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Original Research Article

Nanoparticle for better safety

Synthesis And Characterization Of *Moringa Oleifera* Copper Nanoparticles Shows Toxic Effect On Fish *Cirrhinus Mrigala*

Dr. Chikkappa Udagani[1], Dr. Shivaraj Yallappa[2*], Suman D P[3] and Shruthinag N[4]

[1, 3 & 4]Department of Physics, University College of Science, Tumkur University, Tumakuru-572 103, Karnataka, India.

[2]Department of Zoology, L.V.D. College Raichur, Raichur-584 101, Karnataka, India.*

Abstract: Fish is an healthy food for humans and nutritious diet due to the presence of high protein levels, beneficial fat and various sources of vitamins and minerals. Though the fish is a healthy food, a concern about the toxic effect of copper nanoparticles on fish was reported. The objective of the present work is to study the effect of *Moringa oleifera* CuNPs on fish *Cirrhinus mrigala* fish species. *Moringa oleifera* is a fast growing and drought resistant tree which contains rich amount of nutrients and vitamins -.The initial concentration of the CuNPs colloidal solution was 350 nm by TEM Transverse Electron Microscope micrograph and UV-visible spectrum and X-Ray diffraction. The freshwater fish, *Cirrhinus mrigala* was exposed to lethal concentration of copper nanoparticles synthesized via chemical reduction method using *C* for a period of 4 days exposure, where the value of LC_{50} of CuNPs was obtained 2ppm. For the static renewal of exposure, the lethal concentration of copper nanoparticles were 0.5, 1, 1.5 and 2ppm including control group. Some Changes in fish behaviour, like changes in Erratic swimming, restlessness, surfacing and hyper activity etc, were observed at the time of exposure periods. This work seems to be useful in analysing not only the disadvantages of using CuNPs but also problems related to their use.

Keywords: *Cirrhinus mrigala*, *Moringa oleifera,* LC_{50} CuNPs, Behavioural Changes, Copper nanoparticles.

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***Corresponding Author**

Dr. Shivaraj Yallappa , Department of Zoology, L.V.D. College Raichur, Raichur-584 101, Karnataka, India.

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1. INTRODUCTION

In the recent days, copper nanoparticles of *Moringa oleifera* (CuNPs) are of great interest to for recent research. The pollution of an ecosystems in this condition living organisms shows deformities and defects in early developmental stages, due to variation of proper canalised rainfalls and weather conditions disturbance[1]. CuNPs have found to be useful in many applications such as conductive link[2], thermal transfer nanofluids[3], lubricants[4] electronic materials[5]and anticancer drugs[6]. The CuNPs have gained special interest in the field of nanotechnology mainly due to the fact that synthesis of CuNPs is cost effective in comparison with synthesis of noble metal nanoparticles such as silver and gold[7].The chemical method is a type of I way of synthesis of nanoparticles, however chemical synthesis method involves some of the toxic chemicals which are potentially dangerous for the environment and living organisms[8]. This problem can be avoided by synthesis of nanoparticles via green synthesis route. *Moringa oleifera* is a tree that is sometimes called the Tree of Life or a Miracle Tree, but rather than this being in reference to its potential medicinal usage this is actually refering to how it is a very valuable food crop (it is drought resistant, grows very fast, and is highly nutritive) and even beyond food it serves many benefits in third world countries such as having an ability to be used for some crafts (due to being a tree) and cleaning water. Plant extracts mainly constitute of bioactive polyphenols, alkaloids, proteins, sugars, phenolic acids and terpenoids etc., which reduce the metallic ions into nanoparticles[9,10] There are a number of research reports available on the green synthesis of CuNPs. The CuNPs are synthesized by the reduction of Copper Sulphate and *Nerium oleander* (a shrub) leaf extract as reducing agent[11]. Early reports confirmed that the CuNPs of size 40-70 nm are synthesized by the reduction of copper sulphate and *Lycopersicon Esculentum* fruit juice as reducing agent[12] It has been reported that CuNPs are synthesized using Copper Sulphate pentahydrate ($CuSO_4$ $.5H_2O$) and *Murraya Koenigii* leaf extract[13]. And *Moringa oleifera* is a fast growing and drought resistant tree. Moringa is rich in minerals, vitamins[14] and possess antioxidant properties[15]. In addition to the best available nutrients it also contains a lot of medicinally valuable chemical constituents[16]. *Moringa oleifera* is well named as miracle tree for its incredible applications. Every part of *Moringa oleifera* is beneficial to human nutritionally and medicinally[17]. Many researchers reported the synthesis of silver nanoparticles by the reduction of silver nitrate ($AgNO_3$) using *Moringa oleifera* as reducing agent[18-22].The Moringa oleifera leaf extract has been successfully used to synthesize zinc oxide (ZnO) nanoparticles by the reduction of Zinc nitrate[23], Titanium dioxide (TiO_2) nanoparticles by the reduction of titanium tetraisopropoxide[24] and Vanadium nanoparticles by the reduction of Vanadium (II) Chloride[25]. One among the Indian major carps, *Cirrhinus mrigala* inhabiting the freshwater sources, is widely cultured in ponds and lakes of this region. This fish is largely preferred for nutritive purpose as a low cost protein and vitamin source. Hence, this fish has a good commercial value and also forms an important component of the freshwater ecosystem. *Cirrhinus mrigala* is considered for composite fish culture as such it is prone to pesticides and nanoparticles intoxication resulting from agro industries, agriculture practices, household application, etc. Owing to its high productivity, easy availability and wide consumption by the natives exists. Hence, *Cirrhinus mrigala* was considered as a suitable experimental animal for the study of pesticides and nanoparticles toxicity that affects a wide range of non-target organisms such as fishes and other aquatic living forms. In this present work we have studied the amount of copper nanoparticles effects on these fishes *Cirrhinus mrigala,* fish is one of the most important composite and table purposes used in our country Even though the CuNPs are widely used, there is little information available on the long term toxic effects on human health as well as ecology concerned. The CuNPs at higher concentrations may be toxic and can induce health risks and cause disturbance in the ecosystem. There should be a strong concern about the toxic effect of CuNPs on aquatic animals especially on fishes. The toxicological risks connected with nanoparticles are partially known to every field of studies. A recent studies reported the toxic effect of nanoparticles on fishes were primitively lost their stability to survive in ecosystems, In spite of the numerous studies on fish, more investigations are required to illustrate the risks associated with toxicity of nanoparticles which effects on fishes. And this experiment demonstrates that synthesis and characterization of *Moringa oleifera* copper nanoparticles shows toxic effect on fish *Cirrhinus mrigala* (Hamilton, 1822).

2. MATERIALS AND METHODS

2.1 *Collection and maintenance of experimental animals*

The collection of carp, from the Fisheries Research and Information Center (Inland) Hebbal, Bangalore, Karnataka, India, provided healthy and active 1 month old Common carp, *Cirrhinus mrigala* fingerlings (3 ± 0.21 g; 3 ± 0.25 cm). Large aerated crates were used to transport fish to the laboratory. Before investigation fish were maintained for 30 days in large cement tanks (22 × 12 × 5)feet. Further, carp (50 fingerlings) were acclimatized to laboratory conditions for 20 days at 22 ± 2 °C in 100 L glass aquaria (120 × 45 × 80 cm) containing DE-chlorinated tap water of the quality used in the test. Characteristics of the water were determined by following the methods by the standard protocols of APHA international guideline[26].In this present investigation, the physico-chemical parameters of water such as Temperature, pH, Dissolved oxygen, Carbon dioxide, Hardness, Total alkalinity, Conductivity, Specific gravity, Chlorinity, Calcium and Sodium were estimated..

2.2 *Synthesis of Copper Nanoparticles*

For the synthesis of CuNPs, fresh *Moringa oleifera* leaves were first washed with tap water and then with distilled water. The leaves were placed in cool place for 5 days until all moisture was removed. The *Moringa oleifera* leaves were grounded into powder. 5gm of *Moringa oleifera* leaf powder was taken in a glass beaker and 100ml double distilled water was added to it. The mixture was stirred at constant speed using magnetic stirrer for 20 minutes at a temperature of 80°C. The yellow coloured leaf extract was filtered using Whitman filter paper and stored in a refrigerator. 20ml of *Moringa oleifera* extract was added to 80ml of 0.03M Copper sulphate ($CuSo_4$) solution and stirred for 30 minutes using magnetic stirrer. The colour change from light color to dark color was a primary indication of the reduction of copper (Cu+) ions. The prepared aqueous solution was centrifuged at 5000 rpm for 10 mins at room temperature. The precipitation of copper nanoparticles was collected as pellet in the centrifuge tube. The pellet was washed with distilled water to remove bioactive molecules of the plant extract and

shivarajmetigouda@gmail.com*

the filtrate was oven dried. The dried sample of CuNPs was collected and stored for further work.

2.3 Acute Toxicity Test

The acute toxicity (96 hr LC_{50}) of copper nanoparticles for *Cirrhinus mrigala* was determined in the laboratory using the semi-static method[27]. The carp (10 fingerlings in 20 L of test medium in each replicate) were exposed to varying concentrations of copper nanoparticles and control in replicates. Test medium was renewed for every 24 hr with their respective test concentrations of the toxicant without aeration. Mortality was recorded every 24 hrs and the dead fish(fig 1) were removed when observed, every time noting the number of fish death at each concentration up to 96 hrs for estimation of acute toxicity (LC_{50}). Time of exposure was the repeated measure factor while treatment (concentration and control) was the second factor. The LC_{50} was calculated using probitanalysis[28], which has been recommended by OECD guideline as an appropriate statistical method for toxicity data analysis[29]. After linearization of the concentration response curve by logarithmic transformation of concentrations (log+2), 96 hrs LC_{50} with 95% confidence limits and slope function were calculated to provide a consistent presentation of the toxicity data.

2.4 Characterization of Copper Nanoparticles

2.4.1 UV-Visible Spectroscopy

UV-Visible spectroscopy is very reliable technique for primary confirmation of the formation of CuNPs. UV-Visible spectroscopy is widely used in characterisation of organic and inorganic nanoparticles quantitatively. A sample was irradiated with electromagnetic waves in ultraviolet region and observed light was analysed through the resulting spectrum. It is employed to identify the constituents of substance and to determine their concentration. The sample may be either organic or inorganic, may exist in gaseous liquid and solid form.

2.4.2 X-Ray Diffraction (XRD)

The XRD analysis of the synthesized CuNPs was performed using Bruker, D8 QUEST X-ray diffractometer operated at 40KV/30mA and the scanning speed of 50 min^{-1}.

3. RESULTS

3.1 Acute Toxicity Test

Based on acute toxicity results of the present study of 96 hour LC_{50} of copper nanoparticles (CuNPs) for different life stages of *Cirrhinus mrigala* ranged from 0.5 ppm – 2 ppm (Table 1 and fig 1).The Behavioural manifestations of acute toxicity like erratic swimming, restlessness and surfacing movements were observed in *Cirrhinus mrigala* exposed to higher concentration of silver nanoparticles over the 24 hours. After 24 hours fishes exhibited lathery and erratic swimming movements suggesting loss of equilibrium at higher concentration of silver nanoparticles. At the time of death evanescent hyper activity was also observed.

Table 1: Mortality of *Cirrhinus mrigala* in different concentrations of Copper nanoparticles at 96 hr exposure period.

Conc. of Copper nanoparticles in ppm	Log conc.	No. of fish Exposed	No. of fish alive	Percent mortality
0	0.00	10	10	0.00
0.5	1.05	10	10	0.00
1.0	2.05	10	8	20.00
1.5	3.15	10	7	30.00
2.0	3.25	10	5	50.00

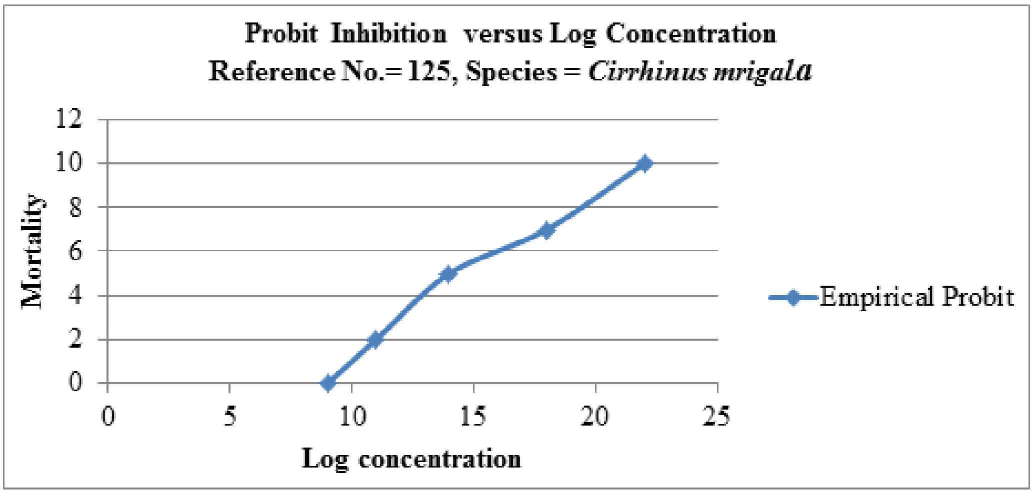

Fig 1. ProbitInhibitation versus Log Concentrations of *C.mrigala* treated with different log concentrations of Copper nanoparticles.

In the present work CuNPs are synthesized through green route by reduction of copper sulphate and *Moringa oleifera* leaf extract as reducing agent. The synthesis of CuNPs via chemical reduction method is confirmed primarily by UV-visible spectrum using the Eppendorf BioSpectrometer kinetic. The Eppendorf BioSpectrometer kinetic provides highly reliable analysis. The recorded UV-Visible absorbance spectrum of CuNPs synthesized and the recorded UV-Visible absorbance spectrum of Copper nanoparticles synthesized using AgNO and NaBH4 and the sharp absorbance peak found at the wavelength region 396 nm to 400nm is the primary confirmation of synthesis of Copper nanoparticles in the colloidal solution. The Gaussian shape curve of UV-visible spectrum shows the well-defined size of Copper nanoparticles. The average size of synthesized CuNPs is determined using the TEM micrograph. The TEM micrograph of synthesized CuNPs. In the TEM micrograph, the scale bar represents 20nm. CuNPs are very nearly spherical and fitted well within the nano-scale. And fig-2 represents the morphology of fish *Cirrhinus mrigala.*

Fig 2. Morphology of Fishes Common carp, *Cirrhinus mrigala.*

3.2 UV-Visible Spectroscopy

Fig.3. shows UV-visible spectrum of CuNPs. Characteristic UV-visible absorption peak found at 350 nm shows the formation of CuNPs. The measured wavelength of UV-visible absorption peak is comparable with the results reported[30-31]. From the study of UV-visible spectrum, it is found that the CuNPs can be synthesised successfully using *Moringa oleifera* extract as reducing agent

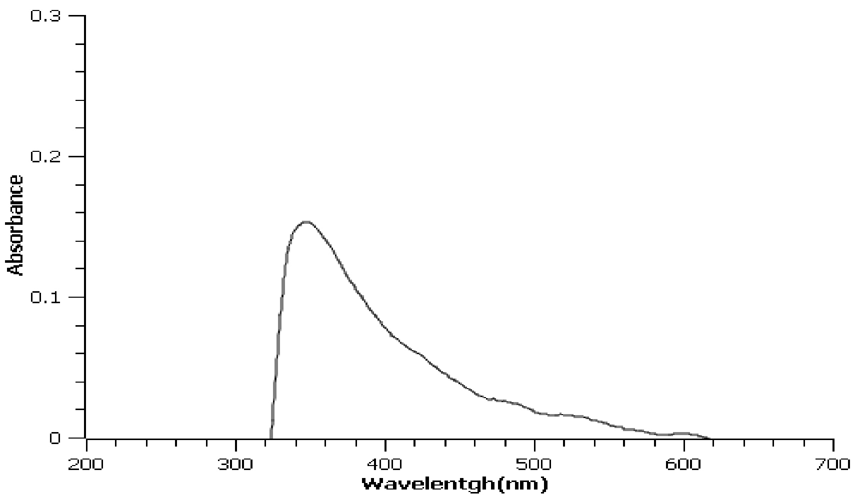

Fig 3. UV-Visible Spectrum of CuNPs using *Moringa oleifera* leaf extract.

3.3 X-Ray Diffraction (XRD)

The XRD is one of the most important characteristic tools in the field of nanotechnology because of its simplicity, reliability, and non-destructive nature. The XRD analysis of the synthesized CuNPs is performed using Bruker, D8 QUEST X-ray diffractometer operated at 40KV/30mA and the scanning speed of 50 min^{-1}. The XRD pattern of CuNPs is shown in Fig.4.

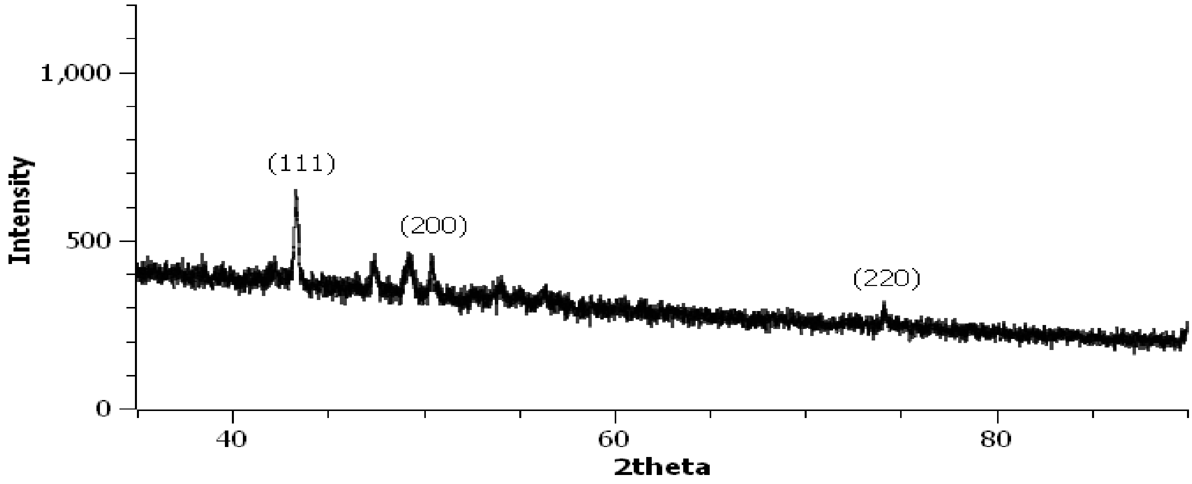

Fig 4. XRD Pattern of Copper Nanoparticles.

The characteristic diffraction peaks of copper are found at 43.7°, 50.7°, and 74.3°. They correspond to the crystallographic planes (111), (200) and (220) of the face-centered cubic (fcc) copper, respectively. Diffraction of X-ray by crystallographic planes is governed by Bragg's law;

$$2d_{hkl}\sin\theta = n\lambda$$

Where, θ is glancing angle of incident X-ray, d_{hkl} is distance between parallel planes from which reflection occurs, n is an integer corresponding to the order and λ is the wavelength of the beam. The inter planar spacing d_{hkl} is calculated using the Bragg's relation;

$$d_{hkl} = \frac{n\lambda}{2\sin\theta}$$

The experimental values of d_{hkl} and lattice parameters are listed in Table-2.

Table 2. Inter planar spacing and Lattice parameter

2Θ	Θ	$Sin^2Θ$	Successive Difference	$Sin^2 Θ$ /C,F	(h, k, l)	Inter planar spacing d_{hkl} in A^0	Lattice parameter, *a* in A^0
43.34	21.67	0.136		3.022	(1,1,1)	2.085	3.61
50.39	25.20	0.181	0.045	4.022	(2,0,0)	1.808	3.61
74.14	37.07	0.363	0.182	8.066	(2,2,0)	1.277	3.61

3.4 TEM analysis of CuNPs

The CuNPs synthesized using *Moringa oleifera* leaf extract as reducing agent is characterized by Tunneling Electron Microscope (TEM), the JEM-1400 available at Indian Institute of Technology, Delhi. The TEM micrograph of the CuNPs is shown in Fig-5. From the TEM analysis it is found that the synthesized CuNPs are nearly spherical in shape. The Fig-6 shows the size distribution of the CuNPs. Size of the CuNPs is 32.215nm within the error of ±0.793.

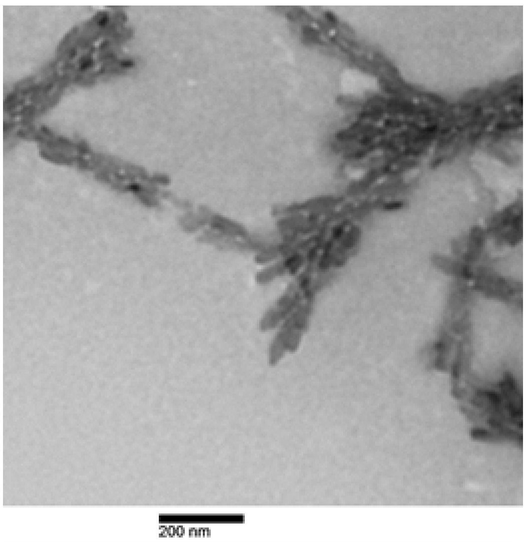

Fig 5. TEM Image of synthesized CuNPs

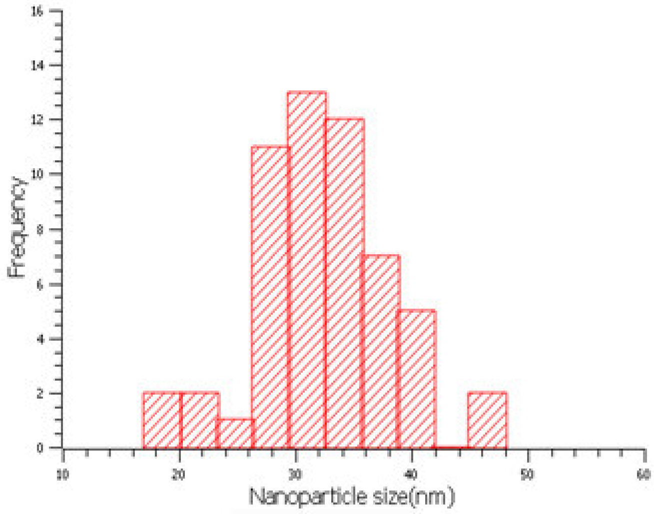

Fig 6. Size distribution of Synthesized CuNPs

In the present study the control fish the behavioural symptoms/signs of the fish in natural manner i.e., they were active with their well-coordinated movements. They were alert at the slightest disturbance, but in the toxic environment fishes were exhibited irregular, erratic and darting swimming movements, hyper excitability, capsizing, attaching to the surface, difficulty in breathing, loss of equilibrium, gathering around the ventilation filter and they slowly became lethargic, hyper excited and restless which we can focused easily at the time of experimental periods and the lethal concentration of LC_{50}value of the CuNPs is 2ppm were dictated. So, in this study the colloidal solution, silver nanoparticles is slightly toxic.

4. DISCUSSION

Many environmental factors which shows different scenario due to vast and drastic changes in today's aquatic ecosystems and in many countries, the registration of synthetic chemicals involves passing a number of tests for environmental risk assessment and hazard classification. Currently, the hazard assessment of chemicals for fish is based on international standards and guidelines (such as the OECD guidelines) with global toxicity endpoints, such as mortality, growth, reproduction impairment, and biochemical changes in exposed fish. The behavioural abnormalities and state of fishes in both the control and experimental fish was recorded at every hour of the first 24 hours. Colloidal silver is one of the most beneficial products of nanotechnology that is effective against many types of pathogenic microorganisms, including bacterial fish pathogens[32]. Plus[33]showed a positive effect of the direct use of silver nanoparticles in reducing fungal infection in trout eggs. However, the commercial application of solution copper nanoparticles (CuNPs) to fish is currently lacking clear safety regulations and toxicology data. According to acute toxicity results of published papers, 96 hour LC_{50} of silver nitrate ($AgNO_3$) for different life stages of rainbow trout ranged from 5.3–20.2 μg/L [34-38]. However based on acute toxicity results of the present study 96 hour LC_{50} of copper nanoparticles (CuNPs) for different life stages of *Cirrhinus mrigala* ranged from 0.5 ppm – 2 ppm. The fish showed marked changes in their behaviour when exposed to various concentrations of copper nanoparticles. Fish exposed to copper nanoparticles had whitish, slimy mucus like substance covering most parts of the body, especially over the pectoral and dorsal fins of the fish. After introduction of Nanoparticles, fish showed interestingly try to jump out of the aquarium to avoid the stress followed by increased swimming, restlessness, surfacing and hyperactivity. In lower concentration of silver nanoparticles the fish showed a slow swimming than the control group. The Behavioural manifestations of acute toxicity like erratic swimming, restlessness and surfacing movements were observed in *Cirrhinus mrigala* exposed to higher concentration of silver nanoparticles over the 24 hours. After 24 hours fishes exhibited lathery and erratic swimming movements suggesting loss of equilibrium at higher concentration of silver nanoparticles. At the time of death evanescent hyper activity was also observed. The reaction and survival of aquatic animal depends not only in the biological state of animals and physicochemical characteristics of water but also on the kind, toxicity, type of exposure to toxicants. The use of AgNPs obtained from synthesis of natural products was applied with positive results[39], for example; when AgNPs was synthetized by *Camellia sinensis* to *Vibrio harveyi* in infected *Feneropenaeus indicus* organisms. In vivo tests demonstrated that 10 μg mL-1 concentration inhibited bacterial growth in 70%. In other study[40] it was used *Bacillus subtilis*, a non-pathogenic organism to synthetize a Nano compound, and it was evaluated their antimicrobial effect on *V. parahaemolyticus* and *V. harveyi*, in infected *Litopenaeus vannamei*. The fishes showed marked changes in their behaviour when exposed to various concentrations of the silver nanoparticles. In a reported study , fishes exposed to silver nanoparticles had whitish, slimy mucous like substances covering most parts of the body, especially over the pectoral and dorsal fins of the fishes[41]and an excess secretion of mucous in fish forms a nonspecific response against toxicants, thereby probably reducing toxicant contact[42]. The results showed a survival of 1% in control group, but with Nano compounds was 90%. Also, was determined that when extract of bacteria and plants were used, was not observed a physiology alteration or toxic effect on studied shrimps. Thus, according to the results of the current study, colloidal silver nanoparticles should also be classified as "toxic" for the juvenile stage of *Cirrhinus mrigala*.

5. CONCLUSION

In this present investigation the direct exposure of freshwater fish *Cirrhinus mrigala* to compound CuNPs revealed that the fish were more sensitive to solution of copper nanoparticles. Furthermore, solution copper nanoparticles should be classified as "very toxic" and "toxic" to the embryo stages and juvenile stage of *Cirrhinus mrigala*, respectively, when that the release of solution copper nanoparticles into the environment or their direct application as an antifungal/antibacterial behaviour conditions during the hatching period of eggs or larval stages should be avoided. Instead, indirect techniques, like the use of filters containing copper nanoparticles, are better for incubation system water treatment also. Further studies are also recommended on the potential use of a fish in different types of biomarker for toxicity assessments by the Eco-toxicologists evaluated of copper nanoparticles and finally this was the agent which shows "slightly toxic" to the entire aquatic ecosystems.

6. AUTHORS CONTRIBUTION STATEMENT

Dr. Chikappa had done the Synthesis and Characterization of Nanoparticles and Dr. Shivaraj have contributed Toxicity and Behavioural study. All authors contributed equally for this development of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none

8. REFERENCES

1. PillaiRaji K, Sareen Sarah John, Toms Joseph C, Chandramohana kumar N and Balagopalan M. Vermifugal, Activity of Bio fabricated Silver Nanoparticles. Research Journal of Recent Sciences. 2012;1:47-51. Available from: https://www.researchgate.net/publication/268221312_Vermifugal_Activity_of_Biofabricated_Silver_Nanoparticles
2. Woo K, Kim D, Kim JS, Lim S, Moon J. Ink-Jet Printing of Cu− Ag-Based Highly Conductive Tracks on a Transparent Substrate. Langmuir. 2009;25(1):429–33. DOI: 10.1021/la802182y
3. Eastman JA, Choi SUS, Li S, Yu W, Thompson LJ. Anomalously increased effective thermal conductivities of ethylene glycol-based nanofluids containing copper nanoparticles. Appl Phys Lett. 2001;78(6):718–20. DOI: 10.1063/1.1341218
4. Tarasov S, Kolubaev A, Belyaev S, Lerner M, Tepper F. Study of friction reduction by nanocopper additives to motor oil. Wear. 2002;252(1–2):63–9. DOI: 10.1016/s0043-1648(01)00860-2
5. Kruis FE, Fissan H, Peled A. Synthesis of nanoparticles in the gas phase for electronic, optical and magnetic applications—a review. J Aerosol Sci. 1998;29(5–6):511–35. DOI: 10.1016/s0021-8502(97)10032-5
6. Halevas EG PA. Copper nanoparticles as therapeutic anticancer agents. Nanomed Nanotechnol J. 2018;2(1):119.
7. Han W-K, Choi J-W, Hwang G-H, Hong S-J, Lee J-S, Kang S-G. Fabrication of Cu nano particles by direct electrochemical reduction from CuO nano particles. Appl Surf Sci. 2006;252(8):2832–8. DOI: 10.1016/j.apsusc.2005.04.049
8. Jeevan Jyoti Mohindru UKG. Green synthesis of copper nanoparticles using tea leaf extract. Int J Eng Sci Res Technol. 2017;6(7):307–11. Available from: http://www.ijesrt.com/issues pdf file/Archive-2017/July-2017/39.pdf
9. Marshall AT, Haverkamp RG, Davies CE, Parsons JG, Gardea-Torresdey JL, van Agterveld D. Accumulation of Gold Nanoparticles inBrassic Juncea. Int J Phytoremediation. 2007;9(3):197–206. DOI: 10.1080/15226510701376026
10. Makarov VV, Love AJ, Sinitsyna OV, Makarova SS, Yaminsky IV, Taliansky ME KN. Green nanotechnologies: synthesis of metal nanoparticles using plants. Acta Naturae. 2014;6(1):20.
11. Gopinath M, Subbaiya R, Selvam MM SD. Synthesis of copper nanoparticles from Nerium oleander leaf aqueous extract and its antibacterial activity. Int J Curr Microbiol App Sci. 2014;3(9):814–8. Available from: https://pdfs.semanticscholar.org/0d2c/3bcbf86100ebf5b5254ec355aea1dcde22b0.pdf
12. B BM and M. Green Synthesis of Copper Nanoparticles Using Solanumlycopersicon (Tomato Aqueous Extract) and Study Characterization. J Nanosci Nanotechnol Res. 2017;1(1):1–5. Available from: http://www.imedpub.com/articles/green-synthesis-of-copper-nanoparticles-using-solanum-lycopersicum-tomato-aqueous-extract-and-study-characterization.php?aid=21066
13. Ashtaputrey SD, Ashtaputrey PD YN. Green synthesis and characterization of copper nanoparticles derived from Murraya koenigii leaves extract. J Chem Pharm Sci. 2017;10(3):1288–91. Available from: https://pdfs.semanticscholar.org/60ef/3a62617a769ce3ec86638c71408187626c1d.pdf
14. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: A review on nutritive importance and its medicinal application. Food Sci Hum Wellness. 2016;5(2):49–56. DOI: 10.1016/j.fshw.2016.04.001
15. Kushwaha S, Chawla P, Kochhar A. Effect of supplementation of drumstick (Moringa oleifera) and amaranth (Amaranthus tricolor) leaves powder on antioxidant profile and oxidative status among postmenopausal women. J Food Sci Technol. 2014;51(11):3464–9.DOI: 10.1007/s13197-012-0859-9
16. Taher MA, Nyeem MA Bin, Ahammed MM, Hossain MM, Islam MN. Moringa oleifera (Shajna): the wonderful indigenous medicinal plant. Asian J Med Biol Res. 2017;3(1):20–30. DOI: 10.3329/ajmbr.v3i1.32032
17. GanatraTejas H, Joshi Umang H, BhalodiaPayal N, Desai Tusharbindu R TR. A panoramic view on pharmacognostic, pharmacological, nutritional, therapeutic and prophylactic values of Moringa oleifera lam. Int Res J Pharm. 2012;3(6):1–7.
18. Sangeetha V P V. Synthesis and characterization of silver nanoparticles using moringa (Moringa oleifera Lam.) pod extract. Trends Biosci. 2014;7(8):675–9.
19. Bhusnure OG, Jadhav PP, Hindole SS, Gholve SB, Giram PS KV. Green Synthesis of Silver Nanoparticle Using Moringa oleifera Lam extract for pharmacological Activity. Int J Pharm Pharm Res. 2017;10(4):59--66.
20. EJ. A-K. Green Synthesis of Silver Nanoparticles Using Leaf Extract of Al-Rawag tree (Moringa oleifera Lamarck) Cultivated in Iraq and Efficacy the Antimicrobial activity. Mesopotamia Environ J. 2016;6:39–48.
21. Shilpashree Mayachar K, Nandini N, and AJA.

Optimization, Characterization and Antibacterial activity of Green Synthesized silver Nanoparticles using Moringa oleifera leaf extract. Res J Chem Environ Sci. 2018;6(5):48–57. Available from: http://www.aelsindia.com/oct_2018/9.pdf
22. Tahany GM M and AE-RA. Environmentally friendly synthesis of silvernanoparticles using Moringa oleifera (Lam) leaf extract and their antibacterial activity against some important pathogenic bacteria. Mycopath. 2015;13(1):1–6.
23. Manokari M SM. Zinc oxide nanoparticles synthesis from Moringa oleifera Lam. Extracts and their characterization. World Sci News. 2016;55:252–62. Available from: https://www.infona.pl/resource/bwmeta1.element.psjd-7b335fcd-9c73-4ad2-90bd-b5bccf4cad8d
24. Vivek Patidar PJ. Green Synthesis of TiO2 Nanoparticle Using Moringa Oleifera Leaf Extract. Int Res J Eng Technol. 2017;4(3):470–3.
25. Aliyu AO, Garba S BO. Green synthesis, characterization and antimicrobial activity of vanadium nanoparticles using leaf extract of Moringa Oleifera. Int J Chem Sci. 2017;16(1):231. Available from: https://pdfs.semanticscholar.org/a256/f1f6d8d1dc8794c7e592422b353a978233c3.pdf
26. APHA. Standard methods for the examination of water and wastewater. 21st ed. Washington DC; 2005.
27. Development O for EC and. OECD Guideline for testing of chemicals (Fish, Acute Toxicity Test). 192AD. TG 203. 1992. Available from: http://www.oecd.org/chemicalsafety/risk-assessment/1948241.pdf
28. OJ F. Toxic effect of profenofos on tissue acetylcholinesterase and gill morphology in a euryhaline fish, Oreochromismossambicus. Probit Analysis . 2nd ed. Cambridge university press, Cambridge; 1953.
29. Lilius H, Isomaa B, Holmström T. A comparison of the toxicity of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and Daphnia magna. Aquat Toxicol. 1994;30(1):47–60.
DOI: 10.1016/0166-445x(94)90005-1
30. Renganathan S, Fatma S, P K. Green synthesis of copper nanoparticle from passiflora foetida leaf extract and its antibacterial activity. Asian J Pharm Clin Res. 2017;10(4):79.
DOI: 10.22159/ajpcr.2017.v10i4.15744
31. Cherimadurai K, Biswas S, Murali R and Thanikaivelan P. Green synthesis of copper nanoparticles and conducting nanobiocomposites using plant and animal sources. RSC Adv. 2014;4:1-11.
DOI: 10.1039/C4RA01414F
32. Soltani M, Ghodratnema M, Ahari H, Ebrahimzadeh Mousavi HA, Atee M, Dastmalchi F RJ. The inhibitory effect of silver nanoparticles on the bacterial fish pathogens. Streptococcus iniae, Lactococcus garvieae, Yersinia ruckeri. Int J VetRes. 2009;3(2):137–42. Available from: https://ijvm.ut.ac.ir/pdf_20639_d90317c93c48f6f61c2db8b97e351992.html
33. Soltani M, Esfandiary M, Sajadi MM, Khazraeenia S, Bahonar AR AH. Effect of nanosilver particles on hatchability of rainbow trout (Oncorhynchus mykiss) egg and survival of the produced larvae. Iran J Fish Sci [Internet]. 2011;10(1):167–78. Available from: http://jifro.ir/files/site1/user_files_eb12be/admin-A-10-1-72-a7480f1.pdf
34. Davies P. Toxicity of silver to rainbow trout (Salmo gairdneri). Water Res. 1978;12(2):113–7.
DOI: 10.1016/0043-1354(78)90014-3
35. Nebeker A V, McAuliffe CK, Mshar R, Stevens DG. Toxicity of silver to steelhead and rainbow trout, fathead minnows and daphnia magna. Environ Toxicol Chem. 1983;2(1):95.
DOI: 10.1897/1552-8618(1983)2[95:tostsa]2.0.co
36. Buhl KJ, Hamilton SJ. Relative sensitivity of early life stages of arctic grayling, coho salmon, and rainbow trout to nine inorganics. Ecotoxicol Environ Saf. 1991;22(2):184–97.
DOI: 10.1016/0147-6513(91)90058-w
37. Hogstrand C, Galvez F, Wood CM. Toxicity, silver accumulation and metallothionein induction in freshwater rainbow trout during exposure to different silver salts. Environ Toxicol Chem [Internet]. 1996;15(7):1102–8.
DOI: 10.1002/etc.5620150713
38. Grosell M, Hogstrand C, Wood CM, Hansen HJM. A nose-to-nose comparison of the physiological effects of exposure to ionic silver versus silver chloride in the European eel (Anguilla anguilla) and the rainbow trout (Oncorhynchus mykiss). Aquat Toxicol. 2000;48(2–3):327–42.
DOI: 10.1016/s0166-445x(99)00029-6
39. Vaseeharan B, Ramasamy P, Chen JC. Antibacterial activity of silver nanoparticles (AgNps) synthesized by tea leaf extracts against pathogenic Vibrio harveyi and its protective efficacy on juvenile Feneropenaeus indicus. Lett Appl Microbiol. 2009.
DOI: 10.1111/j.1472-765x.2009.02799.x
40. Sivaramasamy E, Zhiwei W. Enhancement of Vibriosis Resistance in Litopenaeus vannamei by Supplementation of Biomastered Silver Nanoparticles by Bacillus subtilis. J Nanomed Nanotechnol. 2016;07(01).
DOI: 10.4172/2157-7439.1000352
41. Udagani DRC, Yallappa DRS. Synthesis and characterization of colloidal solution silver nanoparticles effect on behavioural changes and LC50 value of fish Cyprinus Carpio (linnaeus). Int J Pharma Bio Sci. 2019;10(2):68-74.
DOI: 10.22376/ijpbs.2019.10.2.b68-74
42. Shivaraj Y and Asiya N.F.B.Toxic effect of biochemical and morphological changes on carp (Cyprinus carpio) exposed to cadmium chloride. Int J Zool Stud. 2017;2(5):222–8.