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Recovery of freshwater fish from the effect of Engineered Copper Nanoparticles (Cu-NPs) by designed diet

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***Abstract*— Nanotechnology has attained interest due to the needs and applications of nanomaterials in many fields such as industry, agriculture, business, medicine, public health and many others. Engineered nanoparticles can affect fish and is becoming a hot topic, from this point of view; we have synthesized Cu-NPs, noticed their effect in farm fish common carp fish (*Cyprinus carpio*) as animal model for investigation. To reduce the toxicity of nanoparticles on fishes, the protective recovery by designed diet applied on fishes. The fish were divided in to six groups of 10 individuals each and were exposed to copper nanoparticles for different concentrations (LC_{50} and LC_{10} for 24h and 96h respectively) after that, they were fed designed diet for 60 days. The results showed that exposure of common carp fed were recovered with designed free diet notably significant changes in the parameters than exposed to sublethal level of copper nanoparticles with supplementation diet (T2- T5 groups). All selected group were compared with control group of fishes (T1 groups). The supplementations of food were help for reduce the copper nanoparticles toxicity based on biochemical and physiological parameters were studied. The activities of the antioxidants were altered nearly to become the normal levels significantly after the treatment with designed diet.**

Keywords*— Cu-NPs, biochemical parameters, Protective effect, common carp fish *(Cyprinus carpio).

1. INTRODUCTION

Nowadays, the nanotechnology invigorated fast development in research and their possible hazards in aquatic animals have drawn much attention (Liang *et al.,* 2015; Al-Bairuty *et al.,* 2013). The application of nanotechnology has gained a significant priority worldwide. Many engineered nanoparticles have been widely utilized in consumer products. Nanoparticles having different chemical properties with size shape with their chemical environment despite its widespread applications. In the last decade, several studies in finishing steers found that, the adverse effects of these nanoparticles on the environment and organisms have drawn interest (Aasma Noureen and Farhat Jabeen, 2015; Murray, Kagan, and Bawendi, 2000).

Copper nanoparticles (Cu-NPs) are one of the most used nanomaterials due to their antibacterial antimicrobial (antiviral, antibacterial and antifungal), antibiotic treatment substitutes, nanocomposite coating, catalyst, lubricants, inks, metallic coatings of circuits and batteries, for filtration of air and liquid, filler materials for enhanced conductivity and wear resistance and other properties (Gomes *et al.,* 2011; Brownheim, 2011) used for example, in textiles, food storage containers, home appliances, paints, food supplements and so on (Maynard *et al.,* 2006). But copper NPs and its metal oxides have garnered significant consideration due to their negative ecological effects. The potential toxicity of copper nanoparticles (Cu-NPs) has received increasing concern. Metal contamination in freshwater bodies is a matter of serious concern from the human health point of view for fish, forms an integral part of the human diet. The sub lethal toxicity of Cu-NPs on fish were noticed on different body systems of fish as accumulation, stress response, osmoregulation and pathology (Al-Bairuty, *et al.,* 2013; Shaw and Handy, 2011; Cong, *et al.,* 2014; Zhao *et al.,* 2011; Shaw *et al.,* 2012; Wang *et al.,* 2014). Recent data showed, the toxicity of Cu-NPs on digestive enzyme activities, composition of whole-body, and fatty acid are poorly understood. Furthermore, toxicity thresholds can be rather variable in different species (Cong, *et al.,* 2014; Beaumont *et al.,* 2000).

The common carp, *Cyprinus carpio* is a freshwater teleost cultivated fish all over the world, including India owing to its low cost of production, high muscle content and easy rearing. Despite its economic importance, this teleost is referred as an ideal experimental animal model for studying ecology, developmental biology, and evolution (Zhao *et al.*, 201; He *et al.*, 2009). Therefore, the possible health effects and toxicology of Cu-NPs have caused great concerns in both the public interest (Wang *et al*., 2015). The rapid development of aquaculture is accompanied by increasing demands for fishmeal, a scarce and expensive ingredient and also for reduction of the toxicity of nanoparticles on fishes, the protective effects of designed diet applied on fishes (James *et al*., 2008). The present work has been designed to study the effect of the dietary supplements on reduction of Cu-NPs toxicity in the common carp. However, the dosages used in our experiments were lower than other studies. The effect of supplementation of designed food on reduction of copper nanoparticles toxicity based on biochemical and physiological parameters was studied in a freshwater fish, common carp. The activities of the antioxidants were reversed nearly to the normal levels significantly after the treatment with designed diet.

2. MATERIAL AND METHODS

2.1. Fish acclimatization - Common carp *Cyprinus carpio* (15.45 ± 0.32 g) were collected from local Fisheries Consultancy, Jalgaon, India, and transported in an aerated circular container. The fish were acclimatized for the experimental conditions for 15 days. Before experiment, they were fed controlled diet containing the normal requirement for carps, 10 mg/kg (Murthy, 2002; FAO 2011). Glass tanks of 50 liters capacity of filtered tap water at 25 ± 2°C were chosen with aeration was provided in each tank during acclimatization and experiment.

2.2. Fish Diets - Fishmeal (Crude protein, Crude lipid, *Spirulina* powder, Linseed, Soybean, Rice bran, Vitamin - mineral mix) prepared by mixing dry ingredients, then adding water for soft dough. Pellets (1.0 mm diameter) were extruded, and stored at room temp. The experimental diets were compared with a control diet containing 100% fishmeal and a commercial diet.

2.3. Experimental protocol- The fish were divided in to six groups of 10 individuals each and were exposed to copper nanoparticles for different concentrations 0, 0.10, 0.20, 0.40, 0.60 and 0.80 ppm. (LC_{50} and LC_{10} for 24h and 96h respectively) of copper nanoparticles after with the fed designed diet for 60 days. Common carp fed with designed free diet (T1 groups as controlled group) and sublethal of copper nanoparticles exposed T2- T5 groups. Test animals belonging to 2nd, 3rd, 4th, 5th and 6th groups were exposed to 0.20 ppm at sublethal concentration for 24h and 96h of copper nanoparticles and after that, they were fed with supplementation diet of designed diet for about 60 days. A static bioassay method was determined. Probit analysis was followed for the calculation. As per experimental series, a parallel experiment was conducted simultaneously for 24h, 96h, and 60 days to study the impact of designed diet on selected parameters in *Cyprinus carpio.* In the fed treatment, fish were given feed at 09:00 and 16:00 at 5% of their body weight per day.

2.4. Synthesis of CuNPs by chemical reduction method

The copper nanoparticles (CuNPs) were prepared by aqueous chemical reduction method (Liu *et al*., 2012). Freshly prepared 30 ml of 2mM $NaBH_4$ in ice cold water was taken in 50 ml of beaker and kept on a magnetic stirrer at 1800 rpm and a mixture of 10ml of 1mM $CuNO_3$ and 6ml of 1mM NaCl was drop wise added into $NaBH_4$ solution with vigorous stirring in which the color changes from black to green and finally it becomes pale yellow. The prepared CuNPs were stirred for nearly 8 hours for its good stability and then it kept overnight to eliminate surplus hydrogen (More, 2019).

2.5. Biochemical parameters-The estimation of protein concentration was done by Lowry method (1951). Glycogen was estimated following the Anthrone method of Van der Vier (1954). Lipid extractions were performed on minced fish samples (10g each) using the extraction methods of Folch *et al.,* (1957).

2.6. Enzyme assays-The lactate dehydrogenase (LDH) activity by the method of Nachlas *et al.*, (1960) as modified by Pramilamma *et al.*, (1975). Catalase (E.C.1.11.1.6) determined by Claiborne (1985) method. Superoxide dismutase (E.C.1.15.1.1) activity was by the method of Misra and Fridovich (1972).

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3. RESULT AND DISCUSSION

The toxicity of Cu-NPs to common carp was increased with concentration. The feed conversion ratios in the designed diet were significantly better than in the control. Formulated proximate composition of carp designed diets (Table 1). The synthesized Cu-NPs were characterized by using Philips CM-200 SEM and studied their morphology shown in Figure 1 and 2. The syntheses of the CuNPs were followed by UV-visible spectroscopy analysis recorded over a range of 200-800nm shown in Figure 3. Mortality rate were calculated at LC50 at 0.45ppm and sublethal LC10 at 0.20ppm exposed to concentrations of Cu-NPs separately exhibit many biochemical and physiological alterations with recovery at 60 days have been summarized in Graph 1, 2, 3 and 4.

Table 1. Formulation and proximate composition of carp designed diets.

Composition of the diet.	**Control**	**Ingredient (g/kg)**
Fish meal	22.00	22.00
Wheat flour	23.50	15.0
Spirulina	00	11.0
Linseed	00	10.0
Soybean	00	10.0
Rice bran	12.00	12.00
Groundnut oil cake	15.00	15.00
Vitamin - mineral mix	5.00	5.00
Proximate composition (% dry matter)		
Dry matter	90.16	91.75
Moisture	09.84	8.25
Ash	11	11.28

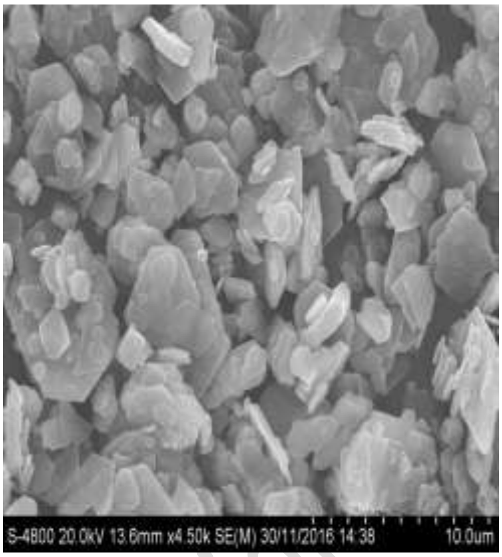

Figure 1. FESEM images of Copper NPs

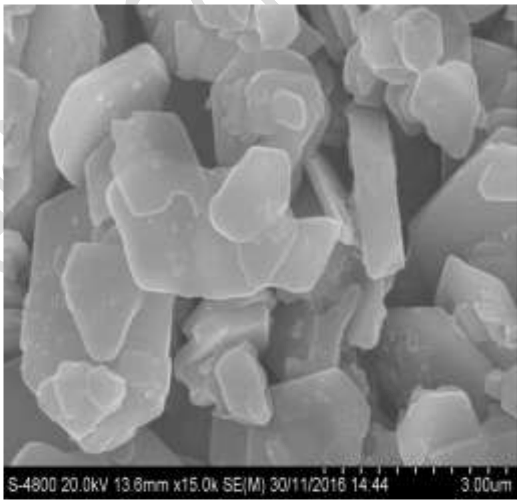

Figure 2. FESEM images of Copper NPs

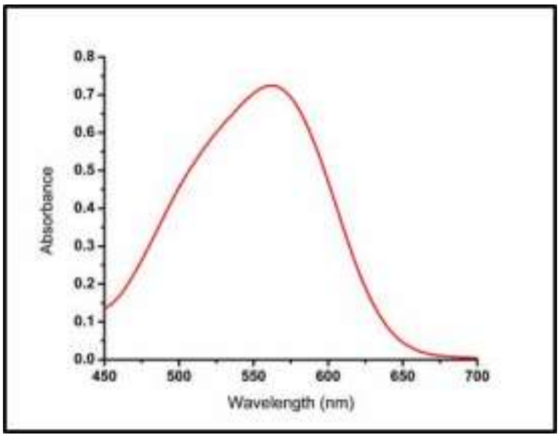

Figure 3. UV-visible spectrum of synthesized CuNPs.

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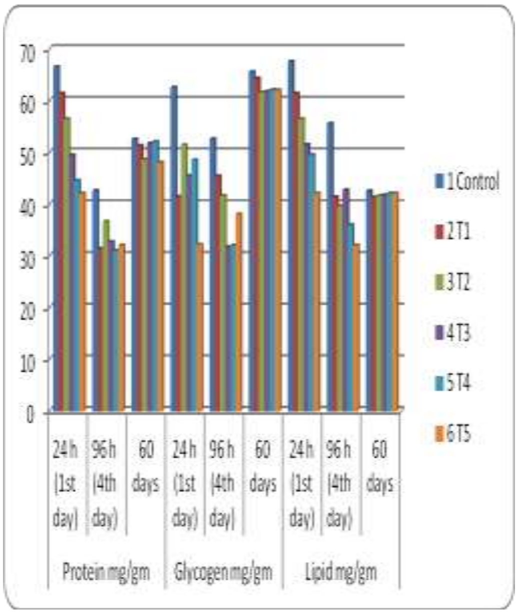
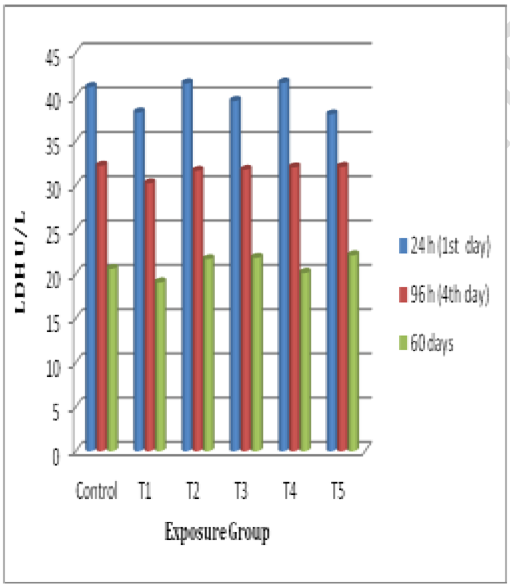
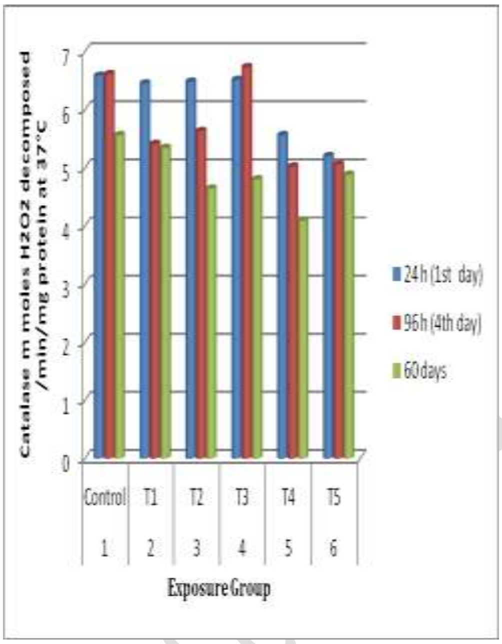


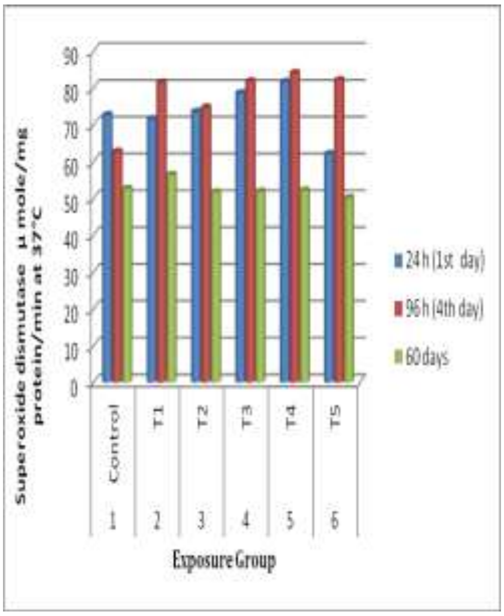
Graph1. Effect of sublethal concentration of Cu NPs for 24 hrs and 96hrs recovered by designed diet for 60 days in biochemical contents of *Cyprinus Carpio*



Graph2. Effect of sublethal concentration of Cu NPs for 24 hrs and 96hrs recovered by designed diet for 60 days in LDH activity of *Cyprinus Carpio*



Graph3. Effect of sublethal concentration of Cu NPs for 24 hrs and 96 hrs recovered by designed diet for 60 days in Catalase activity of *Cyprinus Carpio*



Graph4. Effect of sublethal concentration of Cu NPs for 24 hrs and 96hrs recovered by designed diet for 60 days in SOD activity of *Cyprinus Carpio*

Protein contents of the fish body differed among treatments, but there were no significant differences in lipid than glycogen. In the present study, except for fish fed the designed diet, protein levels in the muscle of fish fed the designed diets was similar reports by Banaee *et al.,* (2011). The reduction of protein at high replacement levels could be due to low digestion efficiency of protein. Spirulina, Soybean and Linseed are rich source of plant protein and fatty acid contents, which may facilitate to enhance growth. A marked altered content of the protein in the tissues of the fish was observed on exposure to Cu-NPs. Chen *et al*., (2013) reported that the reductions in growth performance were noticed gives to two reasons: first, Cu exposure caused increased metabolic expenditure for detoxification and maintenance of homeostasis; second, higher Cu exposure reduced feed intake, which would in turn lead to reduced growth. Similar report were noticed by Lopezalvarado and Kanazawa, (1994); Rodehutscord *et al.,* (1995); Mai *et al.,* (2006); Melegaria *et al.,* (2013). The decreased activity of stress enzyme LDH indicates the alteration for normal homeostasis. Some significant differences were observed throughout the experiment. Decrease concentration of glycogen level as a result of stress, during the process of detoxification of active moieties and their metabolites. Moreover, fish fed the 75 % *Spirulina* / 25 % soybean diet had a lower oxygen consumption, which is used to estimate the oxidation of nutrients in the diet, especially protein (Bureau *et al.,* 2002; Devon and Staff Writer, 2014; Gatlin 2002; Gatlin *et al*., 2007). The roles of this enzyme as observed in the different animals when treated with the different pesticides were reported earlier (Velisek *et al*., 2008).

Enzyme activity (e.g., LDH, Catalase, and Superoxide dismutase) can be used as an indicator of potential feed utilization and growth differences and also serve as an indicator of the digestive capacity in relation to the type of feed offered with aquaculture environments. Dietary supplementation significantly affected on fish. LDH activity was significantly affected between LDH activity and Glycogen. Alterations in the LDH isoenzyme spectrum tissues, induced by toxic conditions, reflect metabolic cellular dysfunction of these tissues. The degree of tissue damage and the LDH isoenzyme pattern reflects which tissues are damaged (Olaifa *et al.,* 2004). Due to the acute toxic stress of Cu-NPs and the breakdown of proteins dominates over synthesis under enhanced proteolytic activity and, indicating a tissue condition favoring anaerobic respiration to meet the energy demands when aerobic oxidation is lowered (Arora *et al.,* 2012). Catalase activities in the tissue were significantly differed between groups but there were no definite trends in catalase activity. SOD activity in fish was significantly higher than in control fish and decreased as the level of supplemented diet (Akhtar *et al.,* 2010). Many studies were noticed that, increase the enzymatic activity of antioxidant biomarkers as superoxide dismutase and catalase (El-Desoky *et al.,* 2013; Viswanada *et al.,* 2011; He *et al.,* 2009; Baker and Davies, 1995; Akhtar *et al.,* 2011).

In conclusion, biochemical and physiological metabolism under metal exposure revealed a complex regulatory mechanism. Dietary supplements of Spirulina powder, Linseed, Soybean, Rice bran reduced the Cu-NPs sublethal toxicity exposed *Cyprinus carpio* and improved food utilization, enzymatic activities with biochemical parameters significantly in a period of time (60 days).

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