

Original Research Article

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An Experimental Study on Cell Mediated Immune Response in Nano Alumina (Aluminium oxide/Boehmite) Treated White Leghorn Chickens

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ABSTRACT

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Nanomaterials can be composed of many different base materials (carbon, silicon, and metals such as gold, cadmium, aluminium and selenium). Generally the nano-particles are part of volcanic eruptions, forest fires, fumes generated during welding, metal smelting, automobile exhaust and other industrial processes. The present study was conducted to understand the effects of nano alumina as cell mediated immune response on forty White leghorn chickens of two week age. The chickens were randomly divided into two groups control (Group I) and treated (Group II). The control group was fed with normal standard recommended feed while treated group exposed to nano alumina through oral route in feed for three months. RO water was provided to both the groups. The cell mediated immune response was seen at the end of experiment, taking random samples from ten birds from each group. The results in this study revealed that delta optical density of LST (Lymphocyte Stimulation Test) values showed no significant change while the mean values of treated group was 18.29 % increased as compared to control group at 90th days post treatment.

Introduction

Nanotechnology is the convergence of engineering and molecular biology, leading to the development of structures and systems that have novel functional properties (Seetharam and Sridhar, 2007). The special physico-chemical properties of engineered nanoparticles are exploited in a broad range of applications as diverse as cosmetics, pharmaceuticals, textiles, electronics, biosensors and catalysts (Handy *et al.*, 2008). Nanoparticles (NPs) have unprecedentedly

different physiochemical characteristics than their bulk materials and thus present possible threats, both medically and environmentally (Ambwani *et al.*, 2015).

Nanotechnology, leads to the development of devices, structures and systems that have novel functional properties with size ranging between 1 and 100 nm by the convergence of engineering and molecular biology (Seetharam and Sridhar, 2007). Nanomaterials (NMs) can be composed of various base materials like- carbon, silicon,

gold, cadmium, and selenium. Nanotubes, nanowires, crystalline structures (such as quantum dots), and fullerenes are different shapes of Nanomaterials. In many types of nanoparticles, 50 to 100% of the atoms can be present on the surface, resulting in greater reactivity of NMs than bulk materials. Nanotechnology was used by ancient Indians is well discussed by Sir Walter Scott in his book "Talisman". In 'Charak Samhita' the concept of reducing size of particles is well explained. Utmost reduction in particle size of metals and non metals is termed as nanotechnology.

Nanomaterials were used as the 'bhasmas' of metals encapsulation with herbal molecules in Ayurvedic system of medicine to cure various human diseases (Chauhan *et al.*, 2010). Particles of nano-size range are part of the volcanic eruptions, forest fires, metal smelting, fumes generated during welding, exhaust from automobiles and many other industrial processes. Aluminium occurs as a natural constituent of drinking water, fruits and vegetables. Aluminium oxide is used for manufacturing sheath tubes, crucibles, dishes, insulators, artificial joints, dental ceramics, catalyst carriers, burner tubes, wear protections, furnace linings, hard facings and machine tools.

Nanostructures can enter the body through various routes such as intra-venous, dermal, subcutaneous, intraperitoneal, inhalation and oral. They enter in the cells of the organ and remain there for an unknown duration before getting excreted or leaving to other organs (Fischer and Chan, 2007). Interaction with biological systems can give rise to toxic effects like allergy (Maynard *et al.*, 2006), tissue and DNA damage (Nel *et al.*, 2006), ROS generation (Meng *et al.*, 2007), fibrosis, deposition in different organs that can lead to organ failure, inflammation and cytotoxicity (Singh *et al.*, 2009). Nanomaterials may

change the mineral concentration beyond the alarming limits. Nano dust, will be a danger to environment, constitutes by nano size particles present in the e-waste (Sharma, 2010). Occupational health studies have shown that finest aluminum powder can cause pulmonary fibrosis under unfavorable industrial conditions. The disease aluminosis has been approved as such, in Germany and workers have been recompensed for the health related issues since 1943 (Kraus *et al.*, 2000). Keeping in view the above facts, the present study has been planned to study the effects of nano alumina intoxication as cell mediated immune response in White Leghorns Chickens.

Materials and Methods

Forty layer chickens of White Leghorns of one week age (average body wt. ~ 85 g) were procured from Instructional Poultry Farm, Govind Ballabh Pant University of Agriculture and Technology, Nagla, Pantnagar, Udham Singh Nagar, Uttarakhand, India and brought to the experimental shed, where they were maintained on deep litter system under proper light, maintaining good hygienic conditions. The chickens of White Leghorns were supplied with *ad-libitum* of standard poultry feed and RO water throughout the experiment. The bedding material of experimental shed was replenished on alternate days during acclimatization period (7days) and then weekly throughout the experiment. The birds were vaccinated against Ranikhet disease at 5th day using F1 strain vaccine (Indovax Pvt. Ltd, Hisar) through oral route. The birds were vaccinated orally for Infectious Bursal Disease at 11th day using live intermediate strain, Bursa B2k (Indovax Pvt. Ltd, Hisar). At 37th day of age, birds were revaccinated for Ranikhet disease using Lasota strain vaccine (Indovax Pvt. Ltd, Hisar) through oral route. Birds were administered a booster dose against Ranikhet

disease at 58th day of age using Lasota strain vaccine (Indovax Pvt. Ltd, Hisar) by wing web prick method. The present study conducted on the chickens was following all the rules and regulation of the Animal Ethical Committee of the University. The birds were randomly divided into two groups of 20 birds each to study the cell mediated immune response of nano alumina. First group was kept as control and given normal standard recommended feed. Second group was fed with maximum tolerable limit (200 ppm) (National Research Council, 1980) of nanoalumina in feed to birds of two weeks age till 90 days post treatment (DPT). The chickens were kept under observation in separate pens. All chickens from each group were sacrificed using standard ethical procedures at the end of trial. Cell mediated immune response was assessed using lymphocyte stimulation test from five chickens from each group at the end of experimental trial using heparinised peripheral blood as per the method of Rai-el-Balhaa *et al.*(1985) and modified by (Chauhan, 1998).

Mitogen

For the stimulation of T cell blastogenesis, Conavalin A (Con-A) was used as the mitogen. Con-A was dissolved in RPMI-1640 cell culture media at a concentration of 5 µg/ml for stimulation of T-lymphocyte cells.

Separation of lymphocytes

Lymphocytes used in this study were obtained from freshly collected blood. Three ml of blood was collected under strict hygienic conditions with the help of disposable syringe from wing vein in sterilized vials containing heparin (125 IU/ml) as anticoagulant. The collected blood was diluted with equal volume of RPMI-1640 media. This diluted blood was then layered carefully over three

ml of histopaque (Hisep LSM 1084) so as to avoid mixing of the blood and histopaque. Then the test tubes were centrifuged at 400 g for 30 minutes. The mixture was separated in three layers, a lower compact layer of RBC, an upper layer of serum & histopaque and a middle layer of lymphocytes and mononuclear cells. This middle buffy layer was carefully aspirated with the help of sterilized Pasteur pipette and further washed with RPMI-1640 twice. The cell viability was examined using trypan blue dye exclusion test (Boyse *et al.*, 1964). The final concentration of the cells was adjusted to be 1×10^6 cells per ml of RPMI-1640 medium. To the final cell suspension (3ml), 10 % fetal calf serum (300µl) was added.

Test procedure

For the estimation of lymphocyte blastogenesis, 96 well, flat bottom tissue culture plates (Cellstar, Grenier bio-one) were used. Each sample was used in triplicate and the mean of these values was used as the delta OD for the given sample. For each sample there was a control, also in triplicate for comparison. In the triplicate wells of tissue culture plate, 100 µl of cell suspension, 50 µl of media and 50 µl of Con-A (5µg/ml) as mitogen were added. 100 µl of media alone was added in control wells.

After loading, the plates were sealed with parafilm (Pechiney plastic packaging) and incubated at 37⁰C for 24 hours in CO₂ incubator (Forma Scientific) with 5% CO₂ pressure. After completion of 24 hours of the incubation period, 10 µl of MTT (5 mg/ml, HiMedia) was added to each of the well (EZcountTM MTT Cell Assay Kit, HiMedia). The plates were again incubated for 4 hours. Cells were observed at periodic intervals under an inverted microscope for the presence of intra cellular needle-shaped, dark purple coloured precipitate. When the purple

precipitate is clearly visible under the microscope, add 100 µl of solubilization solution (EZcount™ MTT Cell Assay Kit, HiMedia) to the wells and stir gently to enhance dissolution of the dark blue crystals and also to stop the reaction of cells with MTT. The plate was then subjected to microspectrophotometer to read the optical density (OD) at 570 nm. Triplicate sample wells were averaged to get the delta OD of a sample. Mean of the control wells was taken as the delta OD of control. The delta OD was plotted on graph as bar diagram for the estimation of T-lymphocyte blastogenesis.

The test compound

Nano alumina, of commercial grade (Sisco Research Laboratories Pvt. Ltd) used in the study was procured from local market. The Nano alumina used as aluminium oxide (Boehmite) with characterization; nano dispersion (50nm), APS (Aerodynamic particle size): 50nm, pH: 4, specific gravity: 1.19, viscosity: 10cps (centi poise), Molecular weight: 59.99.

Statistical Analysis

The data generated during the experiment was statistically analysed by using standard statistical procedures (Snedecor and Cochran, 1994) with the help of SPSS software. The collected data was analyzed by two sample t-test.

Results and Discussion

The present study was undertaken to study the cell mediated immune response of nanoalumina in white leghorn chickens at maximum permissible dose after an exposur for three months. The finding is that the mean values of delta optical density of LST in different groups of experimental chickens at the end of experiment (90th DPT) are presented in Table 1 and Fig. 1 and 2 showing lymphocyte stimulation test. Delta optical density of LST values showed no significant change. The mean value of treated group was 18.29 % increased as compared to control group at 90th DPT.

Table.1 Mean delta optical density of lymphocyte stimulation test (LST) in different groups of experimental chickens (Mean ± SE)

Group	Delta OD at 90 DPT
Control (Group I)	0.82 ± 0.07
Treated (Group II)	0.97 ± 0.08 (18.29%)

Fig.1 Photograph of lymphocyte stimulation test showing different colour intensity with respect to T- lymphocyte blastogenesis

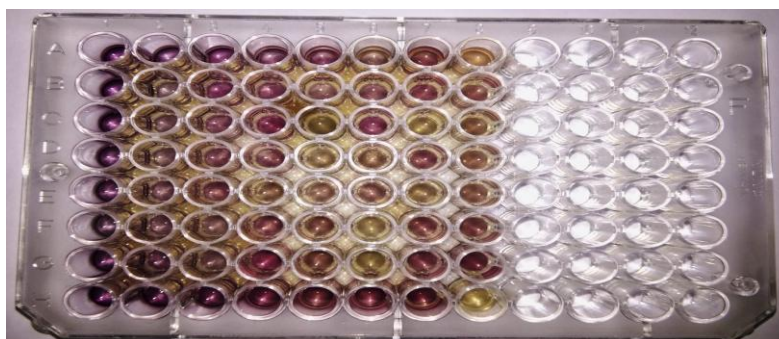
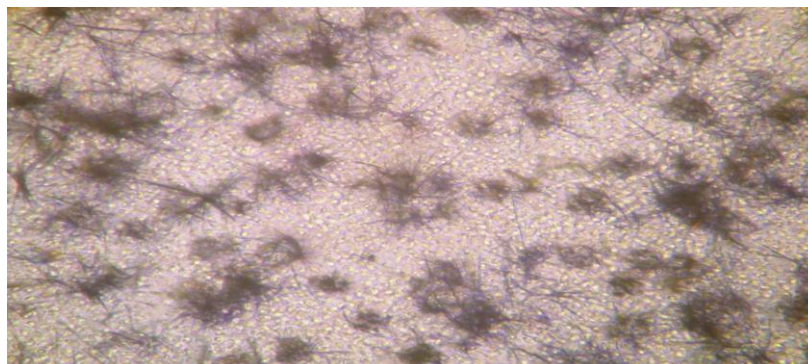


Fig.2 Photograph of lymphocyte stimulation test showing formation of violet colour needle like formazan crystals after two hours of adding MTT dye



The results of present study revealed an increase in cell mediated immune response when compared between treated and control group. However, the results are not significant. Results of present study are in agreement with the result of Athari *et al.*, (2016) in mice, Khalaf *et al.*, (2008) in rats, and Graske *et al.*, (2000) in humans. Where as Hu *et al.*, (2013) in rats, She *et al.*, (2012) in rats, Synzynys *et al.*, (2003), Zhang *et al.*, (2013) in cultured splenic lymphocytes, Zhu *et al.*, (2012) in rats, reported that T and B lymphocyte proliferation and IgG, IgM, IgA decrease in aluminium treated groups. Zhu *et al.*, (2011) reported decrease in IgM and increase in IgA & IgG in aluminium treated group of rats. The cell mediated immune response may not be significant statistically but certainly indicates the pathological effects of nanoalumina. Actually nanoparticles have affinity to bind certain body protein molecules even at lower dose because of its small size as compare to bulk material and then elicit immune response in body, leading to anti immunity and hypersensitivity, which takes time to develop.

Significance

The use of nanomaterials is being enhanced many folds during last decade and scientists continuously, exploring this field for various domestic use besides its applications in

cosmetics, drugs, feeds, cloths, dyes, purifiers, defense etc. with the advancement of nanoscience and nanotechnology, their toxic and pathological effects are not much clear. If was, therefore planned this study to see the effects of nanoalumina at a lower dose rate (200 ppm) in chickens with the main objective to establish the pathology of nanoalumina and develop chicken as model. The effects of nanoalumina were studied for a period of 90 days, as subacute study which revealed that there is cell mediated immune response even at lower dose. It is therefore, proposed that further studies should be carried out in different animal models using varied doses and increased duration to exactly find out the pathological alterations.

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References

- Ambwani, S., Tandon, R., Gupta, A., Ambwani, T.K. and Chauhan, R.S. 2015. Nanoparticles: Utility, Immuno-

- Toxicology and Ethical Issues. *Journal of Immunology and Immunopathology*. 17(2): 68-78.
- Athari, S.S., Pourpak, Z., Folkerts, G., Garssen, J., Moin, M., Adcock, I.M., Movassaghi, M., Ardestani, M.S., Moazzeni, S.M. and Mortaz, E. 2016. Conjugated Alpha-Alumina nanoparticle with vasoactive intestinal peptide as a Nano-drug in treatment of allergic asthma in mice. *European journal of pharmacology*. 791: 811-820.
- Boyse EA, Old LJ and Chouroubnkov I. 1964. Cytotoxic test for determination of mouse antibody. *Methods Med Res*. 10: 39-47.
- Chauhan RS, Sharma G and Rana JMS (2010). Nanotechnology: Ancient Indian Scenario. In: *Nanotechnology in Health and Disease*. (Eds: RS Chauhan, Gagan Sharma and JMS Rana). IBT, Patwadangar. pp. 12-15.
- Chauhan, R. S. 1998. *Laboratory Manual of Immunopathology*. 96 pp. G.B. Pant University of Agriculture & Technology. Pantnagar.
- Fischer, H. C. and Chan, W. C. 2007. Nanotoxicity: the growing need for in vivo study. *Current opinion in biotechnology*. 18: 565-571.
- Graske, A., Thuvander, A., Johannisson, A., Gadhasson, I., Schütz, A., Festin, R. and Glynn, A.W. 2000. Influence of aluminium on the immune system—an experimental study on volunteers. *Biometals*. 13(2): 123-133.
- Handy, R. D., Owen, R. and Valsami-Jones, E. 2008. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. *Ecotoxicology*. 17: 315-325.
- Hu, C., Li, J., Zhu, Y., Bai, C., Zhang, J., Xia, S. and Li, Y. 2013. Effects of Al on the splenic immune function and NE in rats. *Food and chemical toxicology*. 62: 194-198.
- Khalaf, A.E., Morgan, A.M., Mekawy, M.M. and Ali, M.F. 2008. Immunotoxicity following pre-and post-natal aluminum exposure in rats. *Toxicological Research*. 24(1): 51-58.
- Kraus, T., Schaller, K.H., Angerer, J. and Letzel, S. 2000. Aluminium dust-induced lung disease in the pyro-powder-producing industry: detection by high-resolution computed tomography. *International archives of occupational and environmental health*. 73(1): 61-64.
- Maynard, A. D., Aitken, R. J., Butz, T., Colvin, V., Donaldson, K., Oberdörster, G., Philbert, M. A., Ryan, J., Seaton, A., Stone, V. and Tinkle, S. S. 2006. Safe handling of nanotechnology. *Nature*. 444: 267-269.
- Meng, H., Chen, Z., Xing, G., Yuan, H., Chen, C., Zhao, F., Zhang, C. and Zhao, Y. 2007. Ultrahigh reactivity provokes nanotoxicity: explanation of oral toxicity of nano-copper particles. *Toxicology letters*. 175(1): 102-110.
- National Research Council. 1980. *Mineral Tolerance of Domestic Animals*. National Academy Press, Washington, DC.
- Nel, A., Xia, T., Madler, L. and Li, N. 2006. Toxic potential of materials at the nanolevel. *Science*. 311: 622-627.
- Rai-el; Balhaa, G., pellerin, J. L., Bodin, G. and Abdulla, A. 1985. Lymphoblastic read technique. *Comparative Immunology, Microbiology & Infectious Diseases*. 8: 311-318.
- Seetharam, R. N. and Sridhar, K. R. 2007. Nanotoxicity: threat posed by nanoparticles. *Current science*. 93: 769-770.
- Sharma KK (2010). Nanodust particles as environmental pollutants. In: *Nanotechnology in Health and Disease*. (Eds: RS Chauhan, Gagan Sharma and JMS Rana). IBT, Patwadangar. pp. 66-

- 78.
- She, Y., Wang, N., Chen, C., Zhu, Y., Xia, S., Hu, C. and Li, Y. 2012. Effects of aluminum on immune functions of cultured splenic T and B lymphocytes in rats. *Biological trace element research*. 147(1-3): 246-250.
- Singh, N., Manshian, B., Jenkins, G. J., Griffiths, S .M., Williams, P. M., Maffeis, T. G., Wright, C. J. and Doak, S. H. 2009. NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. *Biomaterials*. 30: 3891-3914.
- Snedecor, G .W. and Cochran, W .G. 1994. Statistical Methods (Eighth edition), *Journal of Educational and Behavioral Statistic.*, 19(3): 304-307.
- Synzynys, B.I., Sharetskiĭ, A.N. and Kharlamova, O.V. 2003. Immunotoxicity of aluminium chloride. *Gigiġena i sanitaria*. (4): 70-72.
- Zhang, J.H., Hu, C.W., Zhu, Y.Z., Liu, S.M., Bai, C.S., Han, Y.F., Xia, S.L. and Li, Y.F., 2013. Effects of norepinephrine on immune functions of cultured splenic lymphocytes exposed to aluminium trichloride. *Biological trace element research*. 154(2): 275-280.
- Zhu, Y., Xu, J., Sun, H., Hu, C., Zhao, H., Shao, B., Bah, A.A. and Li, Y. 2011. Effects of aluminum exposure on the allergic responses and humoral immune function in rats. *Biometals*. 24(5): 973-977.
- Zhu, Y., Hu, C., Li, X., Shao, B., Sun, H., Zhao, H. and Li, Y. 2012. Suppressive effects of aluminum trichloride on the T lymphocyte immune function of rats. *Food and chemical toxicology*. 50(3): 532-535.

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