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Short communication

Observation of the Bile Canaliculi of *Puntius javanicus* Liver Affected by Copper

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ABSTRACT

Investigation on *in vivo* effects of copper (Cu) on the ultrastructure of *P. javanicus* liver was carried out using transmission electron microscopy (TEM). The addition of sublethal concentration of 5 mg/L of Cu caused abnormalities on the bile canaliculi (BC) including dilation and elongation compared to control and at lower concentrations of copper with a normal round shape form. Findings from this study support an alternative histological assessment of the effects of Cu concentration on *P. javanicus* liver.

INTRODUCTION

Toxic effects of Cu may affect several parts of the biological system from molecular to the cellular or physiological level [1,2]. Aquatic organisms are the main target of this toxicant, which leached from the agricultural and industrial activities into the water bodies. Moreover, Cu can be bioaccumulated and transferred to other biological parts and organisms through consumptions associated with copper contaminated feed [3,4]. At the cellular level, Cu causes the activation of programmed cell death through the upregulation of reactive oxygen species or ROS [5]. Histological study on fish hepatocyte showed several abnormalities caused by Cu toxicity such as dilation and

congestion of sinusoid, vacuolation, development of macrophage activities, hyalinisation and haemorrhage [6-9]. At the ultrastructure level, the deformation of the peranchymal was observed as well as the fragmentation of endoplasmic reticulum, mitochondrial damage, development of lipid droplet, nuclear rupture and the formation of apoptotic bodies and necrosis [10-12]. These studies demonstrate the marker of Cu toxicity in affected organism through the assessment on liver.

In this present study, toxic effects of Cu were observed to result in changes to the size of bile canaliculi (BC) of *P. javanicus* liver.

MATERIALS AND METHODS

P. javanicus were obtained from the the Inland Fisheries Training Centre, Bukit Tinggi, Pahang, and brought alive and acclimatise in laboratory condition for 15 days (12 hours daylight, 12 hours night). Fish were separated to three groups with nine fish in each group. Two groups were treated with 0.5 and 5.0 mg/L concentration of Cu, respectively, while the untreated group served as the control for the study. At the end of the 96 hours treatment, the fish were killed with their livers removed.

The livers were processed for sample preparation according to the method of Sabullah et al. [5]. 1 mm³ of fish liver was fixed for 20 hours, 10 minutes of triplicate wash and 2 hours of post fixation with 4% of glutaraldehyde, 0.1 M sodium cacodylate and 1% of cold osmium tetroxide. The sample was then dehydrated and infiltrated followed by polymerisation. Thin sectioning was carried out using microtome prior to visualisation under TEM.

RESULT AND DISCUSSION

At the end of 96 hours treatment, no mortality was recorded. Only death sign was observed at the group treated with 5 mg/L Cu through the loss in appetite and a decrease in swimming performance. Both treated samples were visualised using TEM to be compared at the ultrastructure level. **Fig. 1a** displays the treatment at 0.5 mg/L with a normal round shape of BC. On the other hand, when presented at the sublethal concentration of 5 mg/L of Cu, significant alteration of the BC was observed, which was dilated and structurally elongated (**Fig. 1b**).

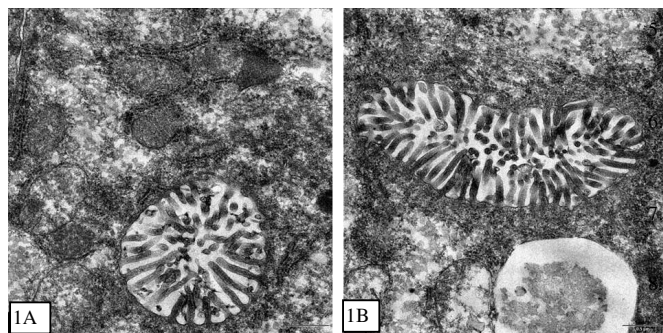


Fig. 1. TEM images of BC of *P. javanicus* exposed to 0.5 mg/L (1a) and 5 mg/L (1b).

It can be considered that Cu is not harmful for BC at low level of concentration and that it can be removed from the hepatocyte as mentioned by Roberts and Sarkar [13]. Thus, there is no doubt that high concentration of Cu may cause harmful effects to the parenchymal cells. This study coincides to the reports of Azumi [14], which stated that the toxic effects of Cu had caused the dilation of BC along with swollen microvilli in dog liver. Other than Cu, lead and arsenic also gave similar effects associated with nuclear damage in the rat liver [15, 16]. In fact, Jattujan et al. [17] have also reported the enlargement of BC and density reduction of microvilli in hamster infected by *Opisthorchis viverrini*.

BC plays a role in collecting bile products secreted by parenchymal which flow to the duodenum [18]. In contrast, abnormal bile canaliculi triggers the activation of programmed cell death caused by the accumulation of bile acids or cholestasis [19]. Hence, it can be concluded that the

development of apoptotic and necrotic cells affected by Cu is not only related to upregulation of ROS as mentioned by Rhee et al. [20], but also the failure of BC secretion to contribute or enhance the process of execution pathway.

CONCLUSION

The present study has ultrastructurally shown the changes of BC in *P. javanicus* liver due to copper exposure, which can be considered as an alternative indicator of Cu toxicity.

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