Chinese Journal of Oceanology and Limnology
Vol. 33 No. 4, P. 846-852, 2015
http://dx.doi.org/10.1007/s00343-015-4082-1

Histopathological alterations in the liver and intestine of Nile tilapia *Oreochromis niloticus* exposed to long-term sublethal concentrations of cadmium chloride*

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Received Mar. 27, 2014; accepted in principle May 19, 2014; accepted for publication Jun. 2, 2014

Abstract Fingerlings of Nile tilapia *Oreochromis niloticus* were exposed to 1.68, 3.36, and 5.04 mg/L cadmium (as $CdCl_2$), which represent 10%, 20%, and 30% of their previously determined 96-h LC_{50}. After exposure for 20 days, sections of the liver and intestine of treated fish were examined histologically. Histopathological changes varied from slight to severe structural modification, depending on the exposure concentration. The hepatic tissues of fish exposed to 10% LC_{50} showed markedly increased vacuolation of the hepatocytes and coarse granulation of their cytoplasm. Abundant erythrocytic infiltration among the hepatocytes was observed in fish exposed to 20% LC_{50}. In the intestinal tissues of fish exposed to all doses, goblet cells proliferated and were greatly increased in size, the longitudinal muscularis mucosa was disturbed and, in the crypts of the sub-mucosal layer, apoptosis increased, indicated by large numbers of degenerated nuclei. Large numbers of inflammatory cells and dilated blood vessels were observed in the intestine of the group treated with 30% LC_{50}.

Keyword: fish; tilapia; pollutant; cadmium; liver; intestine

1 INTRODUCTION

Heavy metal pollutants have been reported in many aquatic organisms (Olojo et al., 2005). These contaminants build up in the food chain and are responsible for adverse effects including the death of organisms (Farkas et al., 2002). Fish are widely used to evaluate the health of aquatic ecosystems because their physiological changes can serve as biomarkers of environmental pollution (Younis et al., 2012). The Nile tilapia *Oreochromis niloticus* is one of the most frequently used freshwater fish in these kinds of toxicological studies (Figueiredo-Fernandes et al., 2006a, b; Abdel-Warith et al., 2011; Younis et al., 2013).

Cadmium (Cd) is non-essential for the normal biological functions of aquatic organisms although they may sometimes be exposed to high levels of this metal in the environment. Chronic sublethal exposure of fish to waterborne cadmium is known to lead to accumulation of the metal, especially in the kidneys, liver, and gills (McGeer et al., 2000). In addition, cadmium toxicity is reflected in effects on growth, reproduction, respiratory functions and osmoregulation (Pratap and Bonga, 1990).

When aquatic animals are exposed to toxic concentrations of cadmium, their organs may accumulate the element (Jalaludeen et al., 2012; Omer et al., 2012), which may then cause biochemical and morphological alterations, particularly in the liver, intestine, gills and kidney (Abdel-Warith et al., 2011; Jalaludeen et al., 2012; Younis et al., 2013).

The liver plays an important role in the metabolism and excretion of xenobiotic compounds and it is known that some toxic conditions can cause morphological alterations in the organ (Van Dyk et al., 2007, 2012; Abdel-Warith et al., 2011; Younis et

* Supported by the Deanship of Scientific Research at King Saud University (No. RGP-VPP-304)
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al., 2013). Depending on the element and its concentration, the fish species, the period of exposure, and other factors, heavy metal exposures have been associated with both increases and decreases in the activities of hepatic enzymes, and with histopathological changes in hepatic tissues (Paris-Palacios et al., 2000; Van Dyk et al., 2007, 2012; Younis et al., 2012).

Monitoring histological changes in fish liver is a highly sensitive method of assessing the effects of xenobiotic compounds in field and experimental studies. However, the uptake of metals occurs mainly through the gills and intestinal epithelium (Mohamed, 2008) and histopathological alterations may also occur in these organs, related to the absorption of toxic metals (Hanna et al., 2005). The present study was carried out to investigate the harmful effects on the liver and intestine following long-term exposure of Nile tilapia *Oreochromis niloticus* to sublethal concentrations of cadmium chloride.

2 MATERIAL AND METHOD

2.1 Experimental fish

Fingerlings of Nile tilapia *O. niloticus* were collected from the fish-seed hatchery of King Abdulaziz City for Sciences and Technology, Mozahmiya, Riyadh, Saudi Arabia. Fish were acclimatized to laboratory conditions for 2 weeks prior to the commencement of the experiments. Water temperature was maintained at 28±1°C by thermostatically controlled heaters, and other water quality parameters were: pH 7.1–8.0, ammonia-N 0.07–0.20 mg/L, nitrite-N 0.15–0.35 mg/L, nitrate-N 4.35–5.77 mg/L, and dissolved oxygen 5.3–6.7 mg/L.

2.2 Experimental design

One hundred and sixty acclimatized fish weighing 28.33±1.12 g were divided into an unexposed control group and three treatment groups exposed to either 1.68, 3.36 or 5.04 mg/L Cd (as $CdCl_2$, purity 98%, Aldrich Chemical Company Inc., USA) for 20 days. These concentrations correspond to 10%, 20%, and 30% of the 96-h LC_{50} for Cd for juvenile *O. niloticus* (16.8 mg/L; Xu and Bai, 2007). Duplicate 80-L glass aquaria (100 cm×50 cm×40 cm) were established for each of the treatment and control groups. Fish were fed twice daily at a rate of 2% of body weight with a 32% crude protein diet. The physiochemical parameters of the water were similar to those during the acclimatization period. Mortalities in each group were recorded daily.

2.3 Histological examination

The livers and intestines of the control and treated fish were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin wax, and processed for routine histological evaluation. Sections (5 μm) were prepared and stained with hematoxylin and eosin, as described by Luna (1968) and Bernet et al. (1999). Histopathological changes were scored according to Dommels et al. (2007). A value of either (-) no change, (+) slight structural changes, (++) moderate structural changes and (+++) severe structural changes was assigned to each investigated section, and then converted to a numerical value for statistical analysis (0, 1, 2, 3, respectively).

2.4 Statistical analysis

Differences among the treatment groups in the scores of histopathological change were analyzed using one-way analysis of variance (ANOVA). Mean values are stated ± their standard deviations and significant differences (5% level) among means tested using Fisher's least significant difference test as described by Snedecor and Cochran (1989).

3 RESULT

3.1 Histopathological changes in the liver

Histopathological changes occurred in a concentration-dependent manner. Clear evidence of hepatic tissue damage was observed in tilapia exposed to 10% and 20% of the LC_{50} of Cd. The liver of these groups had lost its characteristic architecture and clearly exhibited an increased level of vacuolation of the hepatocytes, relative to that of the control fish (Fig.1a, b, d). Erythrocytic infiltration of the liver was more extensive in the 20% LC_{50} group (Fig.1b). In addition, the cytoplasm of the hepatocytes in this group was characterized by densely stained coarse pink granules, and vacuoles. Abundant erythrocytic infiltration was observed in the group exposed to 30% LC_{50} (Fig.1c). Table 1 shows the scores of the histopathological changes in the hepatic tissues of the different groups.

3.2 Histopathological alterations in the intestinal tissues

Cytological damage to the intestinal tissue,

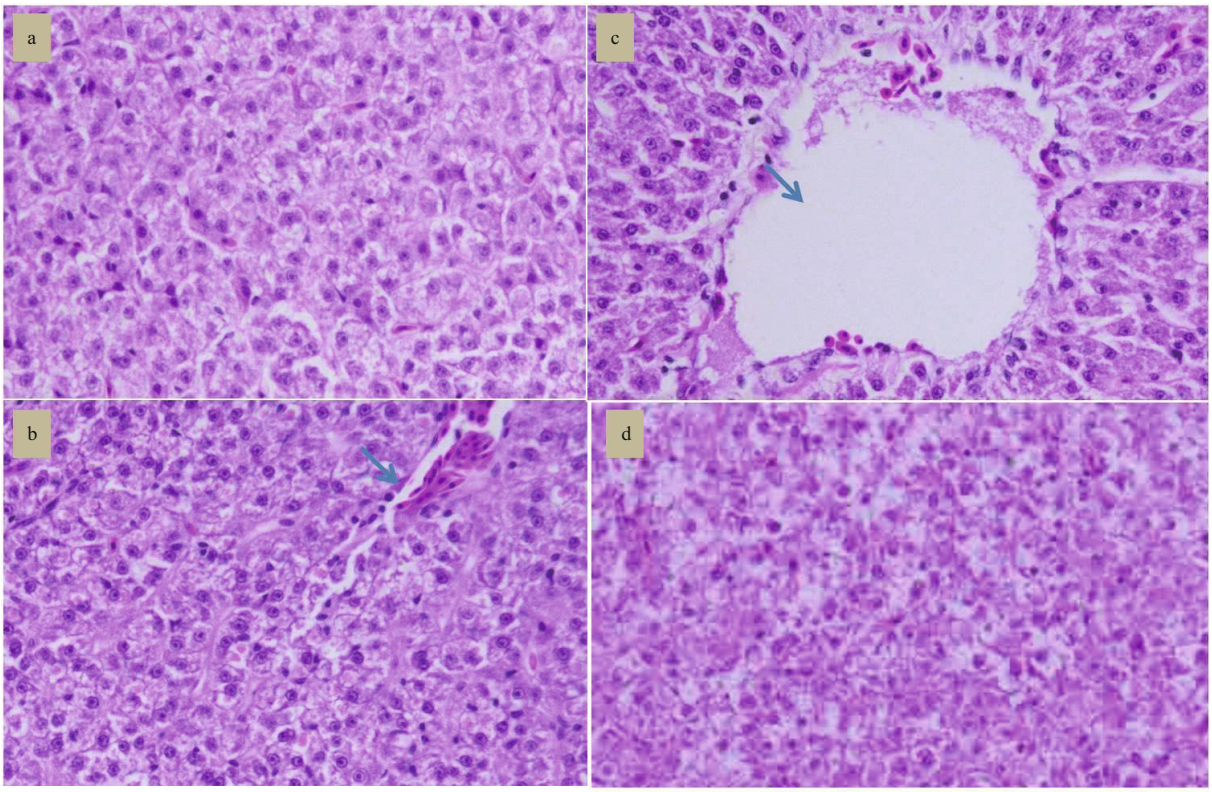

Fig.1 Representative H&E-stained sections (×400) showing histopathological alterations in the liver of Nile tilapia after exposure to different concentrations of Cd (as cadmium chloride) for 20 days

a. 10% of 96-h LC_{50} of Cd; b. 20% LC_{50}, showing a dilated vessel (arrow); c. 30% LC_{50} showing a dilated vessel (arrow); d. liver section of a control fish (×200).

Table 1 Scores of histopathological changes in the hepatic tissues of Nile tilapia exposed to different concentrations of Cd (as $CdCl_2$) for 20 days

Histopathological lesions	Haemorrhage	Infiltration of inflammatory cells	Disintegrated nucleus	Dilated central vein	Vacuolation
Control	-	-	-	-	-
10% LC_{50} of Cd	2.00 ± 1.00^{NS}	2.67 ± 0.577^{NS}	3.00 ± 0.00^{NS}	1.33 ± 0.577^{NS}	2.67 ± 0.577^{NS}
20% LC_{50} of Cd	1.67 ± 0.577^{NS}	2.67 ± 0.577^{NS}	3.00 ± 0.00^{NS}	2.33 ± 1.155^{NS}	2.33 ± 1.155^{NS}
30% LC_{50} of Cd	1.00 ± 0.00^{NS}	2.33 ± 0.577^{NS}	2.33 ± 0.577^{NS}	2.67 ± 0.577^{NS}	1.67 ± 0.577^{NS}

NS: the means are not significantly different at the 5% level among the Cd-treatment groups.

particularly to the enterocytes and villar structures, was evident in Cd-exposed fish. In the groups that were exposed to 10% (Fig.2a, b) and 20% (Fig.2c, d) of the 96-h LC_{50} of Cd, there were higher numbers of degenerated nuclei and apoptotic cells in the crypts of the submucosal layer than there were in the controls. In addition, the longitudinal muscles of the muscularis mucosa were disorganized. The 30% LC_{50} group (Fig.2e, f) showed similar changes and also higher numbers of inflammatory cells and characteristically dilated blood vessels. Table 2 shows the scores of histopathological changes in the ileal tissues for each of the groups in this study.

4 DISCUSSION

Histopathological biomarkers have been primarily used in fish to identify and evaluate the toxic effects of exposure to contaminants (Oliveira-Ribeiro et al, 2006). Several studies have shown a range of changes in the liver of *O. niloticus* resulting from exposure to a variety of toxic chemicals (Figueiredo-Fernandes et al., 2006a, b). The accumulation of metals in the liver of fish is associated with lysis of hepatocytes, cirrhosis and ultimately death (Pourahmad and O'Brien, 2000; Varanka et al., 2001).

Our findings are in agreement with data obtained in

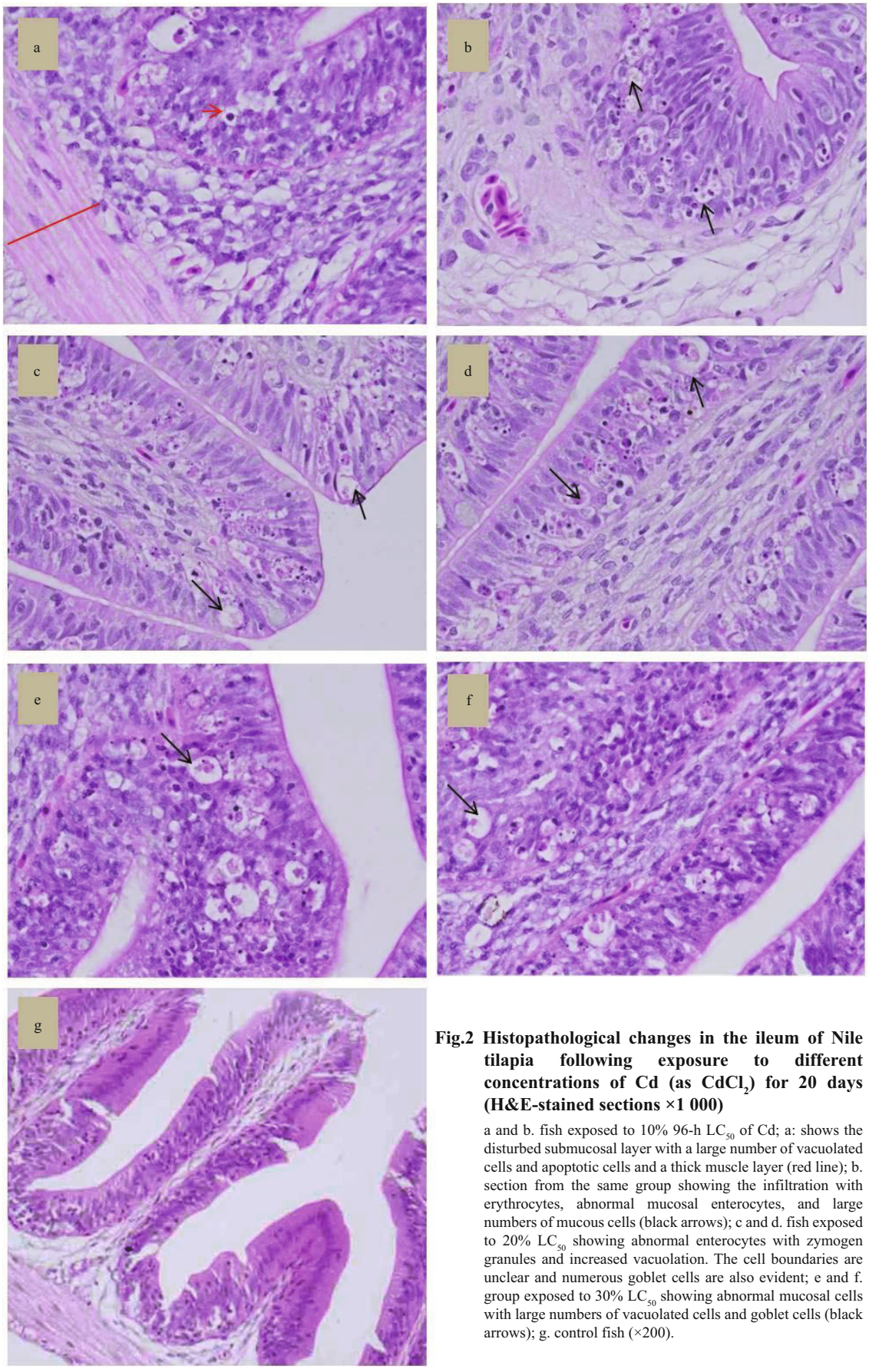

Fig.2 Histopathological changes in the ileum of Nile tilapia following exposure to different concentrations of Cd (as $CdCl_2$) for 20 days (H&E-stained sections ×1 000)

a and b. fish exposed to 10% 96-h LC_{50} of Cd; a: shows the disturbed submucosal layer with a large number of vacuolated cells and apoptotic cells and a thick muscle layer (red line); b. section from the same group showing the infiltration with erythrocytes, abnormal mucosal enterocytes, and large numbers of mucous cells (black arrows); c and d. fish exposed to 20% LC_{50} showing abnormal enterocytes with zymogen granules and increased vacuolation. The cell boundaries are unclear and numerous goblet cells are also evident; e and f. group exposed to 30% LC_{50} showing abnormal mucosal cells with large numbers of vacuolated cells and goblet cells (black arrows); g. control fish (×200).

Table 2 Score of histopathological changes in the ileal tissues of Nile tilapia exposed to different concentrations of $CdCl_2$ for 20 days

Histopathological lesions	Haemorrhage	Infiltration by inflammatory cells	Disintegrated nucleus	Dilated central vein
Control	-	-	-	-
10% LC_{50}	-	2.33 ± 1.155^{NS}	2.33 ± 1.155^{NS}	1.67 ± 1.528^{NS}
20% LC_{50}	-	2.67 ± 0.577^{NS}	3.00 ± 0.00^{NS}	2.00 ± 1.00^{NS}
30% LC_{50}	-	2.33 ± 1.155^{NS}	2.67 ± 0.577^{NS}	1.00 ± 0.000^{NS}

NS: the means are not significantly different at the 5% level among the Cd-treatment groups.

previous studies, which have tended to show increased vacuolar degeneration of hepatocytes, necrotic foci, thromboses in central veins, dilation and congestion of the blood sinusoids, and fibrosis (Soufy et al., 2007; Kaoud et al., 2011; Omer et al., 2012). There is also broad consistency between the current study and the histological responses that have been reported previously in the liver of various fish species exposed to cadmium. In catfish *Clarias batrachus* exposed to 4×10^{-6} and 8×10^{-6} $CdCl_2$ for 30 and 60 days, these included: deshaping of hepatocytes, eccentric positions of nuclei, enucleation, development of cytoplasmic vacuoles, and necrosis of hepatic tissue (Bilal et al., 2011). In *Cyprinus carpio*, atrophy and necrosis of hepatic cells, a decrease in the size of the nuclei and nucleoli, and indistinguishable cell membranes were observed (Figueiredo-Fernandes et al., 2007). The formation of macrophage granulomas was reported in *Carassius auratus* (Tafanelli and Summerfeldt, 1975), and, in *Halobatrachus didactylus*, an increase in connective tissue and hepatocyte nuclei was observed (Gutierrez et al., 1978).

The liver is associated with detoxification and biotransformation processes and these functions, together with its location and rich blood supply, mean that it is one of the organs most affected by water pollutants (Mohamed, 2009). Microanatomical changes in the liver are frequently associated with the response of hepatocytes to toxicants (Van Dyk et al., 2007). Thus the histological alterations identified in liver tissues in this study may have resulted from disruption of various biochemical processes. These vacuolation of hepatocytes may have been a consequence of inhibition of protein synthesis, energy depletion, disaggregation of microtubules, or shifts in substrate utilization, as found by Ajani and Akpoilih (2010). Furthermore, protein inclusion bodies are commonly associated with metal toxicity. The vacuolation of the hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulatory system (Gingerich, 1982). Deficiency of oxygen as a result of gill degeneration is the most common cause of cellular degeneration in the liver (Mohamed, 2001).

The uptake of metals occurs mainly via the gills but may also occur via the intestinal epithelium (Mohamed, 2008). Toxic pollutants enter the digestive tract of fish via the food and water they consume, causing structural and functional deterioration of the intestine (Banerjee and Bhattacharya, 1995). The present study revealed significant damage to the intestines of *O. niloticus* exposed to three different concentrations of cadmium for 20 days. Similar findings were reported by Kaoud et al. (2011) who reported pathological changes in the intestine of *O. niloticus* that were exposed cadmium, including: atrophy in the muscularis mucosa; degenerative and necrotic changes in the mucosa and submucosa with necrotized cells aggregated in the intestinal lumen; and edema and atrophy in the submucosa. Omer et al. (2012) also observed that cadmium toxicity of *O. niloticus* was associated with marked desquamation and congestion of submucosal blood vessels in the intestinal mucosa and infiltration of inflammatory cells.

The histopathological alterations observed in the intestine of *O. niloticus* comprised severe degenerative and necrotic changes in the intestinal mucosa. The edema observed between the submucosa and mucosa may also have been caused by the absorption of toxic heavy metals (Hanna et al., 2005). Epithelial degeneration, inflammatory cell infiltration into the submucosa, and submucosal edema have also been observed in the intestine of tilapia exposed to carbofuran for 8 weeks (Soufy et al., 2007).

5 CONCLUSION

Fish are useful experimental models that have been widely used to evaluate the health of aquatic ecosystems and toxicological pathology. The tilapia *O. niloticus* is recognized as a good biological model

for investigating possible adaptations to pollutants. This study has confirmed the toxic effect of cadmium to *O. niloticus*, when introduced as a water pollutant. At all tested concentrations, cadmium was found to enter the digestive tract via the food and water they consumed, causing the deterioration of structures in the gut and in the hepatic tissues.

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