Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Effect of Chemically and Biologically Synthesized Silver Nanoparticles on Zebrafish Embryos (*Danio rerio*) and Daphnia.

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ABSTRACT

With extensive use of silver nanoparticles (NPs) in various applications comes a greater risk of its release in to aquatic ecosystem. Environmental exposure to nanomaterials is inevitable as they become part of our daily life. However, little is known about their effect on the aquatic species when they exposed to the NPs accidentally. To address this issue, thezebra fish (*Danio rerio*) embryo and daphnia was selected as our experimental model to assess the toxicity of aggregated NPs to the biota in the water column.The main objective of this particular study was to assess the effect of chemically and biologically synthesized silver NPs on zebra fish embryos and daphnia. Toxicity studies were carried out in order to determine the concentration range of nanoparticles for further testing. The embryo of zebrafish and adults of daphnia were exposed to various concentrations of silver NPs. The mortality rate, heartbeat, pigment production was checked after every 24hrs, 48hrs and 72 hrs.In the present study, silver NPswere synthesized using chemical as well as biological method (green synthesis). Chemically and becologically synthesized silver NPs were toxic for the zebra fish embryos at the concentration of 18.34 ppm and 10.08ppm respectively. For daphnia the chemically synthesized silver NPs were toxic up to the concentration of 3.66ppm and above, whereas biologically synthesized silver nanoparticles were toxic till the concentration of 18.34pppm.Therefore, the green synthesis of nanoparticles should be given preference since it does not harm the aquatic life

KEYWORDS: Zebrafish embryos, Daphnia, Silver nanoparticles, Green synthesis.

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INTRODUCTION

The word “Nano” has its origin in the Greek language, meaning “dwarf”**.** Nanotechnology is the study of nanoparticles. Nanoparticlesare particles between 1 and 100 nanometres in size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Nanoparticles possess a very unique optical, thermal, electrical and magnetic properties and has a variety of applications in various fields.

NPs have a large surface area to volume area than their conventional form. The large surface area affects the interaction of the elements and nanoparticles. It is emerging as a rapidly growing field with its application in the field of Science and Technology.[1]

Nanotechnology is undoubtedly one of the most important technologies of the 21st century. Commercial manufacture of nanoparticles has already begun; the market for nanotechnology has been projected to reach $100 billion by 2025. In the UK alone, there are already over 600 micro and nanotechnology companies. Whereas in India, there are more than 50 nanotechnology companies. The nanoparticles are extensively synthesized with an average of 60,000 tons annual production.622 companies of 30 countries produce 1814 Nano products of various application.[2]

Nanotechnology shows great promise in providing solutions to many of today's problems in medicine, energy production, and environmental sustainability.[3]As this technology grows and the number of nanomaterial types and applications increase, so does the likelihood that they will be released into the environment, and in significant quantities, a fact that has been met with increasing concern over their safety in terms of human health and the environment. Sources to the aquatic environment will include waste water discharges, accidental release from factories, and the degradation andwear of products containing NP.[4]

Because of their extremely small size and unique physical properties the behaviour of NP in the environment, their uptake, distribution and effects within the bodies of living organisms are likely to be different when compared to conventional xenobiotics

Silver Nanoparticles (SiNPs) are extensively being used in various fields like biomedical, biotechnology, diagnostic procedure, and gene therapy. SiNPshave a unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaics to biological and chemical sensors.[5]

Silver ions are also being used in the formulation of various medical devices, dental resins composites, bactericidal coating in water filters, and antimicrobial agents in detergents, creams, lotions, soaps, shampoos, toothpaste and other consumer products.[6]

Though there are many benefits of SiNPs there is a danger to of various nontoxicity of silver.[7]

To address this issue, the zebrafish (*Danio rerio*) embryo and daphnia was selected as our experimental model to assess the toxicity of aggregated NPs to the biota in the water column, as these models presents several advantages some of them are as follows:

Advantage of using zebrafish: Transparency and extra-uterine development for direct observation of phenotypic changes during embryonic development.

Advantage of using daphnia: They are ubiquitous; they form an important part of aquatic ecosystem. Daphnia is a keystone species in both ponds and lakes. Due to their important position in food chains, they are used to study the response of ecosystems to environmental change. The reproductive cycle of Daphnia is ideal for genetic study. Generation time ranges from ten to fifteen days.

It has been observed that the concentration level of nanoparticles in fresh water is in the range of (0.4–7%).[8] Therefore, it has become extremely important to assess the impact of nanoparticles on living vertebrate and the invertebrate ecosystem to assess the effects of nanoparticles in fresh water ecosystem.[9]

The present study will be a short acute toxicity study that will be conducted by Fish Embryo Toxicity [FET] test using the OCED guideline [2013] for testing the effect on these organisms.[10]

MATERIALS AND METHODS:

Maintenance of Zebrafish and collection of embryos

In order to carry out the experiment five breeding pairs of Zebrafish (*Danio rerio*) were procured from local commercial aquarium.

The pairs were kept in a charcoal filtered water under semi static conditions in a 10 L glass aquaria. They were exposed to an alternate light/ dark pattern of 14:10 h at 28±1^{o}C along with constantly filtering and aerating their water and replacing 20% of its water daily. The fishes were fed twice with live blood worms.

Collection of the embryos was doneby keeping the fish in a spawning aquarium with a mesh so as to prevent the cannibalization of the eggs. Within half an hour of the light being turned on in the morning spawning was induced. After the egg collection, eggs were immediately rinsed in an E3 medium while the unfertilized eggs were discarded after observing them under light microscope.

Maintenance of Daphnia

Adult Daphnia were obtained from local commercial aquarium. They were kept in fresh water in a medium sized glass tank with constant aeration and were fed twice with green algae.

Silver nanoparticles preparation and characterization

Chemical synthesis of silver nanoparticles

0.01 grams of silver nitrate was dissolved in 100ml of distilled water. 5ml of 1% trisodium citrate was added drop wise and the solution was heated with constant stirring. The clear solution changed to dark yellow colour solution indicating the formation of silver nanoparticles.

Biological synthesizes of silver nanoparticles

20-30 papaya leaves (Carcia papaya) was grinded with motor and pestle after washing them with D/W. The leaves were boiled for 10-15 minutes and the extract was filtered using a muslin cloth. In 5ml of the leaf extract 100ml of silver nitrate solution (Dissolve 0.01 gram of silver nitrate in 100 ml of D/W) was added.

Characterization technique

Characterization of the synthesized nanoparticles was done using UV-visible spectrophotometer (Horiba). Quantification of the prepared silver nanoparticles and zinc oxide nanoparticles was carried out by atomic absorption spectroscopy (Itlab). Further, the morphology of the synthesized nanoparticles was studied using scanning electron microscope equipped with an Energy Dispersive X-ray Analyser. (Icon Analytical).

The main objective of this particular study was to assess the effect of chemically and biologically synthesized Silver Nanoparticles on daphnia and zebrafish.

1. Daphnia and Zebra fish embryos were acclimatized and Fish Embryo Toxicity Test (FET test) was carried out using the OECD guidelines for the testing of chemicals. (OECD, 2013).
2. The toxicity studies were carried out in order to determine the concentration range of nanoparticles for further testing.
3. The Zebrafish embryos and Daphnia were exposed to various concentrations of Chemically Synthesized Silver NPs [10.08 ppm, 2.016 ppm, 0.40 ppm, 0.08 ppm, 0.016 ppm, 0.003 ppm] and Biologically Synthesized Silver NPs [18.34 ppm, 3.6 ppm, 0.73 ppm, 0.14 ppm, 0.02 ppm, 0.005 ppm]
4. The mortality rate and heartbeat were checked after every 24hrs for 3 days. Any gross abnormalities in the morphology were also noted.

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Sophia College, Mumbai with CPCSEA Registration No.1936/Po/Re/S/17/CPCSEA.

OBSERVATIONS AND RESULTS

Characterization of the particle

Silver Nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. As the papaya leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from light yellow to yellowish-brown due to reduction of silver ion, which indicated formation of silver nanoparticles. The bio reduction of Ag+ ions in the solution was monitored in the aqueous component and the spectrum of the solution measured through UV spectrophotometer. The absorption maxima were recorded at 470nm. [Figure 2]

Whereas, in metal Nano particles such as in silver, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band occurring due to the collective oscillation of electrons of silver Nano particles in resonance with the light wave. When the frequency of the electromagnetic field becomes resonant with the coherent electron motion, a strong absorption takes place, which is the origin of the observed colour. Here the colour of the prepared silver nanoparticles is yellow in colour. This absorption strongly depends on the particle size, dielectric medium and chemical surroundings. The absorption peak (SPR) is obtained in the visible range at 430 nm. With the above mentioned concentration. [Figure 1]

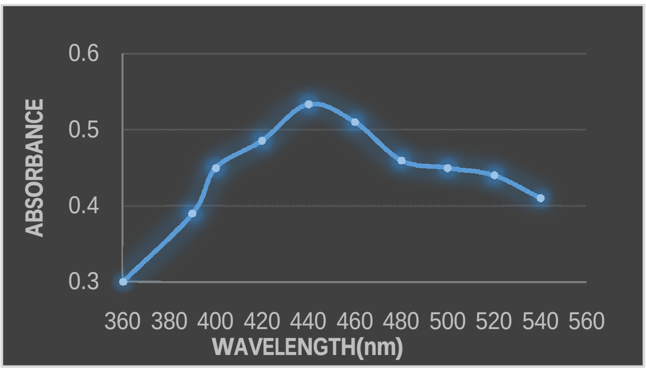

Figure 1: UV absorption spectra of Chemically Synthesized SilverNPs [CLp-AgNPs]

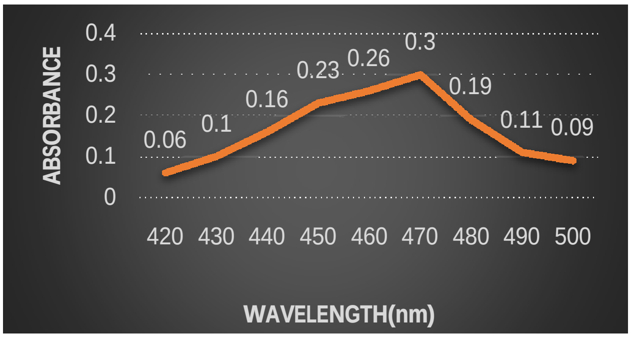

Figure 2: UV absorption spectra of Biologically Synthesized Silver NPs [BAgNPs]

SEM analysis of chemically synthesized Silver Nanoparticle revealed the particle size to be ranging from 62nm to 88nm with spherical morphology. The quantitative analysis using EDX showed the presence of high silver content Whereas, the Biologically synthesized Silver Nanoparticle from *Carcia papaya* leaf revealed the particle size to be form 87nm to 100nm with spherical morphology. The quantitative analysis using EDX showed the presence of high silver conten 43.44%. The spectrum also showed the presence of Carbon, Chlorine, Oxygen of 16.48%. 34.89%, 5.18% respectively.

The Atomic Absorption Spectroscopy studies was done and the concentration of the chemically and biologically synthesized Silver Nanoparticle was found to be 91.7 ppm / 10 ml of the sample and 50.4ppm / 10 ml of the sample respectively.

In order to determine the effect of nanoparticle on zebrafish and daphnia, a serial dilution of the synthesized nanoparticles was carried out and tested on zebrafish embryo and daphnia. Observations were recorded after every 24 hrs. Until the end of the test which include observations of hatchability, heartbeat, pigment production, and coagulation which indicate the development of toxicity. These results were then used to determine the lethality. The dead embryos were removed from the well after every observation.

Table 1: Impact of the synthesized nanoparticles on the mortality of zebrafish embryos:

Dilutions	BAgNps [ppm]	CLp-AgNPs [ppm]	Mortality in chemically synthesized silver nanoparticles	Mortality in biologically synthesized silver nanoparticles
10- 1	**18.34**	**10.08**	5/6	5/6
10-2	**3.66**	**2.01**	6/6	6/6
10-3	**0.73**	**0.40**	6/6	6/6
10-4	**0.14**	**0.08**	6/6	6/6
10-5	**0.02**	**0.016**	6/6	6/6
Control	-	-	6/6	6/6

Table 2: Dilution table: Total number of Daphnia in each well: 6

Well No.	Concentration of the synthesized nanoparticle(ppm)		Volume of Distilled water (ml)
	BAgNPs [ppm]	**CLP-AgNPs [ppm]**	
1	**18.34**	**10.08**	**5**
2	**3.66**	**2.016**	**5**
3	**0.73**	**0.40**	**5**
4	**0.14**	**0.08**	**5**
5	**0.02**	**0.016**	**5**

Table 3: Impact of the synthesized nanoparticles on the mortality of Daphnia:

Well No.	Mortality in chemically synthesized Silver nanoparticles		Mortality in biologically synthesized Silver nanoparticles	
	Day 1	**Day 2**	**Day 1**	**Day2**
1	**-**	**-**	**6**	**6**
2	**5**	**4**	**6**	**6**
3	**6**	**6**	**6**	**6**
4	**6**	**6**	**6**	**6**
5	**6**	**6**	**6**	**6**
Control	**6**	**6**	**6**	**6**

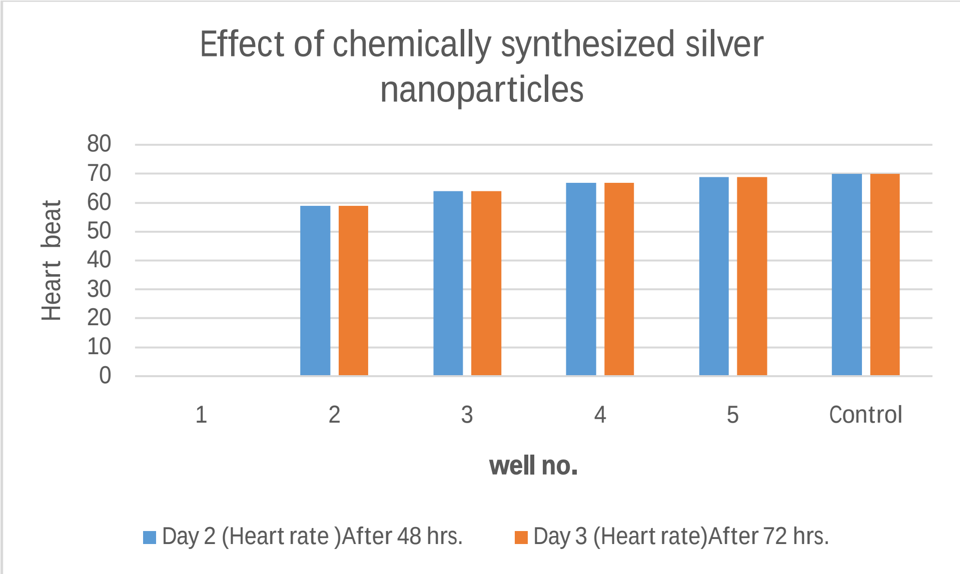

Figure 3: Effect of Chemcially Synthesized Silver NPs on Heart rate of zebrafish embryo

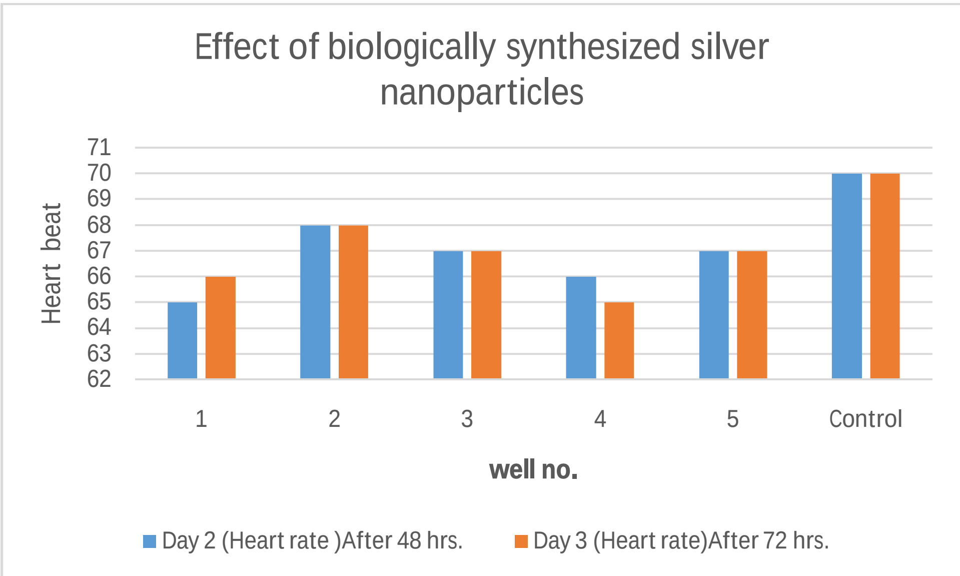

Figure 4: Effect of Biologically Synthesized Silver NPs on Heart rate of zebrafish embryos.

Figure 5: Dead zebrafish embryos observed after 15 hpf exposure to Chemically Synthesized Silver NPs- Failure to hatch.

Figure 6: Dead zebrafish embryos observed after 24 hpf exposure to Chemically Synthesized Silver NPs- Failure to hatch.

Figure 7: Dead Daphnia observed in Chemically Synthesized Silver NPs.

CONCLUSION:

Silver nanoparticles (NPs)have gained considerable interest because of their unique properties, and proven applicability in diverse areas such as medicine, catalysis, textile engineering, biotechnology, Nano biotechnology, bio-engineering sciences, electronics, optics, and water treatment. These NPs have significant inhibitory effects against microbial pathogens, and are widely used as antimicrobial agents in a diverse range of products.

The flexibility of silver nanoparticle synthetic methods and facile incorporation of silver NPs into different media have encouraged researchers to further investigate the mechanistic aspects of antimicrobial, antiviral and anti-inflammatory effects of these NPs.

The eco toxicity studies were carried out on Zebra fish embryos and Daphnia it was found that the silver nanoparticles chemically synthesized were toxic to them at concentrations of 18.34 ppm and above. Whereas, the biologically synthesized silver nanoparticles was toxic to them at concentrations of 10.08ppm and above.

This reinforces our finding that the eco-friendly, green synthesis of nanoparticles are a better alternative to conventional physical/chemical methods used for synthesis of silver nanoparticle and thus has a potential to use in biomedical applications and will play an important role in opt-electronics and medical devices in near future.

ACKNOWLEDGEMENT:

The authors are grateful to Dept. of Life science Sophia College for providing the use of UV-Vis spectrophotometry and the Italab, Mumbai for allowing us the use of Atomic Adsorption Spectrophotometer. The authors are also thankful to ICON Analytical laboratory for providing facilities to carry out scanning electron microscopy.

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