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Effects of hexavalent chromium on structure of liver, intestine and ovary of Zebra fish

Dang Dang Khoa[1,2], Nguyen Thi Phuong Thao[2,3], Le Thanh Long[*2,3]

[1] *Binh Duong University, Thu Dau Mot City, Binh Duong, Vietnam*

[2] *Biotechnology Department, Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, Vietnam*

[3] *Institute of Tropical Biology, Vietnam Academy of Science and Technology, Ho Chi Minh, Vietnam*

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Abstract

The study aimed to evaluate the effects of chromium (vi) on zebrafish's liver, intestines and ovaries on the following days: day 5, day 10, day 20, day 30, respectively. The results showed that the longer the zebrafish exposed to chromium (vi) in water, the more chromium (vi) accumulated in the whole body. Analytical results showed that chromium (VI) accumulation concentration increased with the highest exposure time at day 30 (23.9±1.0mg/kg), this content was 4 times higher than day 5 (6.1±0.4).mg/kg), 3 times higher than day 10 (9.3±0.6mg/kg), 1.5 times higher than day 20 (17±0.8mg/kg). For the control batch, the ratio was 0%, indicating that the amount of chromium (VI) did not accumulate in the fish's body. Based on the results, the damaged intestinal tissue structure increased over time while the adipose tissue decreased and the villi increased densely over time. The structure of zebrafish ovarian tissue changed and the proportion of lipid particles decreased when the fish were exposed to chromium (VI) concentrations for a long period of time. Beside that, the liver tissue structure appeared inflammatory foci and the inflammation became more severe with exposure time.

*** Corresponding Author:** Le Thanh Long ✉ lelong2510@gmail.com

Introduction

Heavy metals have been widely used in large quantities in industrial production and are also toxic to the environment. Heavy metals when exposed will directly affect human health and organisms. Heavy metal exposure can lead to adverse effects on the body's organs (Renieri *et al.*, 2014; Renieri *et al.*, 2019). Zebrafish are classified as a major class of aquatic vertebrates and are an integral component of toxicity testing strategies. In fact, fish may not only differ from vertebrates, but also from most invertebrates in terms of metabolism, espectially the ability to bio-transform chemicals. Furthermore, when in an environment with high levels of contamination and chemical spills, these kind of fish are often the target for assessing contamination. Zebrafish are important in monitoring water pollution, so they are used extensively in the regulation of aquatic toxicity testing (Hill *et al.*, 2005). The evaluation of the impact of chromium (VI) on organism life *in vivo* or *in vitro* has been attracting the attention of many research groups (Jin *et al.*, 2015; Xu *et al.*, 2021; Yan *et al.*, 2023). One of the commonly models for this research direction is the use of aquatic animals as experimental models. However, in order to evaluate the impact comprehensively, the *in vivo* models are more advantages and one of the popular models to evaluate the impact of chromium (VI) is the zebrafish model (*Danio rerio*) (Lu *et al.*, 2017).

Zebrafish (*Danio rerio)* have been used as the popular test subjects because of their high homology with mammals. They are about 70% similar to the human genome as well as having similarities in anatomy and physiology with other vertebrates. The living conditions of zebrafish are suitable with Vietnam's climate, so it is easy to arrange experiments. Moreover, the taking care of zebrafish and embryos is simple, adult zebrafish are small in size, fast growing and short life span. The female fish could give a large number of embryos at a time, which are encased in transparent shells; this is also a sensitive stage in their life cycle. Early embryonic development and organogenesis in zebrafish is very similar to that of other vertebrates, but the rate of development is much faster: after only 3 days, the embryo can develops into a larva. The transparent fish embryos allow to observe the effects of chemicals during early embryonic development (Kimmel *et al.*, 1995), therefore, this model is increasingly receiving the attention of scientists in toxicity testing. In this study, we aimed to estimate the effects of chromium on zebrafish by accessing the chrmoium accumulation, the changes in structure of intestine, liver and ovary.

Materials and methods

Animals

To make sure the zebrafish were healthy before the studies began; they were housed in the lab for a week. A 14h: 10h Light: Dark cycle was used for fish breeding. The fish were fed commercial food each day. Chromium (V) was administered to zebrafish at LD50 (32.3g/L) (Dang *et al.*, 2023). Fish from the control group were raised in a tank without being exposed to chromium.

Hematoxylin and Eosin staining

The liver, intestine, and oocytes were collected after chromium treatment and fixed in 4% paraformaldehyde. The samples were utilized in the University of Medicine and Pharmacy, Ho Chi Minh City, for hematoxylin and eosin staining. Oocyte and intestinal structures were examined under a microscope.

Statistical analysis

Data are presented in the format mean ± SD. The samples were replicated three times, and a one-way ANOVA analysis was used to see how the experimental groups differed from one another. The P value ≤ 0.05 was considered statistically significant.

Results and discussion

Heavy metals are known sources of toxic accumulation in aquatic animal species. Results from various studies on metal accumulation in fish living in polluted water areas indicate a significant amount of different metals can accumulate in fish tissues without causing mortality. Aquatic organisms accumulate chromium (VI) from water and their dietary intake, although evidence suggests that accumulation of chromium (VI) in fish is mostly derived from polluted water rather than dietary sources. The accumulation of chromium (VI) in various fish species has been studied, all of which demonstrate disruptive effects on fish physiology.

In this study, the accumulation of chromium (VI) in zebrafish was analyzed using 250 adult zebrafish exposed to a LD50 concentration of chromium (VI), and samples were collected on days 5, 10, 20, and 30 for analysis. A control group of zebrafish that was not exposed to chromium (VI) was also included.

After 30 days of exposure to chromium (VI) at the LD50 concentration, samples from days 5, 10, 20, and 30 were sent for analysis of the accumulated chromium (VI) concentration throughout the entire bodies of zebrafish at the Viet Tin Testing and Analysis Company. The results of the analysis conducted on adult zebrafish' entire bodies was shown in Fig. 1.

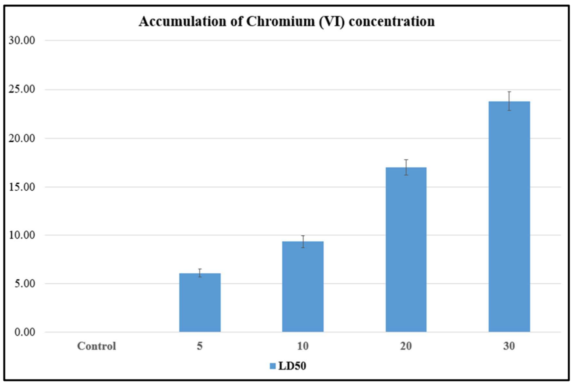

Fig. 1. Accumulation of Chromium (VI) levels in adult zebrafish bodies.

The results in Fig.1 demonstrated that the longer zebrafish are exposed to water containing chromium (VI), the higher the accumulation of chromium (VI) in their entire bodies. At the same LD50 concentration, the analysis results reveal that the accumulation of chromium (VI) increases over time, with the highest level observed on day 30 (23.9±1.0mg/kg). This concentration is four times higher than day 5 (6.1±0.4mg/kg), three times higher than day 10 (9.3±0.6mg/kg), and 1.5 times higher than day 20 (17±0.8mg/kg). In the control group, the accumulation ratio is 0%, indicating that chromium (VI) was not accumulated in the zebrafish bodies.

Aside the study about the accumulation of chromium (VI) in zebrafish bodies, other researchers have assessed the accumulation of various heavy metals in different parts of zebrafish. In a study examining the accumulation of cadmium in zebrafish (Danio rerio) presented by Nguyen Thi Thuong Huyen, the intestines were the site that absorbed the highest and fastest Cd2+ metal (Huyen *et al.*, 2013). The competition between Cd2+ and the calcium presented in bones, which were inversely proportional to each other, resulted in the increasing accumulation of Cd2+ in bones, leading to bone damages like osteoporosis and vertebral deformities. The accumulation of Cd2+ in muscles was the lowest compared to intestines and bones. According to the study conducted by M.N.R Rosli (2018) on heavy metal cccumulation analysis in fish from the coastal region of Terengganu, Malaysia, higher metal accumulation occurred in the liver and gills compared to muscle tissue (Rosli *et al.*, 2018).

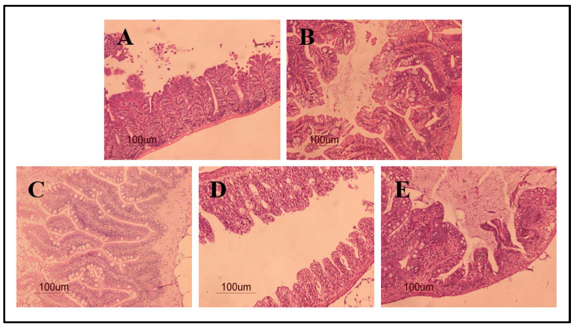

Fig. 2. Cross-sectional images of the zebrafish intestine layers after exposure to Chromium (VI) concentration, magnification 200 (images A, B, C, D, E), scale 100µm. (A) Cross-sectional image of intestine layers in the control group: sparse villi, abundant adipose tissue; (B, C, D, E) Successive cross-sectional images of intestine layers for days 5, 10, 20, and 30, showing decreasing adipose tissue and increasing villi over time.

This study showed that the accumulation levels of non-toxic or essential metals (Cu, Mn, and Zn) in fish were higher than harmful or non-essential metals (Cd). According to Tulasi *et al.*, 1992, exposure of certain freshwater fish species to various lead concentrations led to significant accumulation in blood, kidneys, liver, brain, and relatively less accumulation in muscle tissues and ovaries.

The affection of heavy metal that exposed on zebrafish can potentially cause damage to cellular tissues and alter tissue structures, such as the intestine, liver and ovarian tissue. The results showed the changes in the structure of the zebrafish intestine tissue and liver tisue when exposed to Chromium (VI) at the LD50 concentration over days 5, 10, 20, and 30, as demonstrated in Fig.2 and Fig.3, respectively.

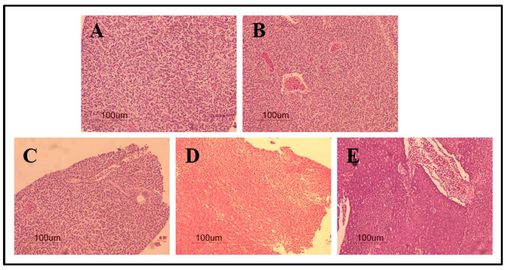

Fig. 3. Cross-sections of the zebrafish liver tissue exposed to Chromium (VI) concentration LD_{50} at different time points: day 5, day 10, day 20, and day 30, magnification 200x (images A, B, C, D, E). (A) Cross-section of liver tissue in the control group: relatively uniform tissue structure. (B, C, D, E) Cross-sections of liver tissue on days 5, 10, 20, and 30, respectively, showing the presence of inflammatory foci and increasing severity of inflammation over time.

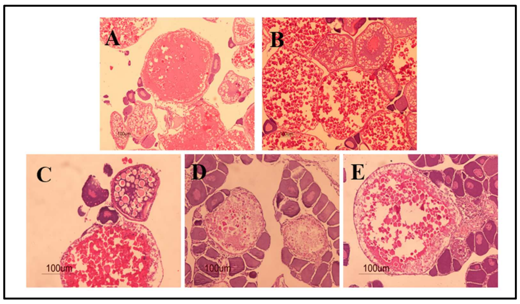

Fig. 4. Cross-sections of the zebrafish ovarian tissue exposed to different concentrations of Chromium (VI), magnification 100, scale 100µm. (A) Cross-section of ovarian tissue in the control group: relatively uniform tissue structure with dense lipid droplets. (B, C, D, E) Cross-sections of ovarian tissue on days 5, 10, 20, and 30, respectively, show a decreasing trend in lipid content in the ovarian follicles over time.

The structure of the ovarian tissue in zebrafish had changes when exposed to Chromium (VI) concentration for longer durations, leading to a reduction in the proportion of lipid droplets (Fig.4). From day 5 to day 30, there was a consistent decrease in the lipid droplet ratio compared to the control group.

The objective of this study was to identify the changes in the intestinal, liver, and ovarian tissue structures of zebrafish when exposed to Chromium (VI). By the obtained results, exposure to Chromium (VI) induced a lot of structural alterations in the zebrafish's tissues. Notable differences were observed in the density of villi in the intestines as well as lipid content in the ovarian follicles. In the liver, inflammatory foci appeared and intensified over the exposure period. Following Tulasi *et al.*, 1992, the exposure of freshwater fish Anabas Testudineus to the lead nitrate was reduced to 5ppm levels for duration of 30 days during the annual reproductive cycle (Tulasi *et al.*, 1992). This issue led to reduce the total lipid, phospholipid content and cholesterol levels in the liver as well as ovarian tissues while the concentration of free fatty acids and lipase activity increased. Seasonal variations of lipid levels in the ovaries, liver, and serum were linked to the annual reproductive cycle in the catfish H. Fossilis. The reduction of lipid content in the liver was related to a decrease in the hepatosomatic index (HSI) during reproductive and spawning phases, while the ovarian lipid level increased significantly during periods of oocyte maturation, corresponding to enhanced gonadosomatic index (GSI). The cholesterol level in serum and ovarian tissue were exhibited decreasingly during the reproductive phase, which were followed by an increase during spawning. The degradation of liver cholesterol initiated before spawning and continued through the reproductive phase, followed by a tendency of recovery. Jinling Cao's study in 2019 demonstrated the heavy metals can hinder zebrafish development and significantly impact reproduction in both genders by disrupting the reproductive gland structure, altering steroid hormone concentrations, and affecting the expression of endocrine-related genes in the HPG of zebrafish (Cao *et al.*, 2019). This study indicated that heavy metals negatively influenced the reproductive endocrine system in zebrafish and might pose a potential threat to fish populations in metal-contaminated water. Changes caused by heavy metal pollutants during the maturation of fish eggs can cause egg toxicity, metal accumulation in eggs, or a direct effect on genomic processes. Egg cell maturation was most sensitive to metal toxicity. Therefore, various disorders caused by heavy metal pollutants during egg development can lead to reduced egg quantity and quality.

According to the study results, intestinal villi increased and adipose tissue decreased with chromium (VI) exposure time. In the study of Chhaya Bhatnagar and colleagues, degenerative effects were evident in the mucosa and villi of the intestine (Bhatnagar *et al.*, 2007). Microscopic changes in the intestinal tissue revealed bacterial degeneration within the intestinal lumen, with up to four OMV chains attached to the intestinal tissue. This attachment was associated with a reduction in intestinal cell and microvilli density, accompanied by accumulation of sloughed-off epithelial tissue within the lumen. The infiltration of degenerated EGC and DC-Like cells was also observed in the necrotic intestinal epithelium. According to El-Sayed Mohamed Younis, histopathological changes observed in the intestines of both Oreochromis niloticus and Lates niloticus indicated severe degeneration and necrosis in the intestinal mucosa (Younis *et al.*, 2013). The presence of heavy metals in the mucosal lining and the lumen could be the result of harmful metal absorption.

The existence of Chromium (VI) and its impact on humans and organisms was unpredictable. The ability to absorb by the respiratory and digestive systems has distributed toxic substances in all parts of the body, affecting the health of humans and organisms. This study identified the presence of heavy metals and their negative effects to the zebrafish's body, demonstrating a significant threat to both organisms and human health. The results obtained from this study would be the basis for contributing to future studies towards improving the environment in order to protect human and animal health.

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Conflicts of interest

The authors declare no conflicts of interest.

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