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Research Article

Genotoxic Effect of Silver Nanoparticles in Silk Worm *Bombyx mori*

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Abstract

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. We demonstrated that use of a natural, low cost biological reducing agent, *Morus alba* leave extracts (aqueous) can produce metal nanostructures, through efficient green nanochemistry methodology, avoiding the presence of hazardous and toxic solvents and waste. Larvae were doused with silver nanoparticles at varying concentrations (1, 10 and 100ppm). Observations revealed there was high mortality rate at 100ppm and moderate mortality rate at 10ppm during metamorphic transitions. In addition to the above evidence there was molecular confirmations were also done through the DNA fragmentation assay in the pupal ovaries and the same was authenticated with *Bm* actin marker gene amplification which also revealed the null amplification in 10 and 100ppm

Keywords: Nanotoxicity; Silver nanoparticles; *Bombyx mori*; DNA fragmentation; *Bm* actin

Introduction

Silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine. Nano silver is a commercial name for pure de-ionized water with superfine silver in suspension. Nanoparticles size is from 5 to 50nm. Most of the silver is in the form of metallic silver nanoparticles. The remaining silver is in ionic form. Because of the small size of the particles, the total surface area of the silver exposed in the solution is maximized, resulting in the highest possible effect per unit of silver [1]. As a result, a very small concentration of silver in Nanosilver provides greater effectiveness inside the body than silver solutions in the colloidal form of many times greater concentration. Nanosilver products are characterized by high percentage of silver metallic form. Silver Nanoparticles (AgNPs) are used in many economic sectors, but they are potentially toxic for living organisms. Nanotoxicology studies of the toxicity of nanomaterials, which can be divided into those derived from combustion processes, manufacturing processes and naturally occurring processes. Insects are experimental models used to estimate the toxicity of nanoparticles. However, the mechanism of AgNP toxicity remains unclear.

The AgNp was synthesized by *Morus alba* L. in biological method. It was diluted by different concentrations such as 50%, 75% and 100% (without dilution). Fresh mulberry leaves (*Morus alba* L.) were rinsed by each concentration and were fed to silkworms, 5th instars and Three feeding/day. In this study silkworm *Bombyx mori* was chosen as a model to measure the toxicity levels [2-4]. *Bombyx mori* is a renewed model to hold its package of innate immunity [5].

Materials and Methods

Biosynthesis of silver nanoparticles

The procedure followed for the preparation of the nanoparticles was the protocol used by Pandiarajan et al. [2]. 5 ml of 5% prepared aqueous solution was added to 95 ml of 1 mM aqueous Silver nitrate (Qualigens – 99.8%) solution separately for the reduction of silver ions. The temperature effects on the synthesis rate and particle size/shape of the prepared silver nanoparticles were studied by reactions that take place in the water bath at 60°C, 90°C and 95°C. The heated mixture of silver nanoparticle solution was then purified by repeated centrifugation at 10,000 rpm for 10 minutes. Then the supernatant is discarded and pellet was redispersed in deionised water to get rid of uncoordinated biological molecules present in it. The activity of centrifugation and redispersion in sterile distilled water was done repeatedly for three times to make sure that there is a better separation of free entities from the metal nanoparticles.

Study design

The study was carried out with 5th instar larva of mulberry silkworm *Bombyx mori* Linnaeus (Lepidoptera: bombycidae). The study was designed to examine the effect of silver nanoparticle synthesized using mulberry leaf extract on the fifth-instar larvae of B. mori. The fifth-instar larvae were chosen for supplementation studies because of the Diatery food consumption of fifth-instar larvae [6].

Selection of the effective concentration of silver nanoparticles

Freshly ecdysed fifth-instar larva were obtained from Sericulture field, Ariyavoor, Tiruchirapalli and used for all the experiments. The larvae were grouped into three groups and coherently two positive controls were also maintained. Each group contains 40 animals. Different concentrations of drug (silver nanoparticle) were prepared in distilled water by dissolving the lyophilized powder (10 ppm, 100 ppm, and 1000 ppm). The Silver nanoparticles treated leaves were used for feeding the V instars larvae of silkworm *Bombyx mori* [6].

Experimental Groups

The fifth instars of *Bombyx mori* larvae fed with the following MR2

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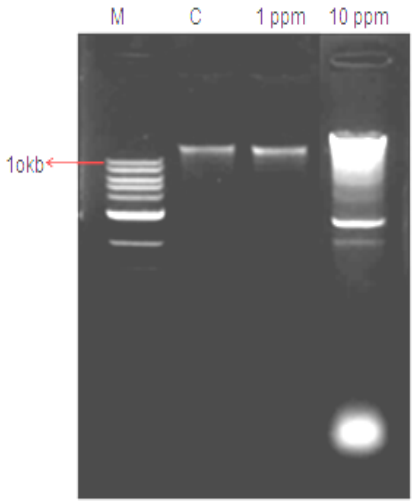

Figure 1: DNA fragmentation assay.

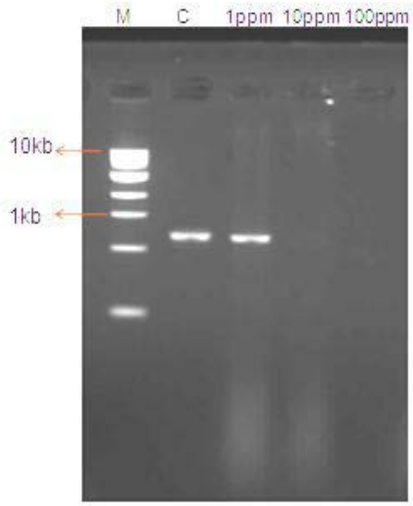

Figure 2: *Bm* actin amplification in Pupal ovarioles at different ppm levels.

mulberry leaves. Control group (C1) larvae fed with normal mulberry leaves, Control group (C2) larvae fed with Leaf extract treated MR2 mulberry leaves group T1 larvae fed with 10 ppm silver nanoparticles treated MR2 mulberry leaves, group T2 larvae fed with 100 ppm silver nanoparticles treated MR2 mulberry leaves and group T3 larvae fed with 1000 ppm silver nanoparticles treated MR2 mulberry leaves.

Genotoxicity analysis

The pupal ovaries of silk worm *Bombyx mori* was subjected DNA fragmentation assay and *Bm* actin amplification (Thermofischer Kit)

Results

The toxic effects of silver nanoparticles on silk worm Bombyx mori, was calculated through the bio-nanoparticles synthesized form *Morus alba* (mulberry) extract was used to Hence B. mori was a monophagous insect; the exact level toxicity created by nanoparticles can be evidenced through its own feed. The biosynthesized silver nanoparticles using *Morus alba* leaves extract proved excellent antibacterial activity. The antibacterial activity is well demonstrated by considerable zone of inhibition. The fifth instar larvae were doused with silver nanoparticles at varying concentrations (1, 10 and 100ppm). Observations revealed maximum larval weight was in 1ppm treatment, pupal weight was maximum in 100ppm. But there was high mortality rate at 100ppm and moderate mortality rate at 10ppm during larval-pupal transition and pupal-adult transition. The present study showed the interpretation of silver nanoparticle based toxicity at the levels of 10 and 100ppm (Figures 1 and 2). The molecular confirmations were done through the DNA fragmentation assay in the pupal ovaries (Figure 1), which exposed the fragmentation at 10 ppm level and at 100 ppm level the DNA was completely ruined out of the gel. The same DNA was authenticated with *Bm* actin marker gene amplification, which also revealed the null amplification in 10 and 100ppm respectively.

Discussion

Insects are one of the most precious creatures in the World; they are notable for their both optimistic and pessimistic paradigm towards the mankind. Due to their diverse behavior, existence and feeding habits, almost no terrestrial food source can escape the consumption by one or more insect species [7]. Silver nanoparticles an accumulative hazard reported by Pandiarajan et al. [2-4]. Sardari et al. [8] suggested that the effect of nanosilver particles on the tissues may cause organ toxicity in rats. Accumulation silver nanoparticles showed tissue damages, bloodshed, cell necrosis and apoptosis in rats. In adult zebra fish, there is evidence of the uptake of silver nanoparticles through the gills that caused silver nanoparticles- specific changes in gene expression and resulted in increased mortality. Increased mortality upon treatment with silver nanoparticles was observed in adult Japanese medaka (*Oryzias latipes*), accompanied by induction of DNA damage [9]. Silver nanoparticles were harmful towards *B. mori* by subsequent ways, lower the resistance to oxidative stress, affected cell apoptosis, and induced cell necrosis by regulating related protein metabolism and metabolic pathways [10].

References

1. Alt V, Bechert T, Steinrücke P, Wagener M, Seidel P, Dingeldein E, et al. An *in vitro* assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. Biomaterials. 2004; 25: 4383-4391.
2. Pandiarajan J, Jeyarani V, Balaji S, Krishnan M. Silver Nanoparticles an Accumulative Hazard in Silkworm: *Bombyx mori*. Austin J Biotechnol Bioeng. 2016; 3: 1057.
3. Pandiarajan J, Balaji S, Mahendran S, Ponmanickam P, Krishnan M. Synthesis and toxicity of silver nanoparticles. In Nanoscience in Food and Agriculture. 2016; 3: 73-98.
4. Pandiarajan J, Krishnan M. Properties, synthesis and toxicity of silver nanoparticles. Environmental Chemistry Letters. 2017; 15: 387-397.
5. Pandiarajan J, Cathrin BP, Pratheep T, Krishnan M. Defense role of the cocoon in the silk worm *Bombyx mori* L. Rapid Communications in Mass Spectrometry. 2011; 25: 3203-3206.
6. Rajathi A, Pandiarajan J, Krishnan M. Effect of RH-2485 on development, metamorphosis and synthesis of major proteins in female silkworm *Bombyx mori*. Biologia. 2010; 65: 903-913.
7. Pandiarajan J, Krishnan M. Comparative bacterial survey in the gut of lepidopteran insects with different bionetwork. Microbiology. 2018; 87: 103-115.
8. Sardari RR, Zarchi SR, Talebi A, Nasri S, Imani S, Khoradmehr A, et al. Toxicological effects of silver nanoparticles in rats. African Journal of

Microbiology Research. 2012; 6: 5587-5593.

9. Wu Y, Zhou Q, Li H, Liu W, Wang T, Jiang G. Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (*Oryzias latipes*) using the partial-life test. Aquatic Toxicology. 2010; 100: 160-167.

10. Meng X, Abdlli N, Wang N, Lü P, Nie Z, Dong X, et al. Effects of Ag nanoparticles on growth and fat body proteins in silkworms (*Bombyx mori*). Biological trace element research. 2017; 180: 327-337.

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