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EFFECT OF CONSUMING UNCOOKED CADMIUM-CONTAMINATED TILAPIA (*Oreochromis* sp.) ON KIDNEY AND LIVER OF WHITE RATS

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Abstract: Cadmium (Cd) is a xenobiotic that can cause numerous health effects to living organisms and is an emerging environmental pollutant due to its anthropogenic activity. The main objectives of this study were to determine the toxicity effect of Cd from fish (*Oreochromis* sp.) in white rats (*Sprague dawley*) and also the level of Cd pollution in raw fish and white rats' organs sample by Inductively-Coupled Plasma Mass Spectrometry (ICPMS). Three groups of designed samples based on 96 h median lethal concentration of Cd exposure were GA-lowest (1.1721 mgl^{-1}), GB-medium (2.3442 mgl^{-1}) and GC-highest (4.6883 mgl^{-1}) while the control groups remained unexposed to Cd. White rats were euthanised at day one, four and seven and liver and kidneys collected for both histological studies and ICPMS readings. A very low concentration of Cd was detected in these organs ($p > 0.05$). However, the effects of low Cd toxicity in liver and kidney were obvious and shown in histological changes as early as day 1. Cd at low levels can bring adverse effect to the health of the white rats.

KEYWORDS: Toxicity; Histology; Inductively Coupled Plasma Mass Spectrometry (ICPMS); pollution.

Introduction

Cadmium (Cd) is a well-known contaminant and is one of the environmental pollutants that has great potential to risk the health of living organisms, especially humans. Cd is also regarded as an emerging environmental pollutant, especially due to its anthropogenic activity (Sant'Ana et al., 2005; van Dyk et al., 2007). Aquatic organisms could be exposed to unnaturally high levels of metals from the pollution (van Dyk et al., 2007). Since it can cause significant changes to the structure and morphology of organs, Cd is pointed out as one of the most toxic heavy metals (Battaglini et al., 1993; Thophon et al., 2002). There are many ways for human and animals to get Cd pollution, for instance through inhalation, consuming contaminated food, skin contact and long-time exposure to its dust (Friberg et al., 1986). Numbers of studies on the heavy-metal pollution in Malaysian have been reported since 1980 (Sivalingam and Ahzura, 1980; Law and Singh,1988). Law and Singh (1991) also found that the level of mercury, lead, copper and zinc contents in the fish collected from the Kelang estuary were significantly higher ($P < 0.05$) than those from the Setiu estuary which is free of industrial pollution.

Numerous studies have shown that chronic exposure of Cd leads to a selective accumulation of Cd in the liver and kidneys (renal cortex) and in some studies up to 75% of the total body burden was found in these organs (Friberg et al., 1986). Cd usually accumulates in soft tissue, especially the kidney and liver (Swiergosz et al., 1996). Histological changes might be produced by Cd toxicity in the kidneys, liver, gastrointestinal tract, testes, heart, blood vessels, bone marrow and pancreas (Domino, 1994; Waalkes et al., 1992, 1994; Selypes et al., 1992; Swiergosz et al., 1996). In liver, Cd causes intralobular fibrosis, cirrohsis, focal mononuclear infiltrates, and proliferation of the smooth

endoplasmic reticulum. According to Ebdon et al. (2001), Cd accumulation increases with age and has an extremely long residence time (over 20 years) in the human body. This chronic exposure is likely to cause proteinuria, glomerular damage, elevated lysozyme and ribonuclease excretion, and reduced creatinine clearance where histological observation has shown degeneration of tubular epithelium, glomerular amyloidosis, and presence of inclusions with soluble crystalline bodies also became more concentrated in the periphery of the liver lobes.According Swiergosz et al. (1996), sublethal doses of Cd may present effects such as reduced growth and anemia.

This paper focusses on the effect of consuming Cd-polluted fish on white rats as less studies have been done on the concentrations of Cd accumulation in the detoxifying organs of mammals.

Materials and Methods

Animals and toxicity test

Tilapia is known among research scientists as a hardy fish, highly resistant to many diseases and infections that are commonly faced by other types or cultured fish. These characteristics have made the fish as the first candidate of choice for such studies. Hybrid tilapia (*Oreochromis niloticus* x O. *aureus*) at the average sizes of 3.5 to 4.0cm and weight between 1.5 to 1.8g were obtained from a local farmer in Terengganu and domesticated and bred in our lab. The fishes were maintained in a fibre tank at 27 ± 0.1°C in aerated water and subjected to a 12 h light: 12 h dark cycle .The acute bioassay procedure was based on standard methods (APHA-AWWA-WPCF, 1998). The experiment was run for 96 hours and was repeated three times. Acute toxicity effect of Cd on hybrid tilapia was determined by the use of Finney's Probit Analysis LC_{50} Determination Method (Finney, 1971). The computer analysis was carried on with LC_{50} 1.00 Software developed by EPA (1999). Tilapias were tested against lower concentrations of cadmium based on data obtained from the acute toxicity test. Fishes were exposed to 25, 50 and 100 % of the 96 hours LC_{50} for another 4 days and these fishes were used as the food for the *Sprague dawley* white rats.

A total of 45 clinically-healthy *Sprague dawley* at the age of 3 months were randomly selected from Animal Unit of Kubang Kerian USM Hospital and kept for 1 week and monitored for any sign of health problems. The white rats were fed with sterile commercial pellet at 25g daily while clean water was available *ad libitum.* The rats were then equally divided into 5 groups and fed with Cd-polluted fish for a period of 7 days with the ratio of 1 rat: 1 fish. The groups were designed as follows: Control Negative (CN) (Fed with unpolluted fresh fish), Control Positive (CP) (Fed commercial rat pellet), Group 1 (Fed with 25 % LC_{50}- Cd-polluted fish), Group 2 (Fed with 50 % LC_{50}- Cd-polluted fish), and Group 3 (Fed with 100 % LC_{50}-Cd-polluted fish) for 7 days. Rats were humanely sacrificed at day 1, 4 and 7 for both metal accumulation and histopathological study.

Analytical Procedures

Briefly, fishes from the sub-lethal toxicity test were cleaned with de-ionized distilled water to remove surface-bound metal before the euthanisation process. The visceral organs, such as kidney, liver, intestines, gills and muscle tissues, were dissected using clean equipment. The tissues were then put onto an oven to dry at 60°C for 72 hours. The dried crushed organs and tissues were placed in a Teflon beaker with a mixture of 1 ml of concentrated perchloric acid (Merck, Germany) and 1 ml of nitric acid (65% m/v) (Merck, Germany). The mixture was left to preliminary digest for 3 hours. Then it was digested at 100°C, placed upon a hot plate until the mixture completely digested. A clean solution was obtained and the mixture was evaporated to nearly dryness. Then, the mixture was topped up to 50 ml with de-ionized water. Cd concentrations for each sample were measured

using ELAN® 6000 Inductively-Coupled Plasma Mass Spectrometer (ICPMS) (PerkinElmer Sciex Instruments, Concord, Ontario, Canada).

Histological analysis

Rats were euthanised and dissected at day 1, 4 and 7. All kidneys and livers were collected and kept separately in 10 % buffered formalin for at least 24 hours to enable the completion of fixation process. The organs were then cut into small pieces and placed in labelled cassettes before being soaked in a tissue processor for dehydration process by a series of alcohol (70%, 90%, 95% and 100%). The tissues were then soaked in xylene, before impregnated with paraffin wax. After processing, the tissues were moulded in melted paraffin wax. Paraffin blocks were then removed from the mould. The blocks were trimmed and sectioned onto slide with standardised thickness 3-4 µm. After sectioning, the sections were put for straitening by floating on water bath with temperature 38-40°C. Then the sections were transferred onto slide and put for drying on hot plate (60°C) overnight. After that, the slides were stained with Haematoxylin and Eosin (H&E) stain. Lastly, slides were mounted with cover slips by using DPX (glue). The slides were then examined and observed under light compound microscope (DMLB Leica) at 10X, 20X and 40X magnifications and pictures were captured using the Image Analyser Software version 2.0.

Statistical Analysis

Descriptive statistical analyses were performed using the software SPSS for Windows (Version 11.0). The data on Cd accumulation was tested for homogeneity of variance and normality and were found normally distributed. The data were analysed by use of two-way analysis of variance (ANOVA). Differences between level means per factor were treated using Tukey`s multiple comparison of means.

Results and Discussion

Finney's Probit Analysis gave 96-h LC_{50} value for the hybrid tilapia exposed to different Cd concentrations as 4.69 mgl^{-1}. Our results are within the range of results reported by several earlier studies. The LC_{50} values of Cd on rainbow trout (*Oncorhynchus mykiss*) for 24, 48, 72 and 96 h were found to be 7.76, 1.95, 0.5, and 0.45 mgl^{-1}, respectively by Oryan and Nejatkhah (1997). Chambers (1995) investigated the effect of acute Cd toxicity on marron, *Cherax tenuimanus* and found the 96-h LC_{50} value as 17.9 (13.4–23.9) mgl^{-1}. Furthermore, Muley et al. (2000) reported the 96-h LC_{50} value of Cd on *Cyprinus carpio* as 121.8 ppm. The 96-h LC_{50} values of Cd on *Salmo gairdneri* and *Xenopus laevis* larvae were reported to be between 80 and 100 mgl^{-1} by Woodal et al., 1988. Many factors are involved in determining the 96-h LC_{50} which include temperature, pH, alkalinity, water hardness, dissolved oxygen and total organic carbon (Wright, 2001).

Overall, the kidney showed more severe histopathological lesions than the liver (Fig. 1 and Fig. 2) as the kidney is known as the first target organ of toxicity clearance (Thophon et al., 2002). Cd can be accumulated even though the concentration exposure level is very low (Koeck et al., 1995; Damek-Poprawa and Sawicka-Kapusta, 2002). The damage in kidneys will increase along with the increase of exposure period. In this study, fibrous material, loose and lining cells and changes of tubular structure were obviously affected by the accumulation of Cd. Novelli et al. (1998) highlighted that the experimental rats showed hepatic and renal damage within 7 days which supports the finding of this study.

According to Prozialeck et al., 2006, the primary target of Cd toxicity in the kidney are the proximal convoluted tubules, glomeruli and also vascular endothelial cells. This is because kidney tissue is one of the most sensitive to the toxic effects of Cd (Prasad and Nath, 1995). Studies by Damek-Poprawa and Sawicka-Kapusta (2002), has shown that kidneys clinically expressed most typical features of Cd toxicity by tubular protenuria, aminoaciduria, glucosuria, phosphaturia, damage to blood vessels, decreased seminiferous tubules' diameter and incorporation of Cd into enzymes. According to Prasad and Nath (1995), development of Cd-induced lesions in kidneys is characterised by proteinuria which is identified as one of the renal dysfunctions. The evidence of proteinuria indicated moderate-to-severe kidney dysfunction. It was suggested by Thophon et al., (2003) that an increase in renal metallothionein (MT) associated with the accumulation of Cd in kidneys may result in the low excretion rate of Cd and tubular epithelial cell necrosis (Chan and Rennert, 1981). Although there were severe histopathological changes in the kidney, the white rats still survived until day 7. This indicated that the rats showed the ability to adapt to the low level of cadmium accumulation in a chronic situation (van Dyk et al., 2007).

In this study, the lesions on the liver were noted on the sinusoids which showed mild congestion and inflammation with a reddish layer covering the empty spaces. Thophon et al. (2002) stated that, in typical cases, sinusoid dilation will be accompanied with blood congestion, hydropic swelling of hepatocytes, dark granule accumulation and lipid droplets accumulation in hepatocytes as the histopathological alterations of Cd toxicity. Cd toxicity can lead to fatty changes which caused vacuolation between hepatocytes, fibrosis and congestion in veins and also severe inflammation in the liver (Thophon et al., 2002). The liver is known as one of the major organs that has the ability to accumulate Cd because it not only acts as a storage organ, but is also the primary site for detoxification mechanisms (Thophon et al., 2003). However, liver tends to accumulate less Cd compared to kidney (Berhard et al., 1992; Ogoshi et al., 1992; Thijs et al., 1992; Swiergosz et al., 1996) due to the low level of Cd exposure. When the level of CD is low, it can bind to glutathione that would then induce synthesis of Cd-binding protein (MT) both of which are thought to serve as intercellular lines of defence against Cd toxicity (Prozialeck et al., 2006). Studies by van Dyk et al. (2007) have shown that the most prevelant histological characteristics identified were hyalinisation of hepatocytes, increased vacuolation associated with lipid accumulation, congestion of blood vessels, and cellular swelling.

According to Habeebu et al. (1998); Kuester et al., (2002); Prozialeck et al., (2006), changes in hepatic vascular endothelial cells are evident and signs of parenchymal necrosis and (or) apoptosis developed after Cd exposure. Studies by Morsey and Protasowicki, 1990 have also shown that Cd exposure caused histopathology changes such as atrophy and necrosis of hepatic cells, decrease or increase in the size of hepatocytes nuclei, and indistinguishable cell membranes of the liver. Cd also caused intralobular fibrosis, cirrohsis, focal mononuclear infiltrates, and proliferation of the smooth endoplasmic reticulum. Van Dyk et al., (2007) suggested that histological changes identified within the hepatocytes may be due to the result of various biochemical lesions caused by Cd toxicity. For example, vacuolation of hepatocytes are associated with the inhibition of protein synthesis, energy depletion, disaggregating of microtubules or shifts in substrate utilisation.

The accumulation of Cd in the body might be less detected when exposed within a short period with low concentration because of the regenerative responses-recovering mechanisms (van Dyk et al., 2007). MT which binds with Cd can regulate Cd into other parts of the body via blood (Prozialeck et al., 2006). However, the histological analysis as discussed before this shows that the organs still suffered from metal toxicity because the re-regulation of MT can bring back the Cd into the detoxifying organs which are the liver and kidney (Prozialeck et al., 2006). The same effects could be found in human tissue after consuming raw fish that has been contaminated with Cd.

Conclusion

There were effects at tissue level on the liver and kidney of white rats fed with Cd-polluted raw fish. Although the level of Cd pollution in fish is low, the effect can still be seen since Cd will accumulate slowly while damaging the respective tissues.

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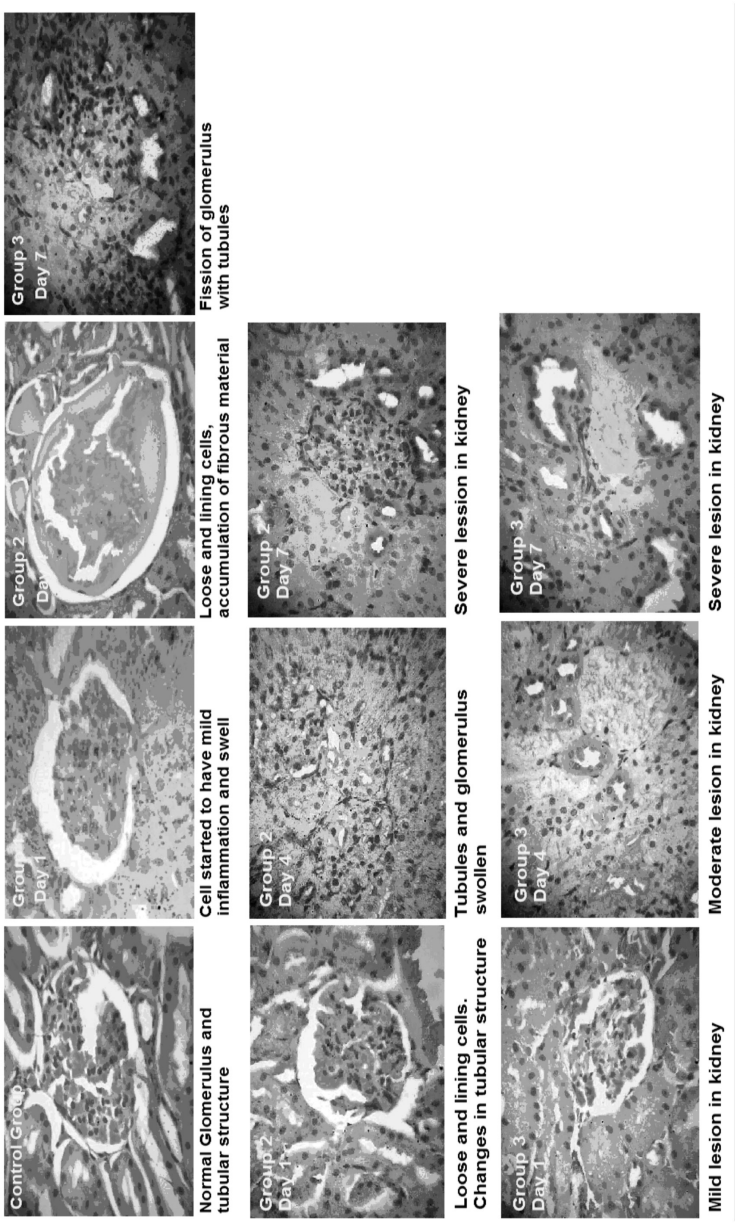

Figure 1. Histology analysis of cadmium effect in white rats`s kidney at 1, 4 and 7 day of consuming cadmium polluted fish.

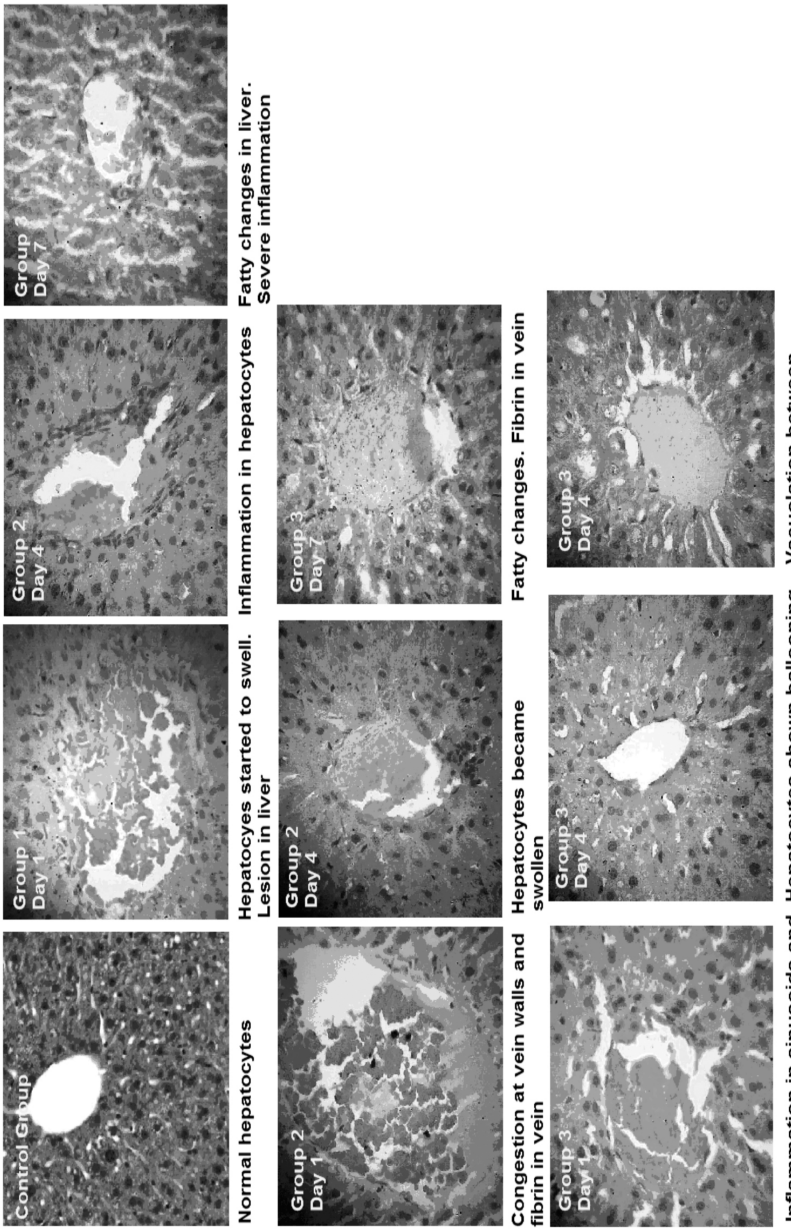

Figure 2. Histology analysis of cadmium effect in white rats`s liver at 1, 4 and 7 day of consuming cadmium polluted fish.

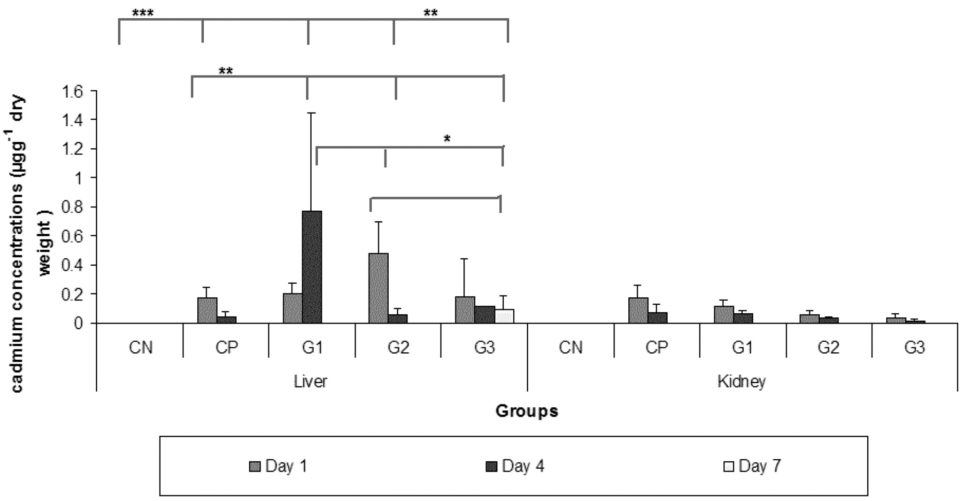

Figure 3. Comparison of Cd accumulation in white rats (liver and kidney) after consuming polluted fish (***p>0.001; **p>0,01; *p>00.05)