent bulbs provided a photoperiod intervals approximate to that in nature. Air pumps provided continuous supply of oxygen. The pH of the aerated water was 7.6-7.8 as indicated by pH meter.

A stock solution of 1 $mg \bullet L^{-1}$ of cadmium was prepared by dissolving reagent grade $CdCl_2$. $2.5H_2O$ in deionized water and was diluted for the experimental doses to bring the concentrations 20, 40 and 60 $\mu g \bullet L^{-1}$ Cd, as there was a control treatment. Each treatment was conducted in two tanks. The dilution was carried out by adding the reagent to the water which filled half of tank to be filled later.

The time intervals for removing the animals from each tank were as follows: 1,4,6,14,17,24,35,55,60 and 73 days. At specific intervals mentioned above 2-3 fishes from each tank were sacrificed. Immediately, the spleen and kidney were fixed in Bouin's fixative, and then processed according to standard technique by preparation of paraffin sections with 5-6 µm in thickness {6}. The sections were stained with hematoxylin and eosin. The above procedure was repeated twice. Identification of kidney components based on {7}.

RESULTS

A. Spleen

The spleen occupies its natural site in the abdominal cavity of grass carp, similar to that known in other fishes.The histological sections examined with onset of investigation revealed an ordinary structure. The different levels of cadmium doses, as well as the capture in tanks, along with periods of exposure impaired the histological structure of spleen in diverse levels. The morphological changes of spleen were clearly visible only among 40 and 60 µg L^{-1} Cd doses.

Control Treatment

Spleen of control treatment showed that small melano-macrophage aggregates surrounded by white pulp started to form after 34 days of capture. The macrophage centres increased in number, although they were found as small yellow spots adjacent to melanin-containing cells in both red and white pulps. At the end of the study, the white pulp was well differentiated from the red, particularly on peripheral zones. The macrophage centres grew in volume rather than their count, along with macrophages increasing, whereas the melanin-containing cells still have the same level.

The most distinctive feature observed was a formation of two enlarged veins which facing each other on peripheral regions of spleen and filled with blood elements, but the arterioles retained their diameter (Fig. 1A).

No necrosis was observed at termination of investigation, but several cases of mitosis were noticed among white pupl cells.

20 µg $\bullet L^{-1}$ Cd Treatment

Histologically, aggregations of melanin-containing cells started to form after seven days as they developed along exposure period in several regions. The macrophage centres increased in number up to 10-11 centre$\bullet mm^{-2}$ after 35 days, attaining about 23 centre$\bullet mm^{-2}$ after 73 days. These centres are mostly small yellow coloured spots which spread mainly close to infected blood vessels.

Twenty four days following exposure, a lack of clear differentiation between the two pulps was noticed , then it disappeared following 73 days at central regions of the organ(Fig. 1B).

Signs of apoptosis were observed after 60 days, and as a result, areas of necrosis or empty spaces were formed among the two pulps. Some regions showed no boundaries among cells during the first month and then continued increasingly along investigation period. The intact

cells together with their nuclei adopted diverse shapes, sizes and stains, as there is pyknotic nuclei. However, the white pulp usually more influenced than red one particularly in relation to lymphocytes which present in the former. Hence, the ingested venules were void from blood elements or with scarce cells, the vein wall adopted darker stain, where the arteries were characterized by usual wall but narrow lumen.

The distinct enlarged veins started to form after 17 days of exposure; that is in comparison with 73 days in control treatment.

40 µg • L^{-1} Cd Treatment

In gross morphology, the spleen was pale in colour and more connected to intestine and less flexible after 60 days. Then 13 days later it seems difficult to remove the shrunk spleen independently.

The two pulps interpenetrated each other during few days of treatment, and so the germinal cells surrounded by marginal zone were clearly affected (Fig. 1C). Simultaneously, the melano-macrophages which surrounded by white pulp started to form as small aggregates but they showed no significant development in counts or size over the next duration. On the contrary, the melanin-containing cells being evident during 35 days as they lasted increasingly till the final days. The macrophage centres greatly increased in size due to this dose of cadmium despite they accounted about 12 centre•0.1mm^{-2} with end of treatment.

The dose also resulted in arising of four (rarely six), large, smoothly walled veins which filled with blood elements and may measuring 175 µm in diameter with long run of treatment.

The arterioles, in general, exhibited very narrow lumen causing the blood cells to pass in single file. The primary arteries and veins were less influenced although some junior vessels were ingested; still, a rudiment of blood cells could be seen inside the lumens. Moreover, the venous sinuses are visible in lowered count by comparison with that in sections related to previous treatments.

The degeneration inside cells was observed as degenerated nuclei arised firstly during first 17 days, but without empty spaces until 35 days. Obvious destruction of tissue came into effect by 55 days, particularly at central regions. Peripheral regions showed variable stained cytoplasm and nuclei, where some cells have irregular or spindle nuclei surrounded by white ring in some instances.

60 µg • L^{-1} Cd Treatment

This dose of the toxicant affected the general activity of fishes and rendered them inert individuals. The spleen appeared, in morphological examination, more impacted than that in lower dose treatments.

The histological sections revealed excessive empty spaces, and so the spleen appeared to be divided into separate lobules. Before the study period ended the spleens were in an advanced state of destruction, accompanied by abundant macrophages, blood cells and melanin-containing cells adjacent to empty spaces.

The most distinguishable feature related to this dose is the reduced number of macrophage centres which attained 10 centres•0.1 mm^{-2} in comparison with 23 and 12 centre•0.1 mm^{-2} in the former treatments. This indicates a reverse relationship between cadmium concentration and macrophage density. The melano-macrophages also followed a similar situation (Fig. 1D).

Twenty four days after exposure the two pulps can not be distinguished clearly, although they differentiate somewhat on the periphery up to 60 days. The splenic blood vessels, in turn, are clearly influenced for they contain less blood cells. The arteries adopted irregular walls, whereas the arterioles and venules became very narrow causing the blood cells to pass in single file. Moreover, some of them were ingested thus causing a failure of blood flow. The venous sinuses also decreased in number along with treatment duration.

The cortex underwent some expressions noticed in the four treatments. In the control treatment the cortex included minute fragments of melanin at end of the experiment, whereas it showed similar changes after 30 days at 60 µg • L^{-1} Cd . In some specimens the cortex tended to separate or being thinner at certain sites after 24 days at 20 µg•L^{-1} Cd (Fig. 1B). It may be lost, as noticed after 55 days at 40 µg • L^{-1} Cd treatment, whereas this occurred after only 30 days at 60 µg • L^{-1} Cd.

B. Kidney

The general morphological and histological structure of the kidney in grass carp seems to be similar to that known in most bony fishes. The distal tubule attains 31-33 µm in its diameter, the epithelial high is 13-14 µm, and the lumen diameter is about 4-5 µm. The extension of proximal tubule I and proximal tubule II are 40 and 62 µm, respectively, and their epithelial high is 11 µm. Mean glomerulus diameter is 50 µm in its widest sector.

Control Treatment

The influence of capture started during 24-35 days where the central regions being more affected than periphery. Sixty days in tanks rendered the proximal tubules (I and II) to adopt a lower epithelial high (5 and 6 µm, respectively). Some of both proximal tubules degenerated, as some collecting tubules seem to be retarded for their function because they lost their ordinary structure. The distal tubules being less affected where the cytoplasm appeared with pale stain, as there was a white internal circle around the tubule; however, they still retain their diameter dimension. The glomerulus showed less circular shape with decreased diameter down to 43 µm. No further variation could be noticed 13 days later except acquisition of hemorrhage in some regions. There are natural macrophage centres as small yellow spots (Fig. 2A).

20 µg • L^{-1} Cd Treatment

After 17 days of exposure, most proximal tubules I appeared with partially or completely plugged lumen, and some of them were actually destroyed. More macrophage centres are seen about the location of destruction with density of 5 centres•0.1 mm^{-2} or higher, but proximal tubules II were less impacted. Several collecting tubules also have partially plugged lumen, but the collecting duct still retains its structure.

Thirty five days later, a number of distal tubules started to take effect, a number of proximal tubules I were degenerated and so they remain more influenced than proximal tubules II which exhibited plugged lumen. Few collecting tubules were degenerated or possessed plugged lumen, while the collecting duct had somewhat degenerated wall (Fig. 2B).

Hematopoietic tissue started to disintegrate through cytoplasmic and nuclear material precipitation in some of paranchymatous tissue. The resulted empty spaces were occupied by macrophage centres which attained 16 centre•0.1 mm^{-2}.

Arrival at later days the distal tubules characterized by disarranged rows of nuclei or degenerated ones within epithelium. Certain proximal tubules II were destroyed, whereas others adopted diverse sizes and pigmentation in their cells nuclei which possessed 2-3 nucleoli. The collecting duct also observed as destructed one. On the other hand, the glomerulus exhibited less circular shape with some vacuoles formed inside it, although it was less affected in relation to volume. The cardinal vein became half filled with blood elements in comparison with onset of experiment; however, some degree of hemorrhage was found .

40 µg • L^{-1} Cd Treatment

After 4 days of exposure, there was partial analysis in paranchymous portion of hematopoietic tissue which increased continuously during the next period. Simultaneously, large empty spaces were formed among the kidney tissues, and in general, there are regions which seem as cynctium or nuclear debris. Finally, the hematopoietic tissue showed excessive disintegration and additional empty spaces were formed throughout the kidney mass (Fig. 2C). The macrophage centres attained 9 centre•0.1 mm^{-2} but they grew is size with period advancement. The glomerulus showed disintegration in its components and more vacuoles were formed.

The well-identified impressions occurred after 14-17 days where distal tubules partially degenerated and seen to be surrounded by white ring which is indeed pale cytoplasm, whereas the nuclei lost their arrangement within tubule epithelium and adopted darker stain. However, some tubules underwent complete destruction for the first time after 35 days while others destroyed with end of period. A number of proximal tubules (I and II) were bereft of natural architecture, besides, there is an increment in affected collecting tubules, when compared with that noticed in previous treatment (20 µg • L^{-1} Cd) during the same duration, where their nuclei displaced from the ordinary position within epithelium. Hence, the collecting duct started to exhibit degenerated circumference and overlapped rows of darker stained nuclei in addition to partial or complete obstruction of lumen in several sections examined. Finally, close to 73 days no tubule or duct was in its natural form.

The glomerulus was the last component resist the degeneration among kidney structure despite it lost the distinguishable shape and contained more vacuoles as it shrank in diameter after 17 days of treatment.

60 µg • L^{-1} Cd Treatment

This concentration of cadmium resulted in obvious reduction of dimensions of different tubules and collecting duct during the first 4 days of exposure, although they retained distinguishable morphological appearance. The distal tubules reduced in diameter down to 25-27 µm with epithelial high of about 10 µm, or it is said that the mass of cells was diminished in favor of nuclei. Moreover, signals of bleeding spreaded throughout the field of sections.

The longer the period up to 35 days there was a continuous damage of different kidney components. Still, no significant increase in macrophage centres but additional empty spaces were spreaded randomly.

The effective impact was noticed after about two months where some distal tubules completely damaged, while proximal tubules I adopted two types of nuclei in epithelium, one of smaller size and darker stain locating closer to lumen and the other of larger size and pale stain locating closer to cell base.

The destruction branded the tubules and collecting duct at the final days indicates a failure of such structures to achieve their functions. The cardinal vein appeared to be practically empty from blood as it exhibited partially degenerated wall, and now, no distinct glomerulus is seen (Fig. 2D). The hematopoietic tissue lacked a distinguishable border, as its components adopted variable stains and dimensions where the necrosis covered larger area which indicate, in turn, a failure in tissue function.

DISCUSSION

The recognizable destruction of splenic white pulp results in lymphocyte reduction which is a response to pollutant stress, i.e., a drop in immunity. {8} found a marked reduction in lymphocyte number at the spleen of fishes exposed to contamination by chemicals. Moreover, {9} suggested that organo-somatic indices reflect the status of organ systems, and so they used that as indicator of well- being of individual organisms.

The enlarged veins on peripheral regions of spleen seem to be a response to the stress of captivity(73 days at control treatment), made worse by pollutant stress (17 days at 20 µg • L^{-1} Cd). Consequently, the pollutant may play a secondary role in the formation of such veins.

The pigments produced throughout spleen, as well as the fine granules in cortex, appear as haemosiderin, which is a common pigment derived from hemoglobin and seen in teleost spleen under starvation and diseases {10}. {11} stated that aging erythrocytes are destroyed by macrophages, mainly in spleen. When some splenic vessels are ingested, as noticed in present study, they cause a certain delay in blood circulation, which in turn, gives arise to staying for more blood cells in spleen. However, the immature form of erythrocytes can often be seen in teleost spleen {12}.

Teleost kidney usually executes the functions attributed to both of bone marrow and lymph nodes in mammals; and so the reduction of hematopoietic tissue results in reduced erythrocytes production.

It appears that kidney of *C. idella* is more resistant to cadmium exposure than *Channa punctatus* (Channidae) as observed by {13}. They found signs of destruction in some proximal tubules after 13 days in 10 µg • L^{-1} Cd treatment. The hematopoietic tissue was affected after

17 days. The glomeruli showed signs of shrinkage and vacuoles formation by 17 and 28 days, respectively, at the same dose, and was destroyed completely by 47 days at 30 µg • L^{-1} Cd treatment. In comparison, signs of destruction started in *C. idella* kidney after 17 days of exposure to 20 µg • L^{-1} Cd. The hematopoietic tissue affected by 34 days, while the glomerulus started in shrinkage by 17 days. However, {13} found that the degree of destruction was linearly proportional to concentration and period, while it seems that the exposure period was somewhat more important for *C. idella*.

In general, the kidney of grass carp seems to be more influenced than spleen to cadmium exposure. This is consistent with {1} who observed that in non-acclimated fishes, tissue total cadmium levels were greater in kidney and gills than liver or muscles (10.7±1.4, 9.0±0.8,4.9±0.6, 0.01±0.002 nmol•mg^{-1} respectively).{1} suggested that chronic exposure to water-born cadmium stimulates the detoxification process. This may explain the tolerance of the present species to prolonged exposure to toxicant. The mitotic figures in spleen indicate an attempt by the fish to replenish deteriorating epithelium.

Three pigment types (lipofuscin, melanin and haemosiderin) were established within hematopoietic organs of *C. idella*. Lipofuscin is represented by the yellow granules pigment {4}, whereas melanin granules within melano-macrophages {10}; haemosiderin usually found in spleen while kidney is devoid from it.

Melano-macrophage centres were accompanied by necrosis in both spleen and kidney, that is to say with destroyed tissues. {4} proposed the protoplasmic degeneration as a major feature of fish melano-macrophage centres. In addition, the majority of pigmented cells within spleen and kidney of teleost are of a phagocytometric nature. Grass carp specimens in our control treatments had less degenerative tissue in comparison with organisms subjected to cadmium doses, as they contained lower density of melano-macrophages. A similar observation was reported for plaice and rainbow trout alevins{14}, suggesting catabolic tissue breakdown is a major factor contributing to the formation of the pigments within melano-macrophage. In the present investigation , size augmentation of macrophage centres in spleen compensated for the increase in counts. It is known that the major phagocytic tissues in fishes have close association with lymphoid elements {15}.

Cytometry has been proposed to detect and enumerate apoptotic cells {16;17}. The dimensions alteration and lesions promoted by cadmium doses in grass carp kidney may also occur as a result of exposure to certain chemical toxicants at similar trends. For instance, {18} stated that exposure to malachite green caused slight dilation of kidney tubules lamina and

alteration of nucleus-cytoplasm ratio, favoring the nuclei in carp fingerling. He mentioned also that copper sulfate caused a necrosis around the kidney tubules and destruction of hematopoietic tissue in cultured fishes, in addition to tubules dilation.

From this study, cytometry can be recommended for small grass carp, but more studies are needed for the large sizes. {3} expected that older specimens of Arctic char accumulate more amount of cadmium as its concentration was positively correlated to age. {19} obtained a similar results on heavy metals concentration in fish of Lake Balaton. Comparison with other species may be strongly influenced by several factors such as size, environment and specific strains of each species tested. Moreover, the two sexes may different in heavy metal accumulation {20}.

CONCLUSION

The structure of the spleen and kidney in grass carp *C. idella* may be used as an indicator for cadmium pollution, although more work needs to be undertaken to prove the cytometric observations noted herein are specific to cadmium and not a general response to heavy metals pollution.. Cadmium reduces the immunity and hemoglobin production, and then fish health as can be revealed morphologically and histologically rather than behavioral monitoring. The spleen is more resistant to destruction than the kidney in this species.

تاثير التعريض طويل الامد للكادميوم على الاعضاء المولدة للدم في سمكة الكارب العشبي (Cyprinidae) *Ctenopharyngodon idella*

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الخلاصة

لوحظ تحطم مكونات الطحال والكلية في سمكة *C. idella* عند اليوم السابع عشر في النماذج المعرضة لتركيز 0.02 جزء/مليون من Cd . وقد تحطم الطحال بشكل حقيقي بعد 73 يوما من التعريض للتركيز 0.06 جزء/المليون من Cd حيث لوحظ عدم تمايز نوعي اللب بشكل واضح ، كما احتوت الاوعية الدموية على اعداد اقل من الخلايا الدموية . اما الكلية فظهر فيها التحطم الكامل بعد 60 يوما من التعرض للتركيز 0.06 جزء/ المليونCd وحيث لم يمكن تمييز حدود النسيج المكون للدم وقد انتشر التنخر خلال مساحة واسعة من الكلية.

كانت درجة التحطم تتناسب خطيا مع التركيز الملوث وفترة التعريض، رغم ان الفترة الزمنية كانت اكثر تاثيرا.
يمكن الاستنتاج ان التغيرات النسيجية في الطحال والكلية تعد دليلا لمدى تلوث المياه بالكادميوم وصحة الاسماك المنتشرة خلالها.

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