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Copper exposure failed to induce caspase-3 activation in gills of the teleost fish, *Oreochromis niloticus*

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Copper, an essential trace element, becomes toxic when its concentration exceeds certain natural levels. Recent in vitro studies demonstrated that copper overload of cells may induce apoptosis through caspase-3 activation [1]. However, the precise mechanism of copper-induced apoptosis in fish is still unclear, even less in *O. niloticus* where no studies have been reported so far. Accordingly, the main aim of this study was to find if copper induces apoptosis in *Oreochromis niloticus* gill, through the caspase pathway. Additionally, we intended to evaluate the potential use of activated caspase-3 expression as a waterborne copper biomarker.
Tilapia (n=60) were *in vivo* exposed to 40 and 400 μg L^{-1} of copper. After 3, 7, 14 and 21 days of exposure, the second gill arch of the right side of each fish was collected and processed to histology. Apoptosis was evaluated by immunohistochemistry using an affinity-purified rabbit anti-caspase-3 active antibody. Apoptotic cells relative volume in gill filament was determined by stereological procedures based on point counting [2, 3] and using the following formula: V_V (structure, reference) = [P(on stained cells) x 100] / P(on reference space). Data was statistical analyzed by two-way ANOVA and the Tukey post-hoc test, with α=0.05 (SigmaStat, SPSS Inc.).
In control and exposed fish, positively stained cells were found isolated and preferentially located in filament epithelium, although some lamellar apoptotic cells were also observed (Fig. 1). The determination of the volumetric density of total immune-positive cells showed that there were no significant differences between control and exposed fish, nor between fish exposed to 40 and 400 μg L^{-1} of copper (Fig. 2). Although in filament epithelium a slight decrease was observed, more evident in fish exposed to 40 μg L^{-1}, in lamellae a no significant increase in the immune-positive cells relative volume was detected, mainly after 3 days of exposure to 400 μg L^{-1} of copper.
Information about the biochemical mechanism of copper-induced apoptosis is controversial and the studies made until now suggest that copper may induce apoptotic cell death through different pathways [1]. The present study reveals that, in *O. niloticus*, the caspase pathway seems not to be activated by continued exposure to copper. On the contrary, the volumetric density analysis indicted a general decreasing trend in exposed fish. Although complementing studies are needed to elucidate if apoptosis is triggered through caspase-independent pathways, the present work also prove that caspase-3 expression in gill is not a reliable biomarker for copper levels in water.

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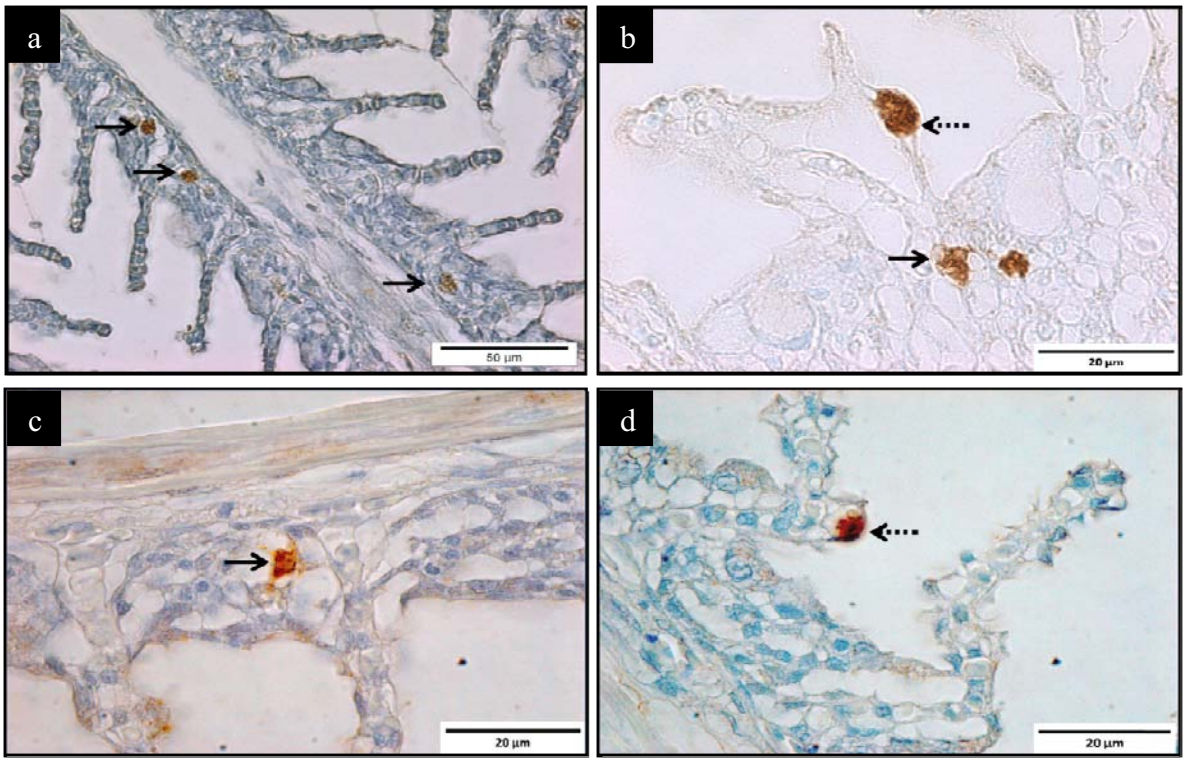

Fig. 1. Photomicrographs of *O. niloticus* gill sections, showing cells immune-positively stained for activated caspase-3. Positively stained cells are present in gill filament (arrow) and in lamellae (dotted arrow). a) and b) gills of fish exposed to 40 µg L^{-1} of copper for 3 and 7 days, respectively; c) and d) show gills of fish exposed to 400 µg L^{-1} during 14 and 21 days, respectively.

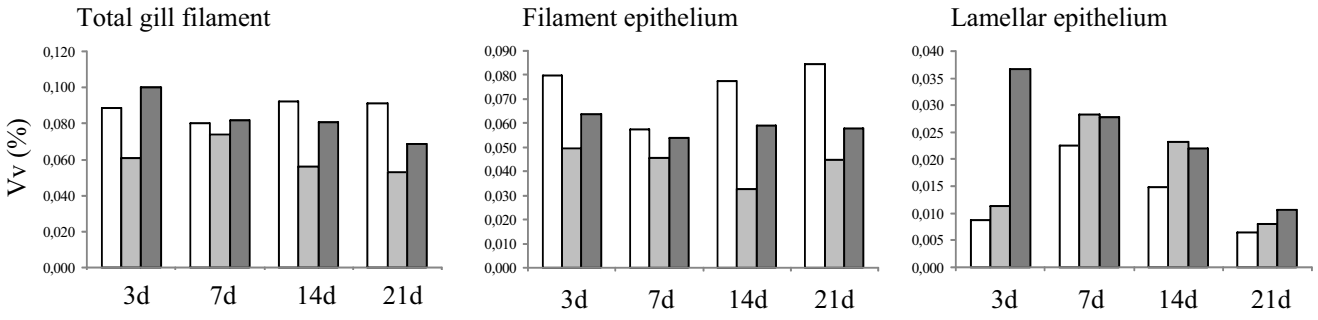

Fig. 2. The graphs show the relative volumes (vertical axis) of immune-positive cells in gill filament according to the days of exposure (horizontal axis). Statistical analysis showed no significant differences in the relative volumes of positively stained cells between controls and exposed fish. White bars, control fish; Light grey, fish exposed to 40 µg L^{-1} of copper; Dark grey, fish exposed to 40 µg L^{-1} of copper.

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