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ORIGINAL ARTICLE

INDUCTION OF MICRONUCLEI IN THE CAT FISH *Clarias batrachus* (Linn. 1755) EXPOSED TO SUBLETHAL CONCENTRATION OF SILVER NANOPARTICLES

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ABSTRACT

In the present decades, the nanoparticles are being synthesized through chemical, physical and biological methods and chemical reduction is more suitable method due to less cost of chemical, ease of control and less byproducts.The extensive use of Ag nanoproducts increases the discharge into aquatic ecosystem. It further contaminated the environment through cement manufacturing, weathering of rocks, burning of fusel fuel, processing of ores, leaching and anthropogenic activities.Fish are more sensitive to many toxicants and are a convenient specimen for aquatic toxicity assays. Fishes are considered to be most significant biomonitors in aquatic systems for the estimation of metal pollution level and they offer several specific advantages in describing the natural characteristics of aquatic systems in assessing changes to habitats. The present investigation has been conducted to understand the effect of sublethal concentration of silver nanoparticles (16.825 ppm; onthe induction of micronuclei*Clarias batrachus* for a period of 10, 20, 30,40, and 50 days. On exposure to sublethal concentration the alterations the in RBCs are micronuclei, fragmented,lobed and buds which increased with the duration of exposure.

Keywords: Fish RBC, Nano toxicity, *Clarias batrachus*, Micronuclei.

1.INTRODUCTION

Silver nanoparticles (Ag-NPs) enter into the environment from cement manufacturing, weathering of rocks, burning of fossil fuel, processing of ores, leaching and anthropogenic activities (Benn and Westerhoff 2008; Awasthi *et al.* 2013; Taju *et al.* 2014). Metallic silver is insoluble, whereas salts (AgCl, AgNO3) are soluble in water and exists in the form of colloidal particles (Wijnhoven *et al.* 2009). Ag-NPs are considered more toxic than other forms because of more readily absorbable. When it reaches the aquatic environment, Ag- NPs most likely to enter the ecosystems produce a physiological response and genotoxicity in animals (Luoma *et al.* 2008; Sohn *et al.* 2015). The other genotoxic effects include DNA double-strand breaks, DSBs, chromosomal aberrations such as acentric and dicentric chromosomes, chromosomal fragmentation and fusions in the treated organisms (Igwilo *et al.* 2006; Ahamed et al. 2008; AshaRani *et al.* 2008; Khan *et al.* 2015a). Recently, the researchers have focused on toxicity of Ag-NPs in aquatic organisms (Monfared and Soltani 2013;Reddy et al. 2013;Taju *et al.* 2014Khan *et al.* 2015;Rajkumar *et al.* 2015;). Limited information regarding the genotoxicity toxicity and cytotoxicity of Ag-NPs is available (Wijnhoven *et al.* 2009 Khan *et al.* 2015;). Moreover, fish blood contained nucleated erythrocytes which can be used to estimate the damages due to environmental pollution. Hence, the study has been designed to understand the sublethal toxicity of silver nano particles on the induction of micronuclei in the red blood cells of the catfish *Clarias batrachus.*

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2.MATERIALS AND METHODS

Collection and maintenance of fish: The fresh water fish *Clarias batrachus* (14 ± 2 cm length and 30 ± 2 g weight) were collected locally from Chidambaram and were brought to the laboratory and kept in a tank size of 60 x 30 x 30 (l x

b x h) cm, filled with tap water for acclimatization for about two weeks. During the acclimatization the fish were fed with minced goat liver on every alternate days. and water in the tank was also renewed daily and aerated to ensure sufficient oxygen supply. For the fish used in experiments, feeding was stopped two days before the start of the experiments to reduce the quantum of excretory products in the tank.

Experimental Protocol: Prior to the commencement of the experiment the median lethal concentration (LC_{50}) for 96 h was calculated by trimmed Spearman karber method (Hamilton *et al.,* 1977) and was found to be 168.25 ppm at 95% confidence limit. For the analysis of sublethal toxicity five groups of 10 fish each were exposed separately to silver nanoparticle (AgNPs) (16.825ppm; solution prepared in tap water. The experimental medium was prepared in tap water having dissolved oxygen 6 ppm, pH 7.5, water hardness 40.44 mg/L and water temperature 28 ± 2 °C (APHA, 2008). Each group of 10 fish were exposed to 50 L of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaria containing 50 L tap water without the addition of AgNPs as controls. Feeding was allowed in the experimental as well as control groups everyday for a period of 3 h before the renewal of the media throughout the tenure of the experiment.

Micronucleus test: A thin smear of blood from each treatment was made onpre-cleaned slide and fixed in methanol for 20 min afterdrying. The slides were air dried and stained with Giemsastaining (6%) for 25 min, washed with tap water, allowedto be dried and examined at 100X magnification undermicroscope (Nikon with DS-L3 camera). Small, circular orovoid and non-refractive bodies with the same staining andfocusing pattern as main nucleus was scored as micronucleiand frequency is each treatment was calculated with thefollowing formula.

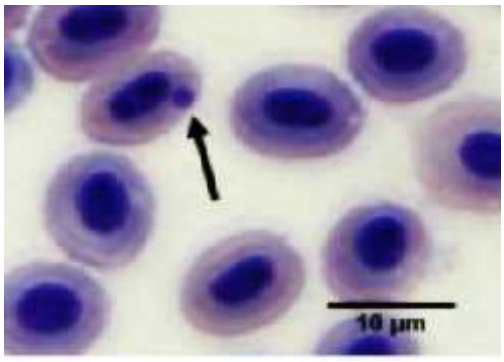
$$MN\,(\%) = \frac{\text{Number of cells with micronuclei}}{\text{Total number of cells scored}} \times 1000$$

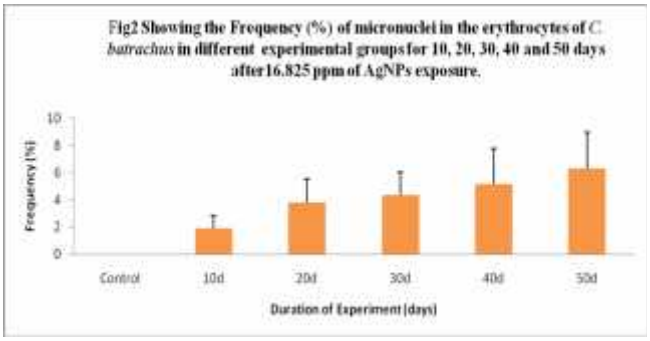
Statistical Analysis: The data obtained were subjected to standard statistical analysis for each sampling time and their respective control groups in different groups. Duncan's multiple range test (Bruning and Kintz, 1968) was performed to determine whether the parameters altered significantly by exposure periods.

3.RESULTS

Mature and normal erythrocytes cells were large, oval andnucleated with 7–15µm in size. The exposed specimens showed significantlyincrease in the frequency of micronuclei when compared to control specimens. The Ag NPs induced micronuclei is shown in Fig 1

On transferring the fish into sublethal concentration of Ag NPs solution the exposed specimens showed a frequency of 1.91 after 10 days. The frequency (%) increased as 3.79, 4.35, 5.16 and 6.31 after 20,30,40 and 50 days respectively (Table 1). Maximum frequencywas recorded after 50 days of treatment (Fig 2)Theinduction of MN gradually increased with the time ofexposures





4.DISCUSSION

Nanoparticles (NPs) are widely used to produce novel materials with uniquephysicochemical properties, which become an environmental concern in the event ofunintended release into the environment. The increasing commercial application of NPs,with at least one dimension of ≤100 nm, is currently showing inventory listings of 1,317nanotechnology-based consumer products in 30 countries (WoodrowWilsonDatabase, 2011), while the production of engineered NPs is expected to reach approximately60,000 tons in 2011 (Jovanović *et al.,* 2011Matranga and Corsi, 2012). The propertiesof NPs that contribute to biological perturbations strongly depend on their size,mineralogy, crystallinity, and surface reactivity which is directly connected to NP
toxicity through redox reactions, production of oxygen or nitrogen free radicals, thedissolution of NPs, release of toxic ions, the sorption and transport of metal ions orxenobiotic pollutants (Choi *et al*. (2010 Bottero *et al.,* 2011).) mentioned that NPs may induce deterimental effects in aquaticsystems as well as aquatic organisms. Despite their widespread use in medicine,cosmetics, renewable energies, electronic devices, and environmental remediation(Fabrega *et al.,* 2011; Matranga and Corsi, 2012), there is limited data on the safety andtoxicity of Ag-NPs especially those relating to its DNA interaction (Flower *et al.,* 2012).The effective levels of Ag-NPs range from µgl–1 to mgl–1 including the variety of aquaticorganism type, particle type, and exposure conditions (Hsin *et al.,*2008Farmen *et al.,* 2012;).

Ag-NPs are widely used in commercial production due to large number of nanoproducts (Khan *et al.* 2015a). In fish,

the micronuclei test of blood erythrocytes is usually preferred because the fish erythrocytes are nucleated in nature.In this study the maximum frequency of MN was recorded on 50 days of exposure to sublethal concentrations of Ag NPs. MNfrequencies in erythrocytes increased with increasing the duration of exposure. Das andNanda (1986) reported a dose-dependent and timedependentincrease in the induction of micronucleiin peripheral blood of *Heteropneustesfossilis.*Yadav and Trivedi (2009) found that frequenciesof micronuclei were increased in *Channa punctata*after 96 h of exposure to heavy metal. Nepomuceno *et al.* (1997) have suggested that thehigher pollutant concentration might inhibit normalcell division, damage erythrocyte chromosomes, andinterrupt DNA duplication, causing micronucleifrequencies to increase. Themicronucleifrequencies tend to even out and fish might promotesome defensive mechanism to reduce some of themetal residues in the body. The percentage of micronuclei increased with the duration of exposure to sublethal concentration of Ag-NPs and this indicates genotoxicity. In conclusion, based on the results obtained from the present study, it was found thatexposure to Ag-NPs produced significant adverse effects on the erythrocytesof catfish, presenting as micronuclei induction. The use of fish as biomarkers for pollution is of increasing importance and canpermit the early detection of waterpollution. These organisms respond to toxic agentsin a way that is similar to that of higher vertebrates,which can allow for the assessment of substances thatare potentially hazardous to humans. Therefore, theneed to understand the toxicological mechanisms of Ag-NPs in fishes as well as todetermine the genotoxic effects of metal nanoparticles is necessary

5.REFERENCES

Ahamed M, Karns M, Goodson M, Rowe J, Hussain SM, Schlager JJ, Hong, Y 2008. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol Appl Pharmacol 233:404–410.

Asha Rani P, Low Kah Mun G, Hande MP, Valiyaveettil S 2008. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 3:279–290

Awasthi KK, Awasthi A, Bhoot N, John P, Sharma SK, Awasthi K. 2013. Antimicrobial properties of electro-chemically stabilized organo-metallic thin films. Adv Electrochem 1:42–47. doi:10. 1166/adel.2013.1013.

Benn TM, Westerhoff P. 2008. Nanoparticle silver released into water from commercially available sock fabrics. Environ Sci Technol 42:4133–4139. doi:10.1021/es7032718

Bottero, J. *et al*. 2011. Manufactured metal and metal-oxide nanoparticles: properties and perturbing mechanisms of their biological activity in ecosystems', ComptesRendus Geoscience, Vol. 343, Nos. 2–3, pp.168–176.

Bruning, J.L. and B.L. Kintz (1968): Computational Handbook of Statistics, Foresman and Company, Dallas, USA, p. 308.

Choi, J.Y. *et al.* 2010. In vitro cytotoxicity screening of water-dispersible metal oxide nanoparticles in human cell lines', Bioprocess and Biosystems Engineering, Vol. 33, No. 1, pp.21–30.

Das R.K. and Nanda, N.K. 1986. Induction of micronuclei in peripheral erythrocytes of fish *Heteropneustesfossilis*by mitomycin C and paper mill effluent 175 (1986) 67-71) MRLett. 0891

Fabrega, J. *et al.* 2011. Silver nanoparticles: behaviour and effects in the aquatic environment, Environment International, Vol. 37, No. 2, pp.517–531.

Farmen, E. et al. 2012.Acute and sub-lethal effects in juvenile Atlantic salmon exposed to low µg/L concentrations of Ag nanoparticles', Aquatic Toxicology, Vol. 108, pp.78–84.

Flower, N.A.L. *et al.* 2012. 'Characterization of synthesized silver nanoparticles and assessment of its genotoxicity potentials using the alkaline comet assay', Mutation Research, Vol. 742, pp.61–65.

Hamilton, M.A.; Russo, R.C.;Thurston, R.V., (1977). Trimmed Spearman Karber method for estimating median lethal concentrations in toxicity bioassays.Environ. Sci. Tech., 11: 714-719.

Hsin, Y-H. *et al.* 2008. 'The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells', Toxicology Letters, Vol. 179, No. 3, pp.130–139.

Igwilo IO, Afonne OJ, Maduabuchi UJ-M, Orisakwe OE (2006) Toxicological study of the Anam river in Otuocha, Anambra state, Nigeria. Arch Environ Occup Health 61:205–208. doi:10.3200/aeoh.61.5.205-208.

Jovanović, B. *et al.* 2011. 'Effects of nanosized titanium dioxide on innate immune system of fathead minnow (Pimephalespromelas Rafinesque,1820)', Ecotoxicology and Environmental Safety, Vol. 74, No. 4, pp.675–683.

Julio Cesar Nepomuceno (1997), Iris Ferrari, Mario Antonio Spano and Alberto Jose Environmental and Molecular Mutagenesis Centeno3 Detection of Micronuclei in Peripheral Erythrocytes of *Cyprinus carpio*Exposed to Metallic Mercury 30:293–297

Khan MS, Jabeen F, Asghar MS, Qureshi NA, Shakeel M, Noureen A, Shabbir S. 2015. Role of nao-ceria in the amelioration of oxidative stress: current and future applications in medicine Int. J Biosci 6:89–109. doi:10.12692/ijb/6.8.89-109.

Luoma SN, Rainbow PS, Luoma, S. 2008. Metal contamination in aquatic environments: science and lateral management. Cambridge Univ Press. doi:10.1111/j.1095-8649.2009.02440_4.x

Matranga, V. and Corsi, I. 2012.Toxic effects of engineered nanoparticles in the marine environment: model organisms and molecular approaches', Marine Environmental Research, Vol. 76, pp.32–40.

Monfared AL, Soltani S (2013) Effects of silver nanoparticles administration on the liver of rainbow trout (Oncorhynchus mykiss): histological and biochemical studies. Eur J Exp Biol 3:285–289

Rajkumar K, Kanipandian N, Thirumurugan, R 2015. Toxicity assessment on haemotology, biochemical and histopathological alterations of silver nanoparticles-exposed freshwater fish Labeorohita. Appl Nanosci 1–11

Reddy TK, Reddy SJ, Prasad, T 2013. Effect of silver nanoparticles on energy metabolism in selected tissues of Aeromonas hydrophila infected Indian major. Carp Catlacatla IOSR J Pharm 3:49–55.

Sohn EK, Johari SA, Kim TG, Kim JK, Kim E, Lee JH, Chung YS, Yu IJ, 2015. Aquatic Toxicity Comparison of Silver Nanoparticles and Silver Nanowires Biomed Res Int:1–16

Taju G, Majeed SA, Nambi K, Hameed, AS 2014. In vitro assay for the toxicity of silver nanoparticles using heart and gill cell lines of Catlacatla and gill cell line of Labeorohita. Comp BiochemPhysiol C PharmacolToxicol 161:41–52.

Wijnhoven SW, Peijnenburg WJ, Herberts CA, Hagens WI, Oomen AG, Heugens EH, Roszek B, Bisschops J, Gosens I, Van De Meent D, 2009. Nano-silver—a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology 3:109–138

WoodrowWilsonDatabase, 2011. Nanotechnology Consumer Product Inventory [online]

Yadav and Trivedi 2009. Chemosphere Sublethal exposure of heavy metals induces micronuclei in fish, *Channa punctata*Volume 77, Issue 11, Pages 1495-150

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