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Research Article

IN VITRO CYTOTOXICITY TESTING OF SILVER NANO-PARTICALS IN LYMPHOCYTE AND SPERM CELLS

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ABSTRACT
Silver Nano-particles because of their interesting characteristics are currently the most widely used nanoparticles. One of the interesting properties is that they display antimicrobial activity. Concomitantly they exert cytoprotective effect on human cells. It is of interest to validate and analyse this property.
In the present studies, silver nanoparticles were synthesised with homogeneous size distribution of 8-10nm in diameter. These were then used to analyse their uptake and consequent cytotoxic effects (interphase death) on human lymphocytes and sperms. Human lymphocytes; somatic cells taken from different individuals (males and females) showed variable response to silver nanoparticles at increasing time intervals i.e. 0, 30 and 60min (One of the individuals was allergic to silver). The sperm cells; germ cells obtained from different individuals (males) showed a different trend of cytotoxicity at the same concentration of silver Nanoparticles. The human sperm cells however exhibited lower cytotoxic response as compared to that of human lymphocytes. The studies highlight considerable differences in the cytotoxic response of human somatic and germ cells to silver nanoparticles.

Key Words: *Silver Nanoparticles, Cytotoxic Effects, Human Lymphocytes and Sperms*.

INTRODUCTION
Nanotechnology deals with structures in the size range of 1-100 nanometre. Nanoparticles are used in many bio applications such as therapeutics, (Kreuter and Gelperina, 2008) antimicrobial agents, (Yoon *et al.*, 2007) transfect ion vectors, (Tan *et al.*, 2007) and fluorescent labels (Su *et al.*, 2008) with their increasing application, there will be increase in the exposure of humans to Nanoparticles. Despite the rapid progress and early acceptance of nanobiotechnology, the potential for adverse health effects due to prolonged exposure to silver nanoparticles at various concentration levels in humans and the environment has not yet been established. However, the environmental impact of nanomaterials is expected to increase substantially in the future. In particular, the interaction of nanoparticles with cell organelles inside the cells is still an enigma, and no metabolic and immunological responses induced by these particles are understood so far.
Some of the applications have however caused huge concern for government and the public, since the potential risks of nano-silver has yet to be evaluated unambiguously. It is hypothesized that the toxic effects of silver nanoparticles are due to the combination of the specific properties of silver nanoparticles and the generation of ions from them. This makes it important to study the effects that silver nanoparticles might have on human health. Due to regulatory constraints and ethical considerations, the quest for alternatives to animal testing has gained new momentum. Therefore, mechanistically based *in vitro* tests addressing specific toxicological questions can yield new information for further research *in vivo*.

MATERIALS AND METHODS
Silver Nanoparticles were synthesized through a chemical reduction of 1mM silver nitrate using 20.5mM trisodium citrate as a reducing agent. (Ikramullah *et al.*, 2011) The preliminary goal was to study the cytotoxic effects (interphase death) of silver nanoparticles for human blood cells while reproductive cytotoxicity was assessed for human sperms *in vitro*. Lymphocytes were isolated from whole blood using

Ficoll Hypaque density gradient method and incubated with different concentrations of silver nanoparticles (1:9, 1:3, 1:1) at different time intervals of 30mins and 60mins. On the other hand, effects of silver nanoparticles on human sperms were assessed through one step eosin-nigrosin staining technique with similar concentrations of silver nanoparticles as mentioned above. The percentage viability was determined. The nanoparticles employed in this study were 8-10nm in size with an absorption maximum at 409nm. The calculated size distribution histogram confirmed the size distribution from electron microscopic observation. (Ikramullah *et al.*, 2011)

RESULTS AND CONCLUSION

Viability assays are vital steps in toxicology that explain the cellular response to a toxicant. Also, they give information on cell death, survival, and metabolic activities. Our experiments unveiled the *in vitro* cytotoxic effects of silver nanoparticles that were screened against human lymphocytes (somatic cells) and sperms (germ cells) presented in table 1A and 1 B and figure 1A and 1 B. We have exploited the microscope based assay to study the effect of Ag-np. The toxicity of silver nanoparticles showed a concentration and time-dependent drop in viability of human and sperm cells as compared to the controls (cells not treated with silver nanoparticles), signifying time and concentration dependent toxicity. The result is in proportion of viable lymphocytes and spermatozoa expressed in integral percentage.

Table 1a: Cytotoxicity of Silver Nanoparticles on Human lymphocytes after normalization.

Sample	Sex	Control % viability	1:9 % viability Treated		1:3 % viability Treated		1:1 % viability Treated	
			30mins	60mins	30mins	60mins	30mins	60mins
1	M	100	97 ±0.6	89 ±0.7	89 ±0.05	52 ±0.05	48 ±0.5	51 ±0.5
2	F	100	81 ±1	73 ±0.6	80 ±0.1	61 ±0.2	39 ±0.5	20 ±0.5
3	F	100	68 ±0.5	66 ±0.5	65 ±0.5	62 ±0.2	27 ±0.4	21 ±0.5
4	M	100	64 ±0.5	54 ±0.5	23 ±0.4	15 ±0.5	0 ±0.5	0 ±0.5
5	F	100	98 ±0.5	97 ±1	93 ±0.4	88 ±0.5	89 ±0.5	87 ±0.3

Table 1b: Cytotoxicity of Silver Nanoparticles on Human Sperms after normalization.

Sample	Control % viability	1:9 treated % viability		1:3 treated % viability		1:1 treated % viability	
		30mins	60mins	30mins	60mins	30mins	60mins
1	100	93 ±0.25	91 ±0.5	89 ±0.5	87 ±0.2	85 ±0.3	81 ±0.2
2	100	76 ±0.1	75 ±0.01	64 ±0.5	63 ±0.6	63 ±0.7	59 ±0.01
3	100	86 ±0.5	83 ±0.01	78 ±0.6	75 ±0.6	76 ±0.5	65 ±0.3
4	100	86 ±0.5	81 ±0.5	84 ±0.2	79 ±0.5	49 ±0.5	42 ±0.04
5	100	86 ±0.3	83 ±0.5	79 ±0.5	76 ±0.4	67 ±0.04	61 ±0.1

It is expected that the bio kinetics of nanoparticles, which is measured as the rate of nanoparticle uptake, intracellular distribution, and exocytosis, contribute tremendously to their toxicity. The nanoparticle size, surface area, and surface fictionalization are major factors that influence bio kinetics and thus toxicity.
Lymphocyte and sperms were exposed to at different dilutions of AgNp (1:10, 1:5 and 1:1) with time period of 30 and 60 min. Variable response of silver nanoparticles was observed for lymphocytes at different time intervals**.** Our results indicated that as the concentration increases with the time, toxicity ranges from 20±0.5 to 51±0.5. One individual showed 100% cytotoxicity even at 30mins incubation for

1:1 dilution. In contrast, another individual appeared to be relatively resistant to cytotoxicity for all the dilutions. Interestingly, the individual having high degree to sensitivity to silver Nanoparticles was found to be allergic to silver.

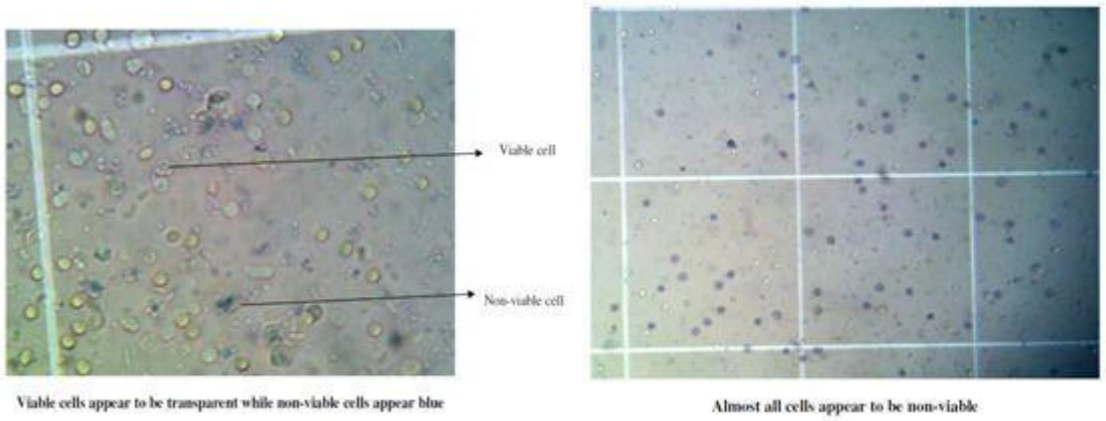

Figure 1a: Viability test using 0.4% Trypan Blue

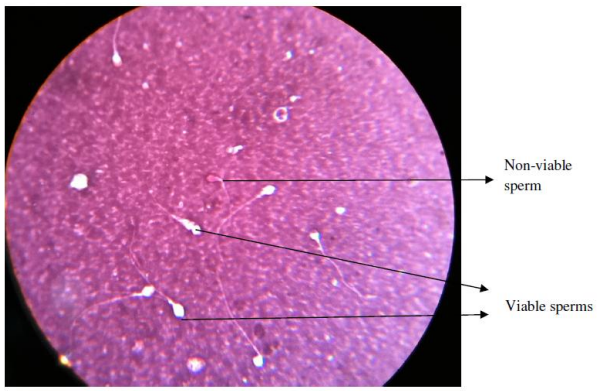

Figure 1b: Viability test using Eosin-nigrosin stain (0.67% Eosin Y, 10% Nigrosin and 0.9% Sodium Chloride)

The trend of cytotoxicity of silver nanoparticles observed for sperm cells that differed from that of lymphocytes at similar concentrations. The toxicity ranges from 93 ±0.5 to 42 ±0.04. The rate of killing appeared to be slower for sperms cells than for lymphocytes.

DISCUSSION

Silver nanoparticles undergo size and shape dependent interactions with bio systems. (Pal *et al.*, 2007; Elechiguerra *et al.*, 2005) Such extremely small nanoparticles have potential to penetrate the cell membrane because of their higher surface area to volume ratio. When size of the silver nanoparticles decrease, the percentage of interacting atoms at the surface increases, and this could probably be one of the reasons why small nanoparticles (1-10nm) are capable of interacting with biological systems. Silver Nanoparticles are widely used in day-to-day products such as lotions, ointments, toothpastes, toys, socks, etc. These day-to-day products utilized by humans in daily life could result in exposure to silver nanoparticles unknowingly thereby resulting in deposition of these particles inside the body causing harm to the normal cells. This provides an insight to study the effects of these particles on human cells.

Since, both lymphocytes and sperms are not actively metabolizing cells; the differential response to silver nanoparticles could be due to differential uptake of these two types of cells. Whether the observed lower cytotoxic response of sperm cells has any implication in the genotoxic effect of silver Nanoparticles is not yet known. The mechanism underline differential response of the lymphocytes and sperm cells to silver

Nanoparticles remains to be validated and investigated in more detail. In the present study, though the sample size was limited; it has brought up interesting leads for future study.

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