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Nephrotoxic effects of copper oxide nanoparticles in *Cyprinus carpio* assessed by histological profiles

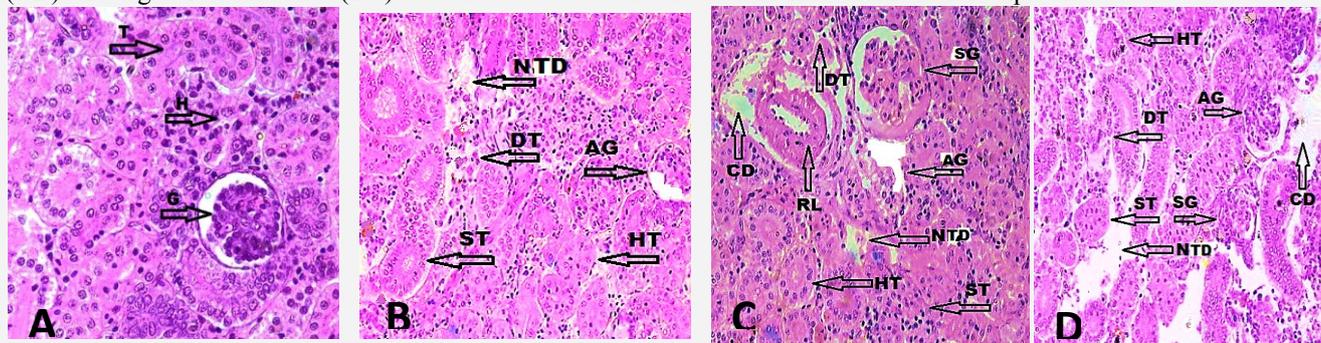
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Application The results of this study provide an insight into the toxicity of Cu-NPs in aquatic organisms. It may help policy makers to suggest preventive measures against the potential toxicity of nanoparticles (NPs) in aquatic organisms.

Introduction Metal oxide NPs such as copper (Cu) have gained significant attention due to their negative ecological effects. A wide range of NPs is being used as biosensor immobilizers for their greater sensitivity and specificity. Due to their low preparation cost and prospective applications, Cu-NPs are intensively used in the industry. Besides, Cu-NPs are also used as one of the main constituents of fungicides, algacide and herbicides. However, they can cause histological alterations and oxidative DNA damage in the living organisms at the cellular level. The use of engineered NPs can result in their release into aquatic environments causing unexpected hazards for aquatic organisms. Therefore, this study assessed the toxicological impacts of Cu-NPs on the kidneys of *Cyprinus (C) carpio*.

Material and methods A fish husbandry system was established prior to the start of experiments to support the health of fish by maintaining the water quality and environment of stock aquariums. *Cyprinus carpio (C. carpio)* of the similar weight (40-45g) were procured from a Fish Seed Hatchery, transported in plastic containers with continuous aeration to the laboratory of GC University Faisalabad, Pakistan. The fish were acclimatized in the tank with 100 L capacity for two weeks prior to the experiment. Un-chlorinated tap water was used and the physicochemical parameters of water were determined by a multi parameter apparatus (HI 9828, HANNA INSTRUMENTS). During the acclimatization period, water temperature was maintained at 25°C, while dissolved oxygen and pH were 6.6–7.6 mg/l, and 6.9–7.5, respectively. During the acclimatization period, the fish were fed with a commercial fish feed. After acclimatization, *C. carpio* from aquaria were randomly transferred into twelve aerated experimental glass aquaria (10 fish/ aquarium) with the same physicochemical parameters as in the acclimatization period and acclimated for 48 hours prior to the experiment. The fish were exposed in triplicate to 0 (Control) or 0.5 or 1 or 1.5 mg/l Cu as Cu-NPs for 14 days. During this study, water in the aquaria was changed daily and freshly prepared solution was added to maintain the concentration of Cu-NPs at a constant level. At the end of the experiment fish from each aquarium were immediately anesthetized into a 200 ppm solution of clove powder and kidneys were collected for histological analysis. Kidney tissues were fixed in freshly prepared Bouin's fixative (15:5:1, saturated picric acid, formalin, and glacial acetic acid) for 12-14 h at room temperature. The fixed tissues were processed with graded alcohol series, followed by xylene and finally embedded in paraplast (P3683, Sigma). Sections of 5 mm thickness were cut using a microtome (Leica Biosystems RM2125 TRS) rehydrated and stained using hematoxylin-eosin. Later, the sections were dehydrated and finally mounted using DPX Mountant. The slides were observed under a light microscope (Nikon Eclipse E200 POL) fitted with a camera and photographed.

Results The results revealed that the Cu-NPs induced significant alterations in the kidneys of *C. carpio* in a dose dependent manner. The alterations in kidneys including necrosis and tubular degeneration (NTD), hypertrophy of tubules (HT), reduced lumen (RL), abnormal glomerulus (AG), shrunk glomerulus (SG), swollen tubules (ST), complete degeneration (CD) and degenerative tubules (DT) were increased with the increase in the dose of Cu-NP exposure.



A= control (normal histology) B - 0.5 mg/l Cu NPs exposure C - 1 mg/l Cu NPs D - 1.5 mg/l Cu NPs

Figure: Microphotograph of *C. carpio* kidney in control and treated groups showing histological alteration (H&E, X400).

Conclusion It is concluded that Cu-NPs were toxic to the fish as indicated by nephrotoxicity in *C. carpio*. Thus it is suggested that the bioavailability of metal NPs by aquatic pollution and subsequent accumulation in fish tissues might constitute a substantial risk to human health and to the environment.