Copper nanoparticles induced oxidative stress and tissue integrity in gills and brain of *Cyprinus carpio*

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Abstract

Copper nanoparticles are widely used in various commercial and industrial products and have potential toxicity. This study assessed the effects of copper nanoparticles on gills and the brain of *Cyprinus carpio*. This study investigated the sub-lethal effects of waterborne copper nanoparticles by involving *Cyprinus carpio* (n=120) of 40±5g weight distributed into four groups with 3 replicates having 10 fish in each group. For this purpose *Cyprinus carpio* were exposed to either 0.6 or 1.04 or 1.6 mg/L of waterborne copper nanoparticles for 14 days. Histopathological and enzymatic studies were carried out. Control fish showed normal histology while in treated groups; gill tissues showed alterations such as necrosis, filament fusion, congestion, the curvature of filaments, gills edema and thickening of the primary and secondary lamella. Enlarged primordial cells, necrosis, and hemorrhage were the changes reported in the brain. These changes were dose-dependent and increased with increasing dose. In this study activity of catalase enzymes decreased as compared to control while activities of lipid peroxide and reduced glutathione increased significantly when compared with control group activities. Study revealed that Copper nanoparticles exposure induced significant toxicological impacts on gills and brain.

Keywords: Toxicity, Histology, Oxidative stress, Fish, Cu NPs

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Introduction

The expression "nanomaterial" depends on the prefix "nano," which originated from the Greek word signifying "dwarf". Exactly, the word nano implies 10^{-9} or one-billionth of a meter (Huang *et al*., 2015). Nanoparticles are small substances having size reaches from 1 to a hundred nm. They may be grouped into various groups depending on their residences, shapes and sizes (Kakakhel *et al*., 2021). The distinctive type incorporates metal nanoparticles, ceramic nanoparticles, and polymeric nanoparticles. Nanoparticles have interesting physical and chemical possessions because of their high surface area and small expanse (Khan *et al*., 2019; Malhotra *et al*., 2020). Nanoparticles are tiny item that acts as a sole unit concerning its delivery and characteristics and varies to a terrific volume from massive substances of comparable structures. The qualities of nanoparticles fluctuate with length, chemical condition, and shape and regardless of their extensive uses nanoparticles, related toxicity has increased considerably in the preceding decade (Gupta *et al*., 2016). NPs have various individual properties like high reactivity, sole optical properties and biocompatibility in contrast with their mass co-related materials because of their high surface area to size ratio. Recently, nanomaterials (NMs) have contributed in the economy of numerous divisions including shopper items, vitality, transportation, beauty care products, pharmaceuticals, antimicrobial specialists and agriculture (Singh *et al*., 2017).

Aquatic contamination with metals is a worldwide natural issue. In most recent years, natural contamination from substantial metals has been seriously analyzed in freshwater biological systems because of the bioaccumulation and toxicity of metals (Wang *et al*., 2014). Heavy metals enter in fish body by three possible ways: by gills, by digestive track, and by body surface. The gills are considered as the significant site for direct uptake of metals from the water though the body surface is normally estimated to take minor part in uptaking of heavy metals in fish (Selda and Nursah, 2012).

The animals living in an aquatic environment get NPs via the gills, digestive tract, olfactory organ, pores and the skin. At the cell level, NPs navigate the cells via endocytosis and after that instigate dangerous consequences for the living organisms. Human beings get contact with nanoparticles by mechanical items, food products, pharmaceuticals, and therapeutic applications; they may experience skin irritation, gastrointestinal wounds, and respiratory problems (Aghamirkarimi *et al*., 2017). Any single physicochemical character of NPs can impact the conveyance, which at a specific degree decides the material lethality. Size surface charge, constituent, and chemistries of the covering could influence NP accumulation and discharge. Clarifying the biodistribution of NPs in organs is valuable to control the change and

alteration of NPs (Yang *et al*., 2017; Sielska *et al*., 2024).

Copper (Cu) is a fundamental component required for ordinary physiological functions in humans and animals, including hemoglobin synthesis, drug digestion, starch digestion, catecholamine biosynthesis, and cancer prevention agent barrier system, But when the entry of Cu surpasses the scope of natural resilience, it can cause unfavorable impacts, including hemolysis, gastrointestinal infections, and liver and kidney damage (Lee *et al*., 2016). Copper nanoparticles, due to their intriguing properties, increase usage in catalysis, cooling liquid or conductive inks, have pulled in a ton of enthusiasm for current years (Dang *et al*., 2011). Cu NPS, due to its excessive conductivity of electricity, excessive melting point, low electrochemical movement conduct and cheap are much in need. The property of copper nanoparticles chiefly relies upon the amalgamation course and their procedure parameters (Tamilvanan *et al*., 2014).

The uses of copper and copper nanoparticles, which depend on earth-inexhaustible and reasonable copper metal, have created lots of enthusiasm for ongoing years, in particular inside the field of catalysis. The convertible change of compound and physical qualities of these NPS utilizing distinctive manufactured procedures and conditions or by means of post engineered synthetic chemical treatment has been to a great extent in charge of the fast development of enthusiasm for these nanomaterials and their applications in catalysis (Gawande *et al*., 2016). The common carp, *Cyprinus Carpio* is a fish living in freshwater farmed in different regions of the earth, because of its minimal attempt of generation, excessive muscles, and simple production. Common carp had an area with the *Cyprinidae* family with a yearly worldwide creation of 3.4 million tons. To stay aware of its worldwide need, carps are raised in monocultures framework which is counterfeited-based and in this way are bound to be presented to different sorts of poisons because of cultivating methodologies. Hence, common carp study has gotten more consideration among specialists as a model to examine toxicity (Lackner, 1998). In addition, *C. carpio* is an important freshwater animal often used in ecotoxicology studies because it is highly sensitive to harmful substances. Tissue changes in the gills of *C. carpio* exposed to copper nanoparticles (Cu-NPs) have been noticed (Sielska *et al*., 2024).

Metabolism of xenobiotic contaminations maybe causes more improvement of reactive oxygen species. Amid oxidation of xenobiotics surrounding oxygen is enacted which might be discharged from the enzymes, harming cell structures and their activity (Sevcikova *et al*., 2011). Metals are an imperative agent of oxidative damage in aquatic species, an association of receptive oxygen species through two components. Redox dynamic metals create receptive oxygen species via redox cycling, at the same time as metals

without redox ability hinder antioxidant protection (Alkobaby and El-Wahed, 2017).

Gills are regarded as a basic part of fish on account that they represent the critical spot for gas transport, discharge of metabolic waste and particle regulation. Due to the large surface area exposed to the outer atmosphere, gills are additionally principle focus to contamination in water. While existing in excessive amount, copper has been accounted for the reason of extreme physiological alteration in gills. Gills physiology is valuable as an advance pointer to the fish healthy condition (Ohkawa *et al*., 1979). Histological changes in gills of fishes are combination or separation of auxiliary lamellae, hyperplasia, lessens secondary lamellae, blood clot in the vascular hub of essential filaments, and cell necrosis. The level of damage was increasingly serious at higher copper nanoparticles values (Aebi, 1974).

Materials and methods

Experimental plan

Copper nanoparticles 50<nm were purchased from Sigma-Aldrich Co., LLC GmbH, Germany in the form of powder. Experimental animal use for this experiment was *Cyprinus carpio* weight about 40+5g provided them with standard living conditions in the aquarium, purchased from Govt. Fish hatchery Peer Mahal, District Toba Tek Singh. This study involve *Cyprinus carpio* (n=120) of 40±5g containing 4 groups with 3 replicates having 10 fish in each group named as A, B, C and D and subject to different concentrations of waterborne Copper nanoparticles as 0g, 0.6g, 1.04g, 1.6 mg/L of pre-calculated LC_{50}. The dose was prepared by adding the calculated amount of copper nanoparticles with 0.1 mL of acetic acid and 10mL of water and sonicated for about 1.5 hours.

Total experimental durations were 14 days. The dose was given at alternate days. After exposure to Cu NPs, samples were collected at 7 days and 14 days of the experiment. After 7 days some fishes were dissected, gills and brain were preserved in 10% formalin for histopathological examination and some preserved in the freezer at minus 40°C for oxidative stress examination. The same process was repeated after 14 days.

Biochemical assays

Histology

Tissues of brain and gills were fixed in sera for 4-5 hours. Components of sera were 10mL glacial acetic acid, 60mL absolute alcohol, and 30mL formaldehyde. After fixation dehydration was done in ascending grades of 80% ethanol throughout the night at ambient temperature 90% of ethanol 2 to 4 hours at core temperature, and 100% of ethanol 2 to 4 hours at core temperature. When the dehydration process was completed of fixed tissues of the brain and gills, cedar wood oil was added to them until they became clear at ambient temperature.

After dehydration tissues of brain and gills were embedded in benzol one for 10 min at core temperature in benzol two for 10 min, in benzol and after that in

paraplast (1:1) for 20 min at temperature of 60°C, then in paraplast one for 12 hours at temperature of 60°C, in paraplast two for 12 hours at temperature 60°C, and in paraplast three for 12 hours at temperature of 60 °C. Tissues of gills and brain which were embedded were then shifted into thaw wax in a bore. Bubbles formed were abolished and was solidified. Wax blocks of paraffin were cut with a knife and then it was arranged on a block of wood for sectioning of tissues.

Tissues of gills and brain which were embedded in paraffin wax were arranged on blocks made of wood and 5um fine sections were clipped with help of microtome. The ribbon with tissues of the brain and gills were stretched and placed in a clean albumenized slides made of glass on Fisher slide warmed at a temperature of 60°C. These slides made of glass were fixed in incubator throughout the night for the execution of removal of bubbles and stretching. Hematoxylin stain was prepared by using 2g hematoxylin, containing 100mL of absolute ethanol, 100 mL of distilled water, 3g of ammonium alum, 0.24g of sodium iodate, 10 mL of acetic acid and 100mL of glycerol. In 100 mL of ethanol two gram of hematoxylin was added. The solution of ammonium alum was formed by dissolving three gram of alum in distilled water and boiling it. The solution of hematoxylin was added to the solution of ammonium alum and then glycerol and sodium acetate was added. In the last step acetic acid was added and was mixed.

Eosin stain was formed by dissolving one gram of eosin in 100mL of 70% of ethanol. Hydration process starts with deparaffinization of slides in xylene throughout the night. Tissues which were removed from paraplast were hydrated in 100%, 90%, 70%, 50%, and 30% alcohol for 2-4 min at core temperature separately. These tissues were washed with tap water. After that, they were dipped in hematoxylin for 2-3 times. And they were again washed with tap water for 5-10 min until tissues of the brain and gills were turned blue color. Sections of tissues were dehydrated in 30%, 50%, 70% and 90% of alcohol for 3 to 5 minutes at ambient temperature separately. After that sections were dipped in eosin and 90% alcohol for one time. They were dehydrated in 100% alcohol for 2 to 5 min at room temperature and then in xylene, approximately 5 to 10 min. After staining process slides were fixed in Canada balsam. After that, they were covered with cover slips and then placed in incubator throughout the night. Xylene was used to remove extra Canada balsam. 5um thin sections of brain and gills of *Cyprinus carpio* were studied under a light microscope at 4, 20, 40, and 100 magnifications and photographed by digital camera.

Oxidative stress

Estimation of lipid per oxidation

Lipid peroxidation (LPO) was estimated by the method of (Ohkawa *et al*., 1979). The reaction mixture contains 0.2 milliliters of 10% tissue homogenate, 0.2 milliliter of 8.1 (%) sodium dodecyl

sulfate, 1.5 milliliters of 20 (%) acetic acid, and 1.5 milliliters of 0.8 (%) aqueous solution of thiobarbituric acid. The pH of 20 (%) CH_3COOH (acetic acid) was adjusted with 1M NaOH of 3.5. The 4-milliliter mixture was made with distilled water and heated at 95°C for one hour in a water bath using a glass ball as a condenser. After cooling in tap water one milliliter of distilled water and 5 milliliters of a mixture of n-butanol and pyridine (15:1) were added and the mixture was shaken vigorously on a vortex mixer. After centrifugation at 4000 rpm for 10 minutes and the absorbance of the upper organic layer was read at 532 nm.

Estimation of catalase (CAT)

Catalase (CAT) was analyzed by following the protocol of (Aebi, 1974). The reaction mixture containing 50 µL of 10% homogenate with 3.0 mL cuvette that contained 1.95 mL of potassium buffer (50 mm, pH 7.0). The reaction was initiated by the addition of 1 mL of 30 mM hydrogen peroxide (H_2O_2). The solution was read at 240 nm for 30 seconds at an interval of 30 seconds. The activity of catalase was expressed as Unit per mL of a homogenate of tissue.

Estimation of reduced glutathione (GSH)

Each homogenate was precipitated with 50% trichloroacetic acid ($C_2HCL_3O_2$), centrifugated at 1000 rpm for 5 minutes. Each supernatant (0.5 milliliter) was taken and mixed with 2.0 milliliter of Tris-EDTA buffer (0.2 M, pH 8.9). The reaction was initiated by the addition of 0.1 milliliters of 0.01 M DTNB (5, 5′-dithiobis-2-nitrobenzoic acid). The solution was kept at room temperature for five minutes and the contents were read at 412 nm on a spectrophotometer. The values of GSH were expressed as µm/g of tissue.

Statistical analysis

The histopathological alternation in brain and gills were not examined statistically. They were analyzed visually to examine any potential difference between treated groups with copper nanoparticles. The data from biochemical assay were examined using Minitab of version 17. ANOVA (analysis of variance) was applied by using Tukey's test at a 95% significance level to compare means of values at $p<0.05$.

Results

NPs are portrayed by their tiny size and substantial surface area with an active group. These qualities increment their chemical reactivity to empower them to enter into living cells. The effects of NPs on human and the surroundings have been advanced by certain researchers and organizations (Abu-Dief *et al*., 2015). Nanoparticles are commonly viewed as risky in a lethality appraisal for many reasons. The tiny size and the moderately vast surface area had been proposed to bring expanded toxicity when contrasted with small particles (Song *et al*., 2011).

The morality of any fish in this experiment at this dose was not reported. The microscopic abnormalities in Gills

and Brain due to the administration of waterborne copper nanoparticles in *Cyprinus carpio* were observed. In this study, micrograph showed a normal histological pattern. No abnormalities were observed in primary and secondary gills lamella. No edema and dilations were observed. Necrosis, Congestion and Gill filament fusion was also not observed. The biomarkers that can be utilized to survey oxidative stress in vivo have been pulling in premium on the grounds that the precise estimation of such pressure is fundamental for the examination of its role in way of life damages just as to assess the adequacy of treatment. Numerous markers of oxidative stress have been proposed (Yoshikawa and Naito, 2002). Fish constitute the biggest group of vertebrates and they occupy a wide range of environment where they are exposed to a vast range of aquatic contaminants. In many expressions, the injurious impacts of contaminants have been associated with the enlistment of oxidative stress (Lushchak, 2016). In this study, dissection was done after 7 and 14 days of exposure with waterborne Cu NPs. Organs were taken and histopathological analysis was done. Mild abnormalities were observed in Copper nanoparticles treated groups such as curvature of filaments, dilations, necrosis, tip degeneration, congestion, filament fusion, and edema. Similar abnormalities were observed after 14 days of exposure but due to the increase in the amount of dose, they were observed at a high rate as compared to mid dissected organs. Abnormalities were observed in group C were dilations, curvature of filaments, necrosis, congestion, tip degeneration, filament fusion, and edema. These abnormalities were observed at a high rate in gills of fish dissected after 14 days of exposure with waterborne Cu NPs. Severe abnormalities were observed in group D such as filament fusion, dilations, curvature of filaments, congestion, necrosis, and edema and tip degeneration. These abnormalities were more in fishes dissected after 14 days of exposure of waterborne Cu NPs (Fig.1).

Histopathological alternation in control and treated groups with waterborne Cu NPs in the brain of *Cyprinus carpio* was also observed. There was no abnormality reported in group A considered as a control group. Control group showed normal histological structures of brain tissues. Exposure to the lower concentration of nanoparticles shows enlarged and binucleated pyramidal cells, vacuolation and necrosis. These abnormalities were also observed in higher rate after 14 days of exposure with water borne Cu NPs. Histological abnormalities such as pyramidal elongation, necrosis, and mild vacuoles were appeared due to increased concentration. Similar abnormalities were reported after 14 days of exposure at a higher rate as compared to mid dissected organs. The severity of abnormalities increased at higher concentration of nanoparticles. Pyramidal swelling, vacuolation, necrosis, and hemorrhage were reported histological abnormalities in group D. These injuries also include blood vessels

changes. These abnormalities were high in fishes dissected after 14 days of exposure with waterborne Cu NPs (Fig. 2).

The activity of catalase showed significant (p<0.05) decrease in group B, C, and D after 7 days of exposure with waterborne Cu NPs. The amount was higher in the control group (1143.78±36.86).

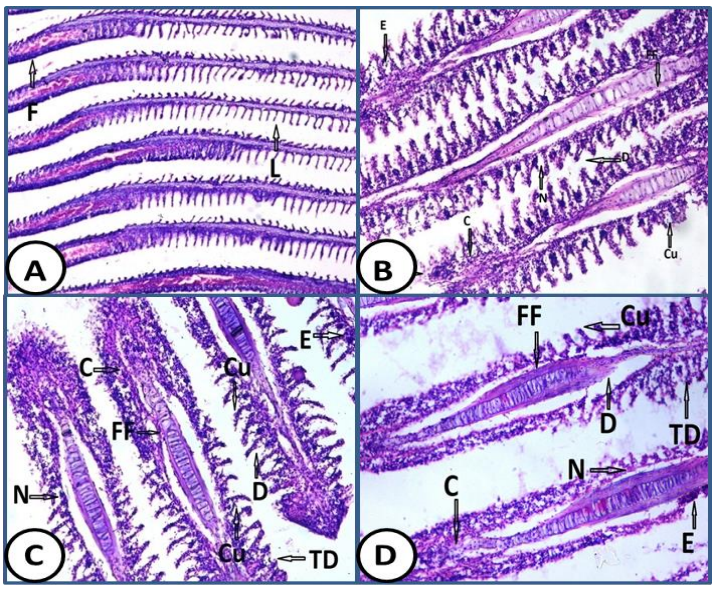

Figure 1: Photomicrograph of gills of *Cyprinus carpio* gills treated with waterborne Cu NPs (D) (H&E; X400) showed (E) Edema in gills, (FF) Fusion of filaments of gills, (D) Dilation in lamellar sinuses,(Cu) Curvature of filaments, (N) Necrosis of lamella, (C) Congestion in lamellar blood, and (TD) Degeneration of tip of filaments.

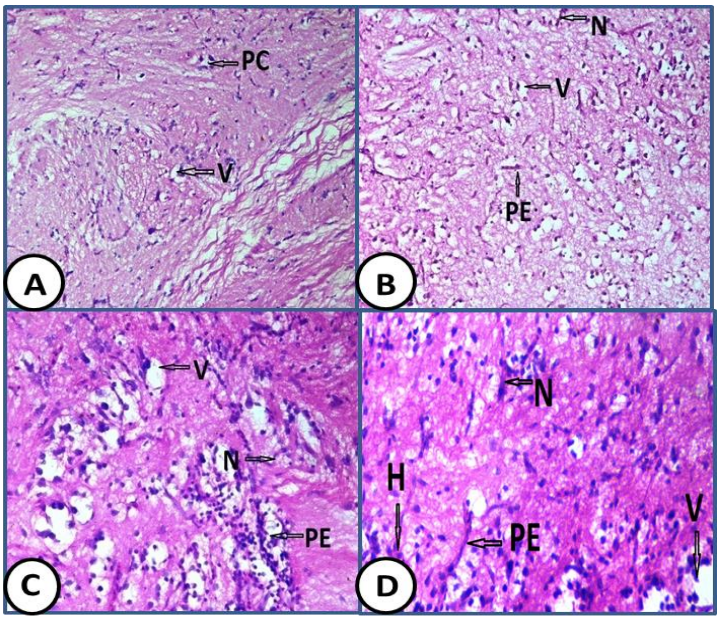

Figure 2: Photomicrograph of brain of *Cyprinus carpio* treated with water borne Cu NPs (D) (H&E; X400) shows Mild (N) necrosis between granular and molecular layer, (V) vacuole formation, (PE) enlarged and binucleated pyramidal cells, (H) hemorrhage (damage blood vessels) in brain tissues.

Similarly, CAT activity showed significant (p<0.05) changes after 14 days of exposure with waterborne Cu NPs in gills of *Cyprinus carpio*. The amount was highest in group A (1128.98±31.27a). In treated groups with waterborne copper nanoparticles amount was highest in group B (236.75±2.33). The LPO activity in gills of *Cyprinus carpio* showed significant (p<0.05) increase after 7 and 14 days of exposure with waterborne Cu NPs (Tables 1and 2).

Table 1: Mean (±SD) values for CAT, LPO and GSH activities in gills of *Cyrinus carpio* after 7 days of exposure with Cu NPs. Values with different letters were significantly different.

Parameters	Control A (0.000)	Cu NPs B (0.6 mg/L)	Cu NPs C (1.04 mg/L)	Cu NPs D (1.6 mg/L)
Catalase	1143.78±36.86^{a}	63.22±2.57^{d}	175.59±2.06^{c}	243.31±2.99^{b}
LPO	3800.55±6.170^{d}	3833.32±4.449^{c}	4616.07±6.170^{b}	5045.29±6.170^{a}
GSH	100.5±1.19^{d}	5915.4±11.95^{c}	11128.2±11.95^{b}	11963.8±8.61^{a}

Table 2: Mean (± SD) values for CAT, LPO AND GSH activities in gills of *Cyrinus carpio* after 14 days of exposure with waterborne Cu NPs. Values with different letters were significantly different.

Parameters	Control A (0.000)	Cu NPs B (0.6 mg/L)	Cu NPs C (1.04 mg/L)	Cu NPs D (1.6 mg/L)
Catalase	1128.98±31.27^{a}	96.29±1.87^{c}	236.75±2.33^{b}	60.80±1.74^{d}
LPO	3811.47±7.506^{d}	4182.16±3.265^{c}	4733.91±7.506^{b}	5760.91±8.091^{a}
GSH	100.5±1.19^{d}	7497.4±8.61^{c}	12190.4±8.61^{b}	13050.2±8.61^{a}

The values were high in group D (5045.29±6.170) treated with waterborne copper nanoparticles. Similarly, after 14 days the activity of LPO was highest in group D (5760.91±8.091) as compared to control (3811.47±7.506). The activity of GSH showed significant (p<0.05) increase after 7 (Table 1) and 14 days of exposure with waterborne Cu NPs (Table 2). The values were highest in group D (11963.8±8.61) as compared to control (100.5±1.19). Similarly, the activity of GSH was highest in group D (13050.2±8.61) and lowest in group B (7497.4±8.61) in treated groups with waterborne Cu NPs after 14 days of exposure.

Discussion

In the present study abnormalities like as curvature of filaments, tip degeneration, necrosis, congestion, dilations, edema, and filament fusion were observed due to the accumulation of waterborne copper nanoparticles in gills of *Cyprinus carpio*. Similar changes were observed in different studies. Histological alterations such as gill edema, a fusion of gill lamellae, curved tips and thickening of secondary and primary gill lamellae were observed when treated with different nanoparticles (Shahza *et al*., 2018).

In gill tissues of the exposed fish to silver nanoparticles hyperplasia of gills epithelium, secondary lamellae aneurism of gills, as well as gills lamellae adhesion were seen, when compared with control fishes in another

study (Monfared *et al*., 2015). In recent studies, several forms of histopathological changes, secondary lamellae fusion, hyperplasia in cells of primary filaments were observed in the tissues of gills of the fish treated with copper nanoparticles. Copper nanoparticles increased values caused the shortened length of secondary lamellae. 0.5 milligram/Liter the dose of Copper nanoparticles caused degeneration of cells of epithelial tissues of gills (Aghamirkarimi *et al*., 2017).

Gills of fish are of great importance because they regulate water in the body of fishes. Toxicants invade in fishes through gills and cause hematological, physiological, and biochemical abnormalities. Abnormalities occur in fish are histopathological abnormalities which are the server. Gills are great indicators of pollution in the environment. Gills are useful in indicating water quality and its pollutants (Bagherzadeh *et al*., 2016).

A study found a series of histological changes found in gills in research in which fish were exposed to different nanoparticles (Ag and Cu), lamellae fusion was found in all groups treated with nanoparticles. Secondary and primary lamellae reduction and secondary lamellae reduction in numbers resulted by increasing concentrations of nanoparticles hypertrophy of epithelium were found in all treated groups and 0.15 and 0.05 milligram Liter−1 copper nanoparticles treated groups, caused secondary lamellae fusion. More anomalies were epithelial layer lifting, degeneration of hyaline, blood vessels dilations, and necrosis in the epithelium (Ostaszewska *et al*., 2016). Some studies showed the effect of copper salts on the gills of fishes. These effects were similar to the abnormalities caused by copper nanoparticles. Exposure of gills to $CuSO_4$ caused hyperplasia, hypertrophy in the epithelium, and a fusion of lamella an aneurism in the lamella. Hyperplasia and mucocytes swelling were most observed.

Results showed an increase in hyperplasia until 0.037 ppm Cu concentration and decreased in 0.075 ppm copper concentration. Aneurism in lamella and infiltration in leukocytes increased in 0.15 ppm Cu concentration while the fusion of lamella, hyperplasia, and hypertrophy in epithelium decreased (Khabbazi *et al*., 2015). Some studies showed comparative study between copper nanoparticles and other nanoparticles. Morphological responses in gills of fishes to metal revelation were found such as an increase in cellularity in lamellar space. Changes in the filament of gills width following exposure of metal significantly varied in metal and their forms. At twenty-four hours, Cu NP exposure caused an increase in the filament of gills width, whereas small alternations were found with Ag nanoparticles (Griffitt *et al*., 2008).

Toxicants in water first effect gills and then other internal organs of fishes. Gills which were damaged cause hypoxia in fishes due to the reduced ability of gills to gases exchange. In the study, no abnormalities were found in

the control group fishes with any fusion of lamella, uplifting, necrosis, edema and congestion. Other scientists reported swollen tips, hyperplasia, and hypertrophy in epithelium and gills lamella shortening (Naddy *et al*., 2015). In our study, *Cyprinus carpio* is treated with copper nanoparticles. Pyramidal swelling, hemorrhage and necrosis were reported histological anomalies in the brain of fishes treated with waterborne copper nanoparticles, whereas no abnormalities were found in control group fishes and show normal physiology of organs. Many studies showed the same effects on the brain of fishes.

Exposures to different nanoparticles have quite similar results. Manifestation to a lower concentration of nanoparticles (Al_2O_3) revealed histopathological changes includes binucleated and enlarged pyramidal cells, necrosis and vacuolations in the brain in freshwater fish *O. mossambicus*. Histological changes severity in the brain at 150 ppm, increased in exposed fishes. Vacuoles were found due to an increase in concentrations. Histopathological alternations such as hemorrhage, pyramidal cells enlargement, and extreme necrosis were found at 180 ppm. These anomalies also included blood vessel alternations on the ventral side, necrosis between the granular and molecular layers. Necrosis of neurons of the cerebrum was severe, which indicates nissl substances lost (Murali *et al*., 2018).

Copper nanoparticles have major impacts on the fish. In the brain, alternations were found in telencephalon nerve cells, changes in the layers of mesencephalon thickness, and blood vessel enlargement on the ventral side of the cerebellum (Al-Bairuty *et al*., 2013). Nanoparticles behave like other virulent metals where the rate of growth and hematology can be saved during exposures sub-lethally, but in case of titanium oxide nanoparticles, this might happen at the expense of organs including spleen and brain (Ramsden *et al*., 2009).

Brain damage in fishes may cause by plastic nanoparticles through food chain was observed. Fishes which were exposed to NPs had less water in brains than in control fishes. The microscopic analysis showed that cerebral lobes gyri were larger in fishes exposed to 53 nm nanoparticles. Morphological alternations in brains of the 53 nm NPs fed fishes showed that fishes brain were affected directly by the plastic-nano and the effects were size dependent (Mattsson *et al*., 2017).

Oxidative damage was analyzed in gills of control and treated fishes with copper nanoparticles in our study. Data shows an increase in values of GSH activity in treated groups with waterborne copper nanoparticles (13050.2±8.61, highest group value) in comparison with the control group (100.5±1.19). The value of CAT (243.31±2.99, highest group value) decrease as compared to control (1143.78±36.86). While values of LPO (5760.91±8.091, highest group value) increase as compared to the control group (3811.47±7.506).

Copper nanoparticles exposures were studied on *Cyprinus carpio*. The levels of CAT, SOD, and GST were examined in gills of treated and control groups. For the activity of catalase, the outcomes found a significant increase in substrate level in the gill of treated groups. In SOD treated groups showed significant (p<0.05; p<0.01) elevation in SOD values in gill compared to control. For GST, the resultant adduct absorbance of CDNB-GSH resulted in significant (p<0.05; p<0.01) increase in gill tissues of treated groups in contrast with control (Gupta *et al*., 2016). In comparative examination between copper nanoparticles and copper oxide bulk found that after thirty days, exposure of *O. niloticus* to both studied values of copper oxide (bulk and nanoparticles) showed a significant elevation in the level of MDA in gills. GSH values decreased significantly in gills at LC_{50}/96 hour of copper oxide nanoparticles in contrast with a control group without any alternations when fishes were given the calculated values of copper oxide bulk particles (Abdel-Khalek *et al*., 2015).

Current study is in line with previous study in which copper oxide nanoparticles in *O. mossambicus* trigger oxidative damage in fish gills. In gills, GR and CAT showed significant changes in activity. CAT activity elevated significantly in FW_5 in contrast with control showed that copper oxide nanoparticles exposure is dependent on context and modulated by salinity in the environment. The activity of GR decreased at SW_5 and FW_5 compared to control in gills. Gills activity of CAT decreased significantly due to copper oxide exposure. The significant changes to the GSH/GSSG ratio were lowered in gills in (FW0.5) treatment (Villarreal *et al*., 2014).

Current study's findings are also in good agreement with the study of Shahza *et al*. (2018), who observed increase in the amount of SOD, LPO, and CAT in the gills with increased dose of copper oxide nanoparticles treatments in *O. mossambicus*. CAT amount elevated with the increasing value of copper oxide nanoparticles in gills. The CAT mean was 12.67±0.153 U/milligram in gills, in contrast, to control mean 2.87±0.058 U/mg in gills. SOD showed the elevating trend in gills where SOD mean was 9.33±0.115 U/mg in gills in contrast with control 6.80±0.100 U/mg. GSH value was more noticed at higher values where GSH mean in gills was 1.32±0.015 and 2.33±0.208 U/mg. The LPO value in gill was 6.70±0.200 nmol/mg. −1exposure (p=0.0467) after 15 d

Conclusions

Water is one of the most valuable resources in nature while aquatic metallic pollution is a global environmental hazard. Copper nanoparticles have wide applications and are used as antimicrobial and antibiotic treatment alternatives, nano-composite coating lubricants and filler materials for enhanced conductivity. But on the other hand in this study, Cu NPs induced histological alterations and oxidative stress in gills and brain of of

Cyprinus carpio in a dose dependent manner.

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