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Research Article

TOXICITY OF COPPER NANOPARTICLE ON HAEMATOLOGY AND BIOCHEMISTRY OF FISH, *TILAPIA MOSSAMBICA*

Sheeba Ali Siddiqui [1]* and C. M. Noorjahan [2]
[1]Ph.D. Research Scholar, PG & Research, Department of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Teynampet, Chennai-600 018, Tamil Nadu, India
[2]Assistant Professor, PG & Research Department. of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Teynampet, Chennai - 600 018, Tamil Nadu, India
*Corresponding Author Email: sheebaalisiddiqui@gmail.com

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ABSTRACT

Present investigation was carried out to study the toxicity of copper nanoparticle on haematology (blood) and biochemistry in different organs (gills, liver and muscle) of fish, *Tilapia mossambica*. Fishes were exposed to different levels of copper nanoparticle for a period of 14 days and control was also maintained simultaneously. Blood samples were collected from experimental and control animals and haematological study was carried out. The results of the haematological study showed that there was a decrease in haematological parameters (total WBC count, basophils, eosinophil, neutrophils, lymphocytes, monocytes, platelet count, total RBC count, haemoglobin, haematocrit, MCV, MCH and MCHC) compared to that of control. Since major biochemical constituents such as carbohydrates and proteins of the body play an important role in body's construction and energy metabolism, the work was further extended to study biochemical estimation in different organs (gills, liver and muscle) of *Tilapia mossambica* after exposure to different levels of copper nanoparticle for 14 days. The results of the biochemical estimation indicated that the carbohydrates and proteins were highly reduced in the gills followed by the liver and muscle and carbohydrate was much depleted than protein when compared to control.

Keywords: Copper nanoparticle, *Tilapia mossambica,* Haematology and Biochemistry.

INTRODUCTION

Nanoparticles are ultrafine unit with dimensions measured in nanometres (nm; 1 nm = 10^{-9} metre)[1]. Nanoparticles exist in the natural world and are also created as a result of human activities. Because of their submicroscopic size, they have unique material characteristics and manufactured nanoparticles may find practical applications in a variety of areas[2]. Nanoparticles are being used for diverse purposes such as medical treatments, in various branches of industry production such as solar and oxide fuel batteries for energy storage, wide incorporation into diverse materials of everyday use such as cosmetics or clothes, optical devices, catalytic, bactericidal, electronic, sensor technology, biological labelling and treatment of some cancers. Due to their exceptional properties including antibacterial activity, high resistance to oxidation and high thermal conductivity, nanoparticles have attracted considerable attention in recent years[3]. Nanoparticles can be synthesized chemically or biologically.

Metallic nanoparticles that have immense applications in industries are of different types, namely gold, silver, iron, copper, alloy, magnetic etc. A copper nanoparticle is a copper based particle of 1 to 100 nm in size[4]. Like many other forms of nanoparticles, a copper nanoparticle can be formed by natural processes or through chemical synthesis[5]. These nanoparticles are of particular interest due to their historical applications as coloring agents and their modern-day biomedical usage. Copper nanoparticles, due to their excellent physical and chemical properties and low cost preparation have been of great interest. Copper nanoparticle (2-5mm) have strong antibiotic activity and are able to decrease the microbial growth by 99.9%. Humans are exposed to various nano-scale materials since childhood and the new emerging field of nanotechnology has become another threat to human life[6]. Because of their small size, Nanoparticle find their way easily to enter the human body and cross the various biological barriers and may reach the most sensitive organs[7].

Nanotoxicology is a study of impact of manufactured nanomaterial's on living organisms and environment. Thus this study aims to present an overview of production and use of copper nanoparticles are likely to result in release into aquatic environment which can lead to health hazards. The toxicological evidence of copper nanoparticle is still lacking, and hence present investigation was carried out to study the toxicity of copper nanoparticle on different haematological parameters. Since major biochemical constituents, carbohydrates and proteins of the body play's an important role in the body's construction and energy metabolism, so biochemical estimation of carbohydrates and proteins of fish, *Tilapia mossambica* exposed to copper nanoparticle was also studied and compared with control on 7th and 14th day of exposure.

MATERIALS & METHODS

Copper nanoparticle: Commercially available copper nanoparticle was purchased to study the toxic effect of copper nanoparticle on haematology and biochemistry of different organs of the fish, *Tilapia mossambica*.

Collection of fish: Healthy *Tilapia mossambica* fishes were collected from hydrobiological research station, Chetpet , Chennai and maintained in the laboratory[8].

Methods

Toxicity of copper nanoparticle on *Tilapia mossambica*: Study of the toxic effect of copper nanoparticle (10 ppm, 20ppm and 40ppm) on *Tilapia mossambica* were carried out for a period of 14 days at an interval of 7 days.

Haematological estimation: Estimation of the haematological parameters (total WBC count, basophils, eosinophil, neutrophils, lymphocytes, monocytes, platelet count, total RBC count, haemoglobin, haematocrit, MCV, MCH and MCHC) of the fish, *Tilapia mossambica* after exposure to different levels (10ppm, 20ppm and 40ppm) of copper nanoparticle for 7-14 days was carried out.

Estimation of haemoglobin, total count of WBC, total count of RBC and differential count of WBC was carried[9,10]. Biochemical estimation of carbohydrates and protein present in different organs like gills, liver and muscle of fish, *Tilapia mossambica* were carried out[11,12].

RESULTS & DISCUSSION

The results of the estimation of haematological and biochemical parameters are presented in the tables 1,2,3 & 4. In control group, the hematological parameters were found to be normal but when fish, *Tilapia mossambica* exposed to different concentrations of copper nanoparticle for 7-14 days showed significant reduction in the haematological parameters to that of control group. In addition to the total WBC count, basophils, eosinophil, neutrophils, lymphocytes, monocytes, platelet count, total RBC count, haemoglobin, haematocrit, MCV, MCH and MCHC was decreased significantly by elevation in the copper nanoparticle concentrations. On 7th day of exposure of fish to different concentrations of copper nanoparticle the value of total WBC count (3100 thousand/cu.mm), total RBC count (5.12 million/cu.mm), haemoglobin (9.42 g/dl), platelet count (1.06 cmm), lymphocytes (83 million/cu.mm) were decreased when compared to control values such as total WBC count (4566.66 thousand/cu.mm), total RBC count (6.832 million/cu.mm), haemoglobin (13.7333 g/dl), platelet count (1.2166 cmm), lymphocytes (92 million/cu.mm) and the value of eosinophil (2 million/cu.mm), neutrophils (4.333 million/cu.mm), monocytes (11 million/cu.mm), haematocrit (22.8 g/dl), MCV (80.1 fl/red cell), MCH (19.8 pg/cell) and MCHC (30.1 g/dl) were increased in the experimental fish when compared to that of control values such as eosinophil (1.033 million/cu.mm), neutrophils (3.333 million/cu.mm), monocytes (4 million/cu.mm), haematocrit (4 g/dl), MCV (0.4 fl/red cell), MCH (0.305 pg/cell) and MCHC (2 g/dl) and basophils levels (0) were found to be normal in both control (0) and experimental fish.

On 14th day of exposure of fish to different concentrations of copper nanoparticle the value of total WBC count (4100 thousand/cu.mm), total RBC count (5.26 million/cu.mm), haemoglobin (11.2 g/dl), platelet count (1 cmm), neutrophils (3 million/cu.mm), haematocrit (19.5 g/dl), MCV (76.2 fl/red cell), MCH (19.2 pg/cell) and MCHC (27.6 g/dl) were decreased in experimental fish when compared to that of control values for total WBC count (5500 thousand/cu.mm), total RBC count (6.81 million/cu.mm), haemoglobin (13.8 g/dl), platelet count (1.26 cmm), neutrophils (10 million/cu.mm), haematocrit (23.4 g/dl), MCV (80.9 fl/red cell), MCH (21.3 pg/cell) and MCHC (33 g/dl). Whereas the value of basophils (1 million/cu.mm), eosinophil (2 million/cu.mm), lymphocytes (83 million/cu.mm) and monocytes (25 million/cu.mm) were increased in experimental fish when compared to that of control for basophils (0 million/cu.mm), eosinophil (1 million/cu.mm), lymphocytes (76 million/cu.mm) and monocytes (13 million/cu.mm). The results of the study revealed that there was a significant decrease in haematological parameters compared to that of control which may be due to transient changes in haematology and depletion of plasma Na^+ which occurs after treatment with copper nanoparticle that affects in branchial Na^+/K^+-ATPase activity in cells and leads to depletion of plasma and ion concentrations suggest that copper nanoparticle are an ion regulatory toxicant[13].

The biochemical estimation indicated that the carbohydrates and proteins were highly reduced in the gills followed by the liver and muscle and carbohydrate was much depleted than protein when compared to control. Carbohydrates and proteins which constitutes the major biochemical constituents of the body play an important role in the body construction and energy metabolism[14]. The results of the estimation of carbohydrates present in different organs of the fish showed that the carbohydrate content present in the gills, liver and muscle was found to be gradually reduced in fish, *Tilapia mossambica* exposed to different concentrations of copper nanoparticle for 7-14 days. It was found to be lowest (0.137±0.002) in gills on 14th day exposure to 40ppm concentration in comparison to control (0.345±0.002). Whereas low carbohydrate content was recorded in liver (0.063±0.002) on 14th day exposed to 40ppm concentration in comparison to control (0.151±0.002) and in muscle it was found to be lowest (0.063±0.002) on 14th day exposed to 40ppm concentration in comparison to control (0.587±0.002). The results of the estimation of Protein, revealed that the protein content present in the gills, liver and muscle was found to be gradually reduced when fish, *Tilapia mossambica* exposed to different concentrations of copper nanoparticle for 7-14 days. It was found to be lowest in gills (0.107±0.003), liver (0.103±0.050) and muscle (0.002±0.003) of fish on 14th day exposed to 40ppm concentration in comparison to that of control gills (0.199±0.002), liver (0.124±0.003) and muscle (0.006±0.001) of fish. Among the two biochemical constituents tested in the different organs (gills, liver and muscle) of the fish, *Tilapia mossambica*, carbohydrate was more depleted followed by protein. This is because carbohydrates are important fuel reserve stored in large quantities and during extreme starvation, considerable amount of carbohydrate would be extracted from the tissue[15]. Moreover, among the three organs of the *Tilapia mossambica*, the gills are mostly affected organ as the gills are the first organ to come in contact with toxic substances present in the effluent and they do not have any kind of covering and vulnerable to the toxic stress of copper nanoparticle followed by liver and muscle[16].

Table 1: Haematological parameters of *Tilapia mossambica* exposed to control, 10ppm, 20ppm & 40ppm of Copper nanoparticle on 7th day

Parameters	Control (Tap water)	10 ppm	20ppm	40 ppm
Total WBC count (thousand/cu.mm)	4566.66	4400	4000	3100
Total RBC count (million/cu.mm)	6.823	5.1366	5.91	5.12
Haemoglobin (g/dl)	13.7333	11.9	10.166	9.42
Platelets count (cmm)	1.2166	1.98	1.26	1.06
Basophils (million/cu.mm)	0	0	0	0
Eosinophils (million/cu.mm)	1.033	1.033	1	2
Neutrophils (million/cu.mm)	3.333	4.333	4	3
Lymphocytes (million/cu.mm)	92	87.333	84	83
Monocytes (million/cu.mm)	4	11	9	11
Hematocrit (g/dl)	4	18.633	19.2	22.8
MCV (fl/red cell)	0.4	72.266	78.4	80.1
MCH (pg/cell)	0.305	18.9	19.6	19.8
MCHC (g/dl)	2	28.233	29.5	30.1

Table 2: Haematological parameters of *Tilapia mossambica* exposed to control, 10ppm, 20ppm &40ppm of Copper nanoparticle on 14th day

Parameters	Control (Tap water)	10 ppm	20ppm	40 ppm
Total WBC count (thousand/cu.mm)	5500	4800	4400	4100
Total RBC count (million/cu.mm)	6.81	5.78	5.28	5.26
Haemoglobin (g/dl)	13.8	12.1	11.9	11.2
Platelets count (cmm)	1.26	1.12	1.06	1
Basophils (million/cu.mm)	0	1	1	0
Eosinophils (million/cu.mm)	1	2	1	2
Neutrophils (million/cu.mm)	10	7	3	4
Lymphocytes (million/cu.mm)	76	72	83	69
Monocytes (million/cu.mm)	13	18	12	25
Hematocrit (g/dl)	23.4	19.5	20.7	22.5
MCV (fl/red cell)	80.9	76.2	78.7	79.2
MCH (pg/cell)	21.3	19.2	19.5	19.8
MCHC (g/dl)	33	27.6	28.9	30.9

Table 3: Carbohydrate content of different organs (Gills, liver and muscle) of *Tilapia mossambica* exposed to control (Tap water), 10ppm, 20ppm and 40ppm of Copper nanoparticle on 7th and 14th day

Samples	Organs mg/100mg of tissue					
	7th day			14th day		
	Gills	Liver	Muscle	Gills	Liver	Muscle
Control	0.345±0.002	0.345±0.006	0.587±0.002	0.345±0.002	0.345±0.006	0.587±0.002
10ppm	0.345±0.001	0.344±0.003	0.346±0.001	0.310±0.015	0.308±0.015	0.287±0.001
20ppm	0.345±0.003	0.344±0.002	0.346±0.003	0.149±0.002	0.143±0.002	0.169±0.001
40ppm	0.344±0.002	0.343±0.002	0.341±0.002	0.137±0.002	0.063±0.002	0.151±0.002

± Standard deviation

Table 4: Protein content of different organs (Gills, liver and muscle) of *Tilapia mossambica* exposed to control (Tap water), 10ppm, 20ppm and 40ppm of Copper nanoparticle on 7th and 14th day

Samples	Organs mg/100mg of tissue					
	7th day			14th day		
	Gills	Liver	Muscle	Gills	Liver	Muscle
Control	0.199±0.002	0.124±0.003	0.006±0.001	0.199±0.002	0.124±0.003	0.006±0.001
10ppm	0.191±0.002	0.120±0.003	0.006±0.002	0.144±0.004	0.119±0.003	0.006±0.002
20ppm	0.174±0.004	0.120±0.002	0.005±0.002	0.123±0.002	0.108±0.002	0.005±0.002
40ppm	0.169±0.004	0.114±0.050	0.004±0.003	0.107±0.003	0.103±0.050	0.002±0.003

± Standard deviation

CONCLUSION

Thus the present study confirmed that copper nanoparticle have toxic effects on haemotology and biochemistry of fish, *Tilapia mossambica.* Hence guidelines should be established by government for the safe handling and use of nanoparticles in research laboratories and proper disposal procedure to be carried out while promoting the benefits of the use of nanoparticles in various fields.

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