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IN-VITRO GENOTOXIC EFFECT OF COPPER IN NILE TILAPIA FINGERLINGS (*Oreochromis niloticus*)

By

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Cu	-	Copper
Cu^{2+}	-	Copper in ionic form
ppb	-	parts per billion
ppm	-	parts per million
HNO_3	-	Nitric acid
HCL	-	Hydrochloric acid
H_2O_2	-	Hydrogen Peroxide
^0C	-	degree Celsius
g	-	gram
mg	-	milligram
cm	-	centimeter
µg	-	microgram
mg L^{-1}	-	miligram per liter
µl	-	microliter
%	-	percentage
bp	-	base pair

ABSTRACT

The effects of prolonged exposure of copper through sub-lethal toxicity test using Nile tilapia (*Oreochromis niloticus*) fingerlings was investigated using five nominal copper concentrations (control, 0.1819, 0.3638, 0.7276 and 1.0914 mg L^{-1}). Throughout 21 days of exposure, tilapia fingerlings were harvested at 7, 14 and 21 days for mean copper accumulation detection through ICP-MS, investigation of cell damage through Single Cell Gel Electrophoresis and determining DNA structure alteration through RAPD-PCR analysis. The 96-h LC_{50} value for Nile tilapia fingerlings was calculated to be 1.819 mg L^{-1}. Accumulation of copper was studied on four different parts; muscle-tissue, viscera, gills and whole fingerling body. The uptake of copper varied in an order from highest concentrations; viscera > whole body > gills > muscle-tissue. Highest Cu^{2+} concentration accumulated by viscera was 690.4904 μg g^{-1} at 21 days. At cell level, damage induced by Cu^{2+} in tilapia fingerlings showed a significant increase in DNA tail percentage in 7 and 14 days of exposure, indicating DNA damage was observed at all concentrations compared with control (P<0.05). A gradual decrease in the mean DNA tail percentage was observed at 21 days indicating repair of the damage DNA. The mean DNA tail percentage showed a dose-related increase and time dependent decrease after the treatment with Cu^{2+} when compared to control. RAPD pattern displayed some changes in polymorphism band patterns in exposed Nile tilapia fingerlings. The disappearance of bands was found in highest Cu^{2+} concentration (1.0914 mg L^{-1}) for primer OPB 7 at band molecular size of 500 bp in 14 and 21 days of exposure. Disappearance of bands proved that DNA fragment of exposed tilapia were altered signifying that DNA damage had

occurred. Therefore, results in the present study proved that Cu^{2+} is significantly known as genotoxic compound to Nile tilapia fingerlings as damage in cell level and DNA fragment alteration was detected.

ABSTRAK

Kesan Genotoksik Kuprum Ke Atas Anak-Anak Ikan Nile Tilapia (*Oreochromis niloticus*)

Kesan pendedahan logam kuprum melalui ujian ketoksikan kronik yang menggunakan anak-anak ikan Nile tilapia (*Oreochromis niloticus*) telah dikaji dengan menggunakan lima jenis kepekatan logam kuprum (kawalan, 0.1819, 0.3638, 0.7276 and 1.0914 mg L^{-1}). Sepanjang pendedahan selama 21 hari, anak-anak ikan tilapia dibunuh pada hari ketujuh, keempat belas and hari kedua puluh satu untuk menentukan jumlah logam kuprum yang telah terakumulasi melalui analisis ICP-MS, menyiasat kerosakan yang berlaku pada peringkat sel melalui kaedah Single Cell Gel Electrophoresis dan menentukan perubahan yang berlaku pada struktur DNA melalui analisis RAPD-PCR. Nilai 96-h LC_{50} bagi ujian penentuan ketoksikan akut logam kuprum telah dikira sebagai 1.819 mg L^{-1}.Akumulasi logam kuprum telah diselidik menggunakan empat bahagian berlainan iaitu tisu, visera, insang dan seluruh bahagian badan anak ikan tilapia. Jumlah akumulasi logam kuprum yang dianalisis, didapati mengikut turutan tersebut; visera > seluruh badan > insang > tisu. Kepekatan logam kuprum yang paling tinggi dikesan pada visera iaitu sebanyak 690.4904 μg g^{-1} pada hari kedua puluh satu. Kerosakan yang disebabkan oleh Cu^{2+} pada bahagian sel ikan tilapia telah menunjukkan peningkatan dalam peratus ekor DNA pada hari ketujuh dan keempat belas, menandakan kerosakan DNA wujud untuk semua kepekatan Cu^{2+} berbanding dengan sampel kawalan (p<0.05). Penurunan secara beransur-ansur dalam peratus ekor DNA juga telah diperhatikan pada

hari kedua puluh satu, menandakan pemulihan berlaku pada DNA yang rosak selepas dianalisis melalui Single Cell Gel Electrophoresis. Kehilangan jalur dikesan pada kepekatan logam Cu^{2+} (1.0914 mg L^{-1}) bagi primer OPB 7 untuk jalur molekul yang bersaiz 500 bp di hari keempat belas dan kedua puluh satu. Kehilangan jalur membuktikan bahawa struktur DNA telah mengalami perubahan. Oleh itu, keputusan dari kajian ini membuktikan bahawa Cu^{2+} sudah terbukti sebagai agen genotoksik terhadap anak-anak ikan tilapia kerana kerosakkan pada peringkat sel dan pada strukur DNA dapat dikesan.