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Cytotoxicity of Silver Nanoparticles Synthesized With the Assistant of Microwave

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ABSTRACT

Silver nanoparticles (AgNPs) are broadly designed for various application. In the current research silver nanoparticles (AgNPs) have been examined for their poisonous outcome on human lymphocyte and chicken embryo fibroblast cell line. AgNPs were synthesized by a simple and easy method from $AgNO_3$ solution with glucose exposed to microwave radiation. The typical size of AgNPs were 30 nm with spherical shape. The cells which cultured with diverse concentrations of silver were examined for their viability using MTT assess, a photometric technique to conclude cell metabolism. The results show that the cell viability of the both cell type were diminished significantly ($P<0.05$) in dosage dependent mode, and the silver nanoparticles presented higher cytotoxicity to CEF than to human lymphocyte at the same concentration and time point..

Introduction:

In recent times nanotechnology have revolted the technological development and portended to be real one of the major technologies in the current century (1). Nanotechnology deals with structures in size vary of 1-100 nanometer (2). AgNPs have established notice owing to their physical, chemical and biological properties that credited to the catalytic activity and bactericidal effects and set up applications in nanobiotechnology investigate (3).

Gradually the attention in nanotechnology investigate is rapidly growing with the enlargement of diverse types of nanoparticles that used in diverse appliance, like treatment, diagnostic test, biosensors and additional products (5).

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AgNPs have been extensively used in numerous applications such as antimicrobial agents (4), in catheters (6), wound dressings as a topical creams to stop injury disease (7), anticancer agents (8), in clothing and cooking manufacturing (9) and as transfect ion vectors (10).

Silver nanoparticles can absorbed through the respiratory tract and arrive at the lymph stream and the blood circulation (11), for that reason they can be allocate all over the chief organs of the body, particularly the brain, kidney and liver (12). A number of researchers reveled that (AgNPs) are capable of bypass throughout blood-brain-barrier (13,14), as well as during cell membrane (15).

One of the most main nanoparticles properties in nanobiotechnology is there toxicity which can be inferred by size, shape, solubility, the chemistry of surface, chemical composition, and the activity of the

surface (16). Nonetheless AgNPs can promote a toxic reply in diverse mammalian cell lines, silver nanoparticle exposure resulted in diminished the function of mitochondria plus reducing the spermatogonial stem cells viability of the mice (17).

Chairuangkitti etal investigated in vitro toxic outcome of AgNPs on human lung carcinoma (A-549) cells and reported a visible connection to the creation of reactive oxygen species (18). AshaRani etal examined the toxic outcome of silver nanoparticles on human cells and genes, their consequences reveled dysfunction of mitochondria in addition to initiation of the reactive oxygen species caused by silver nanoparticles which led to DNA injure plus chromosomal abnormality (19).

As silver nanoparticles are now the most broadly used nanoparticles and their possible hazardous effects as shown by the previous studies so this investigate was doing to evaluate the cytotoxicity of biosynthesized Ag-NPs up on exposure to the human lymphocyte in comparison with CEF cell line. The observed results play a vital role in understanding the interactions between AgNPs and cell lines, by means of positive and ill effects on this cell line and determining the properties and concentration of nanobioparticles for further applications in future.

Material and method:

Synthesis of AgNPs

The silver nanoparticals were synthesis in line with the Tollens' method(20). AgNPs were 30 nm with spherical in shape.

Cell cultures:

Two types of cell cultures were used in the study, prime chicken embryo fibroblast cell (CEF), prepared from ten day aged chicken embryos maintained according to practice method, and human lymphocyte cells were preserved in RPMI media and the two cell types used for measuring of cytotoxicity of silver nanoparticles (21).

MTT assess:

The MTT lessening colorimetric assess was done in line with procedure mentioned by Mossmann (22) by way of a number of alterations. in short, following incubation, the nanocolloid accomplished media were exchanged with new serum rid media contain 0.7 mg/mL of MTT (3-(4, 5 dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma - Aldrich) , cells were incubated for three hours on 37ºC. Then, the cells were washed in the company of Phosphate buffer saline and 100 µL of DMSO (dimethylsulfoxide) were put in every well. later than 10 min of calm shaky, whole suspension was done. Optical density was read by a wavelength of 570. The viability of handle cells was expressed as a percentage of control cells viability.

Statistical analysis:

The Statistical analysis system SAS (2012) program was used to effect of concentration in study parameter (ANOVA),least significant difference LSD test was used to significant compare between means in this study(23).

Results:

Cytotoxicity of Ag-NPs on CEF and lymphocyte:

Cell viability of CEF and lymphocyte was quantified by means of MTT assay table 1 and 2 displays the cell viability of CEF and lymphocyte after (72) hours of exposure to Ag-NPs at concentration of 3.1-100 µg\ml.

Table1: Effect of Ag-NPs in Lymphocyte Viability: Ag-NPs synthesis by glucose and microwave

Concentration (µg/ml)	Mean ± SE
Control	0.216 ± 0.021
100	0.133 ± 0.010
50	0.185 ± 0.013
25	0.167 ± 0.003
12.5	0.180 ± 0.004
6.5	0.142 ± 0.004
3.1	0.184 ± 0.015
LSD value	0.0367 *
*(P<0.05)	

Table2: Effect of Ag-NPs in CEF Viability: Ag-NPs synthesis by glucose and microwave

Concentration (µg/ml)	Mean ± SE
Control	0.123 ± 0.010
100	0.085 ± 0.002
50	0.095 ± 0.007
25	0.108 ± 0.012
12.5	0.109 ± 0.007
6.5	0.115 ± 0.007
3.1	0.145 ± 0.005
LSD value	0.0245 *
*(P<0.05)	

The cell viability were decreased in a dose dependent manner (P<0.05). Cell viability of CEF were significantly lower than that of lymphocyte (P<0.05) at the same concentration and time point. (Table3).

Table3: Compare between CEF and Lymphocyte in Ag-NPs effect.

Concentration (µg/ml)	Mean ± SE		LSD value
	CEF	Lymphocyte	
Control	0.123 ± 0.010	0.216 ± 0.021	0.0645 *
100	0.085 ± 0.002	0.133 ± 0.010	0.029 *
50	0.095 ± 0.007	0.185 ± 0.013	0.041 *
25	0.108 ± 0.012	0.167 ± 0.003	0.035 *
12.5	0.109 ± 0.007	0.180 ± 0.004	0.024 *
6.5	0.115 ± 0.007	0.142 ± 0.004	0.0247 *
3.1	0.145 ± 0.005	0.184 ± 0.015	0.046 NS
*(P<0.05)			

This outcome point toward that CEF might be further susceptible to Ag-NPs treatment once relative to lymphocyte. Under inverted microscopy, Ag-NPs were seen in the cytoplasm of two cell types, solitary or clustered NPs were close to the cell membrane and internalized to cells, the NPs were scattered all over the cytoplasm, other than they were not seen in the nucleus of the two cell types,(Figures 1).

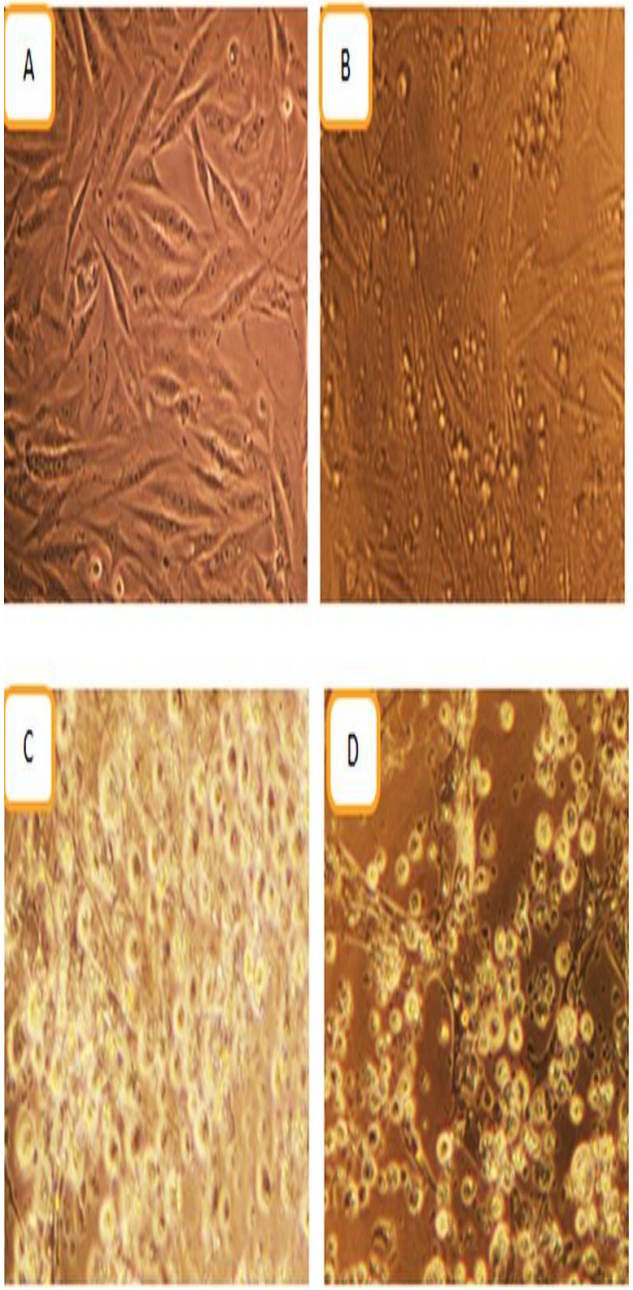

Figure (1) : Inverted micrographs of CEF before (A) and after (B) and human lymphocyte cells before (C) and after (D) treatment with Ag-NPs-1 at 30 μg/mL for 72 h. (100x).

Discussion:

The outcome of this study show that cell viability of CEF was more susceptible to Ag-NPs than lymphocyte provide facts intended for the cell type dependent reaction in biomaterials cytotoxicity check. Likewise Peng *etal* (24) reported that Ag-NPs decreased in human embryonic stem cell-derived fibroblast (EBF) viability further apparently when compared to L-929 cells. Ikramullah etal (2) indicate that the human sperm cells exhibited lower cytotoxic response as compared to that of human lymphocyte. The toxic effect of nanoparticles is greatly dependent on the organs, and more particularly the type of the cell, this is owing to the distinction in cell physiology (for example epithelial or lymphoid), proliferation state (tumoral or resting cells), membrane characteristics and phagocyte characteristics among different cell types (25). For example cancer cell are more resilient towards nanoparticle toxicity than normal cell due to an increased rate of proliferation and metabolic activity (26).

The chief nanoparticle consume possibilities in to the human body were through the skin, through the respiratory tract or through the gastrointestinal tract this uptake depended on not merely on the particle size plus charge but as well on the type of the cell (27).

This tiny nanoparticles have potential to penetrate the cell membrane because of their elevated surface area to volume ratio when size of the silver nanoparticles diminish, the percentage of interacting atoms at the surface enlarge giving them capable of interacting with biological system(2). in this manner nanoparticle shape, size and surface alteration take a central role in the allocation in the organism (1).

The dissimilar response of lymphocyte and CEF to silver nanoparticles could be due to dissimilar uptake of these two types of cells. However, embryos can be more sensitive to pollutants and environmental impacts than adults tissue (28) and this could probably be one of the reasons why cell viability of CEF were significantly lower than that of lymphocyte. Tedja *et al.* (29) found that the diverse level of biological reaction must be mainly qualified to the diversity in the total cellular particle consume among diverse cell types. Therefore, the cell-type-specific reaction of cells toward Ag-NPs at this point might be owing to that CEF are karyotypically plus genetically normal also show a stronger ability to consume NPs.

Reference:

1. Wagner S., Bloh J., Kasper C., and Bahnemann D. (2011); Toxicological issues of nanoparticles employed in photocatalysis. Green. 1(2): 171-188.

2. Ikramullah A., Salve D., Pai G., Rathora M., and Joshi D.S. (2013); In vitro cytotoxicity testing of silver nano-particles in lymphocyte and sperm cells. Indian Journal of fundamental and Applied Life Sciences. 3(1): 44-47.

3. Firdhouse M.J. and Lalitha P. (2015); Biosynthesis of silver nanoparticles. Journal of Nanotechnology. Volume 2015. Article ID 829526, 18 pages.

4. Sayantan M.N.V., Satheesh M., and Kumud U. (2016); Formulation and evaluation of bio-nanoparticulated drug delivery of Rivastigmine, World Journal of Pharmaceutical Sciences. 4(5): 264-272.

5. Yoon K.Y., Park J.H., and Hwang J. (2007). Susceptibility, constants of Escherichia coli and Bacillus subtilis to silver and copper

nanoparticles. Science Total Environment. (373): 572-575.

6. Samuel U., and Guggenbichler. (2004); Prevention of catheter related infections: the potential of a new nano-silver impregnated catheter. International Journal of Antimicrobial Agents. 23(1): 75-78.

7. Tian J., Wong K.K.Y., Ho C.M etal., (2007); Topical delivery of silver nanoparticles promotes wound healing. Chem Med Chem. 2(1): 129-136.

8. Krishnaraj C., Muthukumaran P., Ramachandran M., Balakumaran, and Kalaichelvan. (2014); Acalypha indica Linn: biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against MDA-MB-231, human breast cancer cells. Biotechnology Reports. 4: 42-49.

9. Vigneshwaran N., kathe A.A., Varadarajan P.V., Nachane R.P., and Balasubramanya R.H. (2007); Functional finishing of cotton fabrics using silver nanoparticles. Journal of Nanoscience and nanotechnology. 7(6): 1893-1897.

10. Tan W.B., Jiang S., and Zhang Y. (2007); Quantam- Dom based nanoparticles for targeted silencing of HER2\ neu gene via RNA interference. Biomaterials (28): 1565-1571.

11. Hussain N., Jaitley V., and Florence A.T. (2001). Recent advanced in the understanding of uptake of microparticles across the gastrointestinal lymphatics. Advanced Drug Delivery Reviews. 50(1): 107-142.

12. Tang J. xiong L. wang S.etal.,(2009). Distribution , translocation and accumulation of silver nanoparticles in rats .journal of nanoscience and nanotechnology.9(8):4932.

13. Kim J.S.(2006)Toxicity and tissue distribution of magnetic nanoparticles in mice.Toxicologcal sciences.9(1).338-347.

14. Sharma H.S., Hussain S. Schlager J.Ali S.F. and Sharma A.(2010). Influence of manoparticles on blood – brain barrier permeability and brain edema formation in rats . Acta Neurochirurgica . Supplement .106:359-364.

15. Kashiwada S.(2006).Distribution of nanoparticles in the see . through medaka (oryzias latipes). Environmental health perspectives .114(11):1697.1702.

16. Al-Ogaidi I. Salman M.I. Mohammad F.I. Aguilar Z. Al-Ogaidi M. Hadi Y.A. Abd Al-Rhman R.M.(2017). Antibacterial and Cytotoxicity of Silver Nanoparticles Synthesized in Green and Black Tea.

17. Braydich-stolle L.Hussain S.Schlager J.J.and Hofmann M.C.(2005). In vitro cytotoxicity of nanoparticles in mammalian germline stem cells .Toxicological Sciences.88(2):412-419.

18. Chairuangkitti P.Lawanprasert S.Roytrakul S.etal.(2013).Silver nanoparticles induce toxicity on AS94 cells via ROS-dependent and ROS-independent pathways. Toxicology in vitro.27(1).330-338.

19. Asharan.P.V.Mun G.L.K. Hande M.P. and valiyaveettil S.(2009). Cytotoxicity and genotoxicity of silver nanoparticles in human cells . ACS Nano.3(2):279-290.

20. Yin Y. Li Z. Zhong Z. Gates B. Xia Y. Venkateswaran S. (2002). Synthesis and Characterization of stable aqueous dispersions of silver nanoparticles through the Tollens process. J. Mater.Chem. Issue 3.

21. Freshney RI. Culture of Animal Cells: A Manual of Basic Technique. (2005).New York: Wiley-Liss,

22. Mosmann T.: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. (1983), 65, 55–63.

23. SAS.2012. Statistical analysis system. User's Guide. Statistical. Version 19thed. SAS. Inst.Inc.cory.N.C.USA.

24. Peng H., zhang X., wei y,Liu W.,Li S.,yu G., FU X., cao T., and Deng X.(2012). Cytotoxicity of silver nanoparticles in human embryonic stem cell. Derived fibroblast and an L-929 cell line . journal of nanoparticles. Article ID 160145. 9 Pages.

25. Diaz B. Sonchez .Espinel C.Arruebo M.etal.(2008). Assessing methods for blood cell cytotoxic response to inorganic nanoparticles aggregates .small.4:2025-2034.

26. Nan A.Bai X.Son SJ Lee SB,Ghandehari H.(2008). Celluar uptake and cytotoxicity of silica nanotubes .Nano Lett.8:2150-2154.

27. Zhang L.W.and. monteiro Riviere N.A.(2009).Mechanism of quantum dot nanoparticles culture uptake. Toxicological Sciences 110(1):138.155.

28. Malaczewska J., and Siw(CK) A.K.(2015)commercial metalbased nanocelloids evaluation of cytotoxicity. Bull Uet Inst pulawy.59-115-122.

29. Tedja, R., Marquis C., Lim M., and Amal R., “Biological impacts of TiO2 on human lung cell lines A549 and H1299: particle size distribution effects,”(2011).Journal of Nanoparticle Research, vol. 13, no. 9.

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سمية جزيئات الفضة النانوية المصنعة باستخدام المايكرويف

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الخلاصة:

تستخدم جزيئات الفضة النانوية في تطبيقات عديدة تم دراسة التأثير السمي لجزيئات الفضة النانوية على نوعين من الخلايا الطبيعية وهي خلايا اللمفاوية للإنسان وخلايا جنين الدجاج المولدة للالياف. صنعت جزيئات الفضة النانوية بحجم 30 نانوميتر وبشكل كروي وبطريقة بسيطة باستعمال محلول من نترات الفضة والكلوكوز المعرض للمايكروويف. تم دراسة نسبة بقاء الخلايا المعاملة بتراكيز مختلفة من جزيئات االفضة النانوية باستخدام طريقة الـ MTT وهي عبارة عن طريقة طيفية لقياس ايض الخلايا. اظهرت النتائج ان نسبة بقاء الخلايا لكلا النوعين تقل وبصورة معنوية كلما زاد تركيز جزيئات الفضة النانوية ($P<0.05$), كما اتضح ان التأثير السمي لجزيئات الفضة النانوية هو أعلى معنوياً ($P<0.05$) على خلايا المولدة للالياف لجنين الدجاج مقارنة بالخلايا اللمفاوية للانسان.