Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 21(2) May-August 2023]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology, Pantnagar

PANTNAGAR JOURNAL OF RESEARCH

Vol. 21(2) May-August, 2023

CONTENTS

Effect of aluminium nano particles on humoral immune response of wistar rats

SHODHAN K.V, SEEMA AGARWAL* and R S CHAUHAN

Department of Pathology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar-263 145 (U. S. Nagar, Uttarakhand)
**Corresponding author's email Id: seema_patho@rediffmail.com*

ABSTRACT: Animals and man are inadvertently exposed to various man-made or natural forms of nano particles due to their presence in the micro-environment. Limited investigations have been carried on effect of aluminium nanoparticles on immune response and health status of animals and man. Therefore, present study was designed to study the effect of aluminium nano particles on humoral immune response of Wistar rats. For this study, a total of 35 rats of six-week age were divided randomly into two groups of 20 rats in Group I (control) and 15 rats in Group II (treated). The control group fed with normal standard recommended feed while treated group exposed to aluminium nano particles orally at NOAEL dose viz.6 mg/kg BW per day for a period of 90 days. Humoral immune response was assessed by hemagglutination (HA) and hemagglutination inhibition (HI) assays and also by performing ELISA from the serum separated from blood of 5 rats from each group at 30th, 60th and 90th DPT. The antigen used was reconstituted commercial Ranikhet Disease Vaccine, R2B strain vaccine Mukteshwar (Indovax Pvt. Ltd., Hissar). There was increase in humoral immune response in treated group but the results were not significant statistically.

Key words: Aluminium, health, humoral immune response, nano particles, Wistar Rats

Nanotoxicology deals with the interactions of nanoparticles with biological systems with an attention on the relationship between the nanoparticles physicochemical properties like; shape, size, surface chemistry and aggregation with development of toxic responses in biological system (Maynard *et al.*, 2006). Human exposure to nanoparticles is mainly via inhalation, dermal absorption, subcutaneous, gastrointestinal tract absorption and intravenous route (Oberdörster *et al.*, 2005). Nanomaterials in comparison to their micro-scale material, impose different biological effects, due to their unique physicochemical properties such as small size and relatively larger specific surface area (Nel *et al.*, 2006). According to Oberdorster *et al.* (2005), the size of the particles is not only the factor that causes changes in the biological activities of materials at the nanoscale but also the characteristics like; size, distribution, crystal structure, agglomeration state, shape, surface area, porosity, chemical composition, surface chemistry and surface charge are linked to nanotoxicity. The high surface reactivity of the nanoparticles is mainly responsible for negative health and environmental impacts (Colvin, 2003). Nanoparticles because of the smaller size can easily undergo cellular uptake as well as transcytosis across endothelial and epithelial cells into the blood and lymph circulation and thus can easily reach sensitive target organs such as bone marrow, brain, spleen, lymph nodes and heart (Colvin, 2003; Campbell *et al.*, 2004). Nanoparticles (NPs) with size less than 20 nm enter circulatory system and thus, may cause cardiovascular diseases including atherosclerosis and high blood pressure, thrombi formation due to either disruption in the function or structure of vascular endothelium or by activation of the blood coagulation cascade (Yildirim *et al.*, 2013). Unlike bulk materials, nanoparticles pass through various biological barriers i.e., blood–brain barrier (BBB), blood–testis barrier, placental barrier (Zoroddu *et al.*, 2014; Bakand and Hayes, 2016; Muoth *et al.*, 2016). Thus, NPs accumulate in various tissues and organs inducing negative health effects (Almeida *et al.*, 2011). Nanoparticles also undergo translocation along dendrites and axons of neurons gaining access to the CNS and ganglia (Oberdorster *et al.*, 2005; Nel *et al.*, 2006). Thus, different nanoparticles gets deposited in their target organs, penetrate through cell membranes, and gets lodge in mitochondria or nuclei. This triggers a range of injurious responses at the cellular, subcellular, and protein levels. Nanoparticles interacts with

lymphocytes and other immune cell types leading to inflammation, immunomodulation and hypersensitivity (Ambwani *et al.,* 2015). Overall, above facts makes it necessary to study toxicity and impact of nanoparticles on environment in a systematic manner. Keeping in view the above facts and due to the scarcity of information about the effect of Al_2O_3-NPs, the present study has been planned to observe the effects of nanoalumina in humoural immune response of Wistar rats.

MATERIALS AND METHODS

Five weeks old Wistar Rats of both sexes were procured from Laboratory Animal Resources, Indian Veterinary Research Institute, Izatnagar, Bareilly, India. The rats were kept in Experimental Animal House of Department of Veterinary Pathology, Pantnagar and were fed standard recommended feed and RO water ad-libitum from 0 day upto 90 days of course of experiment. Rats were kept in cages, two rats per cage, in the rats room, which was maintained at a temperature of 21.0°C–23.1°C and good hygienic conditions. All rats were acclimatized for 7 days. The rats were immunized with Ranikhet Disease Vaccine R2B strain at every 15 days interval from 0 day (six week of age) upto 90 days of course of experiment period. The present study conducted according to the rules and regulation of the Animal Ethics Committee of the University. Total 35 rats were divided into 2 groups to study the effects of nano aluminium in humoral immune response. Control group comprised of 20 rats and Test group comprised of 15 rats. The latter were administered nano aluminium by oral gavaging at NOAEL dose of 6.0 mg/kg body weight for 90 days (Park *et al.*, 2015). 5 rats each from both group were sacrificed on day 0 (only control rats), 30, 60 and 90 days post treatment. All rats from each group were sacrificed using standard ethical procedures. Humoral immune response was assessed by hemagglutination (HA) and hemagglutination inhibition (HI) assays and also by performing ELISA from the serum separated from 5 rats from both group at 30th, 60th and 90th DPT using HA-HI titer as described by Allan and Gough (1974). Indirect ELISA was performed for antibody assay in the serum sample collected at 0, 30, 60 and 90 DPT as per the standard procedure described by Joshi and Chauhan (2012). The antigen used was reconstituted commercial Ranikhet Disease Vaccine, R 2 B strain vaccine Mukteshwar (Indovax Pvt. Ltd., Hissar).

The test compound, aluminium oxide (Boehmite), of commercial grade was purchased from Sisco Research Laboratories Pvt. Ltd for the study. It has a nano dispersion of 50 nm and M.W: 59.99, pH 4, Specific gravity: 1.19, Viscosity: 10cps (Sisco Research Laboratories Pvt. Ltd.). The nanoparticles were diluted in distilled water and working samples of recommended dose formulation was prepared daily during the entire period of study. The nanoparticles were homogenized by vortexing just prior to administration and were given orally once daily for 90 days.

HA Procedure: In this procedure, 50µl NSS was added to each well of U-bottom 96 well micro titre plate except wells of first column. In first 3 wells of first column 100µl of 1/10 virus sample (10 µl virus + 90 µl NSS), in next three wells 2/10 virus sample and in last 2 wells of first column 3/10 virus sample was added. The constituents of each well were mixed and 50µl was transferred to the next well on its right. Again, the constituents were mixed and 50µl was transferred down the length of the plate and 50µl was discarded from the second last well. Then 50µl of 0.5% RBCs suspension was added to each well and mixed gently. Then the plate was leave at room temperature for 30 minutes.

The titre (HA titre) was calculated as simple number of the highest dilution factor that produced a uniform reddish color (matt) across the well.

1 Hemagglutination Unit (HAU) = highest dilution giving complete hemagglutination.

HI Procedure: Constant virus concentrations and diluted serum were used in this procedure. 50 µl PBS was added each well. Then 50 µl of serum was added from each sample into the respective wells in the first column of V-bottom 96 well micro titre plate using micropipette. After mixing the 50 µl of its

content was transfer to the next well on its right. Then transfer of 50 µl was repeated down the length of the plate and 50µl from the last well was discarded. After that, 50 µl virus suspension containing 4 HA unit virus was added to each well and was mixed well by manual shaking and the plate was incubated at 37°C for 1 hr. Finally, 50 µl of 1 % chicken erythrocytes added to each well and after gentle mixing the plate was kept at 40C for 30-60 minutes for the settlement of RBCs. Haemagglutination inhibition appeared with button formation. Matt formation indicates lack of haemagglutination inhibition. The results were observed after 1hr.

HI titre = The reciprocal of the end point showing complete inhibition of haemagglutination is directly expressed as HI titre of the serum.

Enzyme Linked Immunosorbant Assay

Indirect ELISA was performed for antibody assay in the serum sample collected at 0, 30, 60 and 90 DPT as per the standard procedure described by Joshi and Chauhan (2012).As per the procedure, 100µl of the antigen diluted in 0.05 M carbonate bicarbonate buffer of pH 9.6 was coated into the wells of the polysterene plate and incubated at 4°C under refrigeration for overnight. Next day, the content was discarded and the plate was washed 3 times for 5 minutes each using washing solution which constitute 0.2M PBS containing 0.05% tween-20 solution. After each washing, the plate is tapped gently against any soft cloth or paper to get rid of unabsorbed antigen on the well. The unbounded sites were then immobilized using 5% skim milk solution following incubation of the ELISA plate for 1 hour at 37°C. After this, entire content was removed from the plate via washing 3 times for 5 minutes each and tapping of the plate after each wash as already described. After this 100µl of serum (1:1000 dilution) was added to the polysterene plate and incubated at 37°C, followed by washing and taping procedure as described earlier. Following the protocol, 100µl of Anti rat IgG conjugate (1:1000 dilution) was added in each well and incubated for 1 hour at 37°C, followed by washing and taping procedure as described earlier. Finally, Orthophenylene diamine dihydrochloride (OPD) substrate was used as 8 mg OPD dissolved in 15 ml of 0.1 M citric acid phosphate buffer containing 5µl 30% hydrogen peroxide. 100 µl of this freshly prepared substrate was added to each well and then the

plate was incubated. The reaction was stopped after 30 minutes by stopping reagent using 100 µl of 1M H_2SO_4. The absorbance was then read at 450 nm using UV-Vis spectrophotometer.
Calculation for ELISA values was done by the following formula:

ELISA value = OD of Test /OD of Control

RESULTS AND DISCUSSION

Hemagglutination inhibition test (HI)

Mean HI titre of the experimental rats from GI (control) and GII (treatment) groups were calculated at 30 days intervals and is expressed as log2 and presented in Table 1 and Fig1. Mean HI titre of GI group was 9.2±0.37, 8.2±0.37 and 8.4±0.24 at 30th, 60th and 90th DPT, respectively. Mean HI titre of GII group was 7.4±0.24, 7.6±0.4 and 9.4±0.24 at 30th, 60th and 90th DPT, respectively. Upon comparison of values between the two groups, there was decrease in the mean HI titre of GII by19.57%, 7.32% at 30th and 60th DPT, respectively and increase by 11.90% at 90th DPT in comparison to the GI. This change in the mean HI titre of GII was statistically significant on 30th and 90th DPT and non-significant on 60th DPT as compared to GI group. Upon comparison of values within the GII

Table 1: Mean hemagglutination inhibition titre (HI titre, log2) in different groups of experimental rats at different time intervals (Mean ± SE)

Day Post Treatment	Mean Hemagglutination Inhibition titre (log2) (Mean ± SE)	
	GI Control	GII Treatment
30	$9.2a±0.37^{a}$	$7.4±0.24^{bB}$ (-19.57%)
60	$8.2±0.37$	$7.6±0.4^{B}$ (-7.32%)
90	$8.4±0.24^{b}$	$9.4±0.24^{aA}$ (11.90%)

groups at different DPT, there was significant increase in mean HI titre at 90th DPT in comparison with 30th and 60th DPT.

Different small alphabetic letters (a and b) indicate significant (P<0.05) difference when compared horizontally between the GI and GII group at particular DPT. Different capital alphabetic letters (A and B) indicate significant (P<0.05) difference when compared vertically within the GI and GII group between particular DPT. (DPT= Days post treatment).

Mean ELISA values of the experimental rats from GI (control) and GII (treatment) groups were calculated and presented in Table 2 and Fig2. Mean ELISA values of GI group were 0.95±0.00, 1.10±0.04 and 1.10±0.13 at 30th, 60th and 90th DPT, respectively. Mean ELISA values of GII group were 0.99±0.07, 1.19±0.07 and 1.22±0.03 at 30th, 60th and 90th DPT, respectively. Upon comparison of values between the two groups, there was increase in the mean ELISA values of GII in comparison to the GI by 4.17%, 8.25% and 11.29% on 30th, 60th and 90th DPT, respectively. However, this change in the mean delta OD value of GII was statistically non-significant on 30th, 60th and 90th DPT as compared to GI group. Upon comparison of values within the groups at different DPT, there was significant increase in mean ELISA values at 60th and 90th DPT in comparison with 30th DPT.

Different capital alphabetic letters (A and B) indicate significant (P<0.05) difference when compared vertically within the GI and GII group between particular DPT. (DPT= Days post treatment)

The experiment demonstrated a significant increase in the HI titre of the nanoalumina treated group in comparison with the control group at 90th DPT. Within treatment group, HI titre had significantly increased on 90th DPT. This indicated that there was a significant increase in the level of antibodies in the serum of the nano alumina treated group. Also, there was a significant increase in the ELISA values in the nano alumina treated groups at 60th and 90th DPT in comparison with 30th DPT in treatment group. This indicated that there was a significant increase in the IgG level in the serum of nanoalumina treated group. These results are in confirmation with the results of Zhu *et al.* (2011) on aluminium chloride treated Albino rats (orally for 120days) which showed increase in the IgG, IgA and IgE levels. Aluminium is said disrupt ferric ion homeostasis and thus affect lymphocyte proliferation (Perez *et al.*, 1999). Nanoalumina because of its high surface reactivity, liberate Al3+ (Burklew *et al.*, 2012) which in turn liberate the ferric ion from transferrin (Mahieu *et al.*, 2000). It also inhibits ceruloplasmin which is necessary for the

Table 2: Immunoglobulin (IgG) assay using ELISA in different groups of experimental rats at different time intervals (Mean ± SE)

Day Post Treatment	Mean ELISA value (Mean ± SE)	
	GI Control	GII Treatment
30	0.95±0.00	$0.99±0.07^{B}$ (4.17%)
60	1.10±0.04	$1.19±0.07^{A}$ (8.25%)
90	1.10±0.13	$1.22±0.03^{A}$ (11.29%)

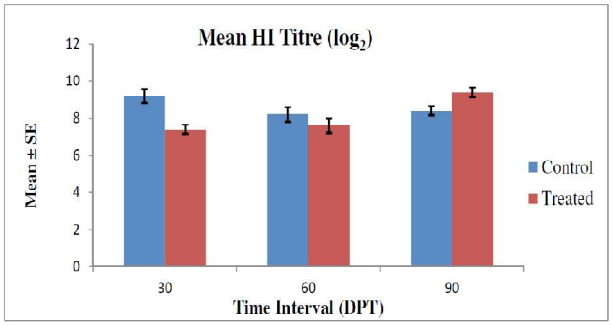

Fig 1: Mean hemagglutination inhibition titre (HI titre, log2) in different groups of experimental rats at different time intervals

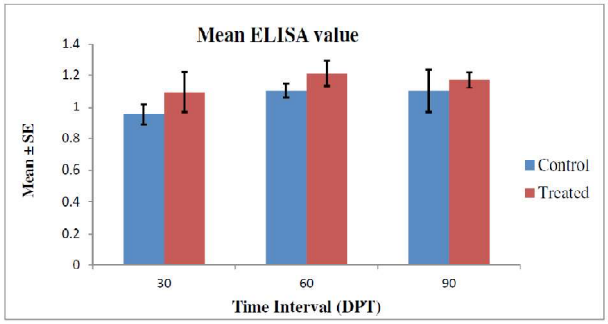

Fig 2: Mean ELISA values in different groups of experimental rats at different time intervals

incorporation of the ferric ion into the transferrin (Chmielnicka *et al.*, 1994). This results in reduction in the uptake of ferric ion necessary and thus inhibits its proliferation. Aluminium bound to transferrin, inhibited the proliferation of human peripheral lymphocytes stimulated by PHA-M in a dose-related way (McGregor *et al.*, 1990). But spleen being the hemocatheretic organ, has enough of iron because of hemoglobin catabolism which will be available for cell differentiation (Lauricella *et al.*, 2001). Also, the lymphocytes showed an increase in the number of surface transferrin receptors constitutes, an adaptation in the cells to overcome the iron deficit caused by the aluminium (Perez *et al.*, 1999). The long 90 days long exposure to nanoalumina may have favoured the adaptation mechanism to kick in and thus the cells are able to show the cellular proliferation. An increase in humoral immune response and in cell mediated immune response was also reported in nano alumina treated white leghorn chicken group when compared to control group (Kuntal *et al.,* 2017). An increase in humoral immune response in wistar rats treated with Zinc nano particles reported. (Pathak, Abhivyakti 2018).

ACKNOWLEDGEMENTS

The authors are thankful to Dean, College of Veterinary and Animal Sciences, Dean, College of Post Graduate Studies and Director, Experiment station G. B. Pant University of Agriculture and Technology, Pantnagar for providing facilities required for conducting the research work.

REFERENCES

Allan, W. H. and Gough, R. E. (1974). A standard haemagglutination inhibition test for Newcastle disease. (1). A comparison of macro and micro methods. *Veterinary Record*, 95(6): 120-123.

Almeida, J.P., Chen, A.L., Foster, A. and Drezek, R. (2011). In vivo biodistribution of nanoparticles. *Nanomedicine,* 6: 815–835.

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.

Bakand, S. and Hayes, A. (2016). Toxicological considerations, toxicity assessment and risk management of inhaled nanoparticles. *International Journal of Molecular Sciences*, 17: E929.

Burklew, C.E., Ashlock, J., Winfrey, W.B. and Zhang, B. (2012). Effects of aluminum oxide nanoparticles on the growth, development and microRNA expression of tobacco (*Nicotiana tabacum*). *PloS one*, 7(5): e34783

Campbell, A., Becaria, A., Lahiri, D.K., Sharman, K. and Bondy, S.C. (2004). Chronic exposure to aluminum in drinking water increases inflammatory parameters selectively in the brain. *Journal of Neuroscience Research,* 75:565–572.

Chmielnicka, J., Nasiadek, M. and Lewandowska-Zyndul, E. (1994). The effect of aluminium chloride on some steps of heme biosynthesis in rats after oral exposure. *Biological Trace Element Research,* 40(2): 127

Colvin, V.L. (2003). The potential environmental impact of engineered nanomaterials. *Nature Biotechnology,* 21:1166–1170.

Joshi, A. and Chauhan, R.S. (2012). *Immunological techniques: Interpretations, Validation and Safety Measures*. Kapish Prakashan, Gurgaon (Haryana). Pp: 11-17, 38, 44.

Kuntal, N.S. (2017). Pathology of nano alumina in chickens. Thesis, MVSc. G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Lauricella, A.M., Garbossa, G. and Nesse, A. (2001). Dissimilar behavior of lymph cells in response to the action of aluminum. In vitro and in vivo studies. *International immunopharmacology*, 1(9-10):1725-1732.

Mahieu, S., Contini, M.C., Gonzakez, M., Millen, N. and Elias, M.M. (2000).Aluminium toxicity: haematological effects. *Toxicology Letters*, 111: 235–242.

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.

McGregor, S., Naves, M., Oria, R., Vass, J.K., Brock, J.H. (1990). Effect of aluminium on iron uptake and transferrin-receptor expression by human erythroleukaemia cells. *Biochemical Journal,* 272: 377–82

Muoth, C., Aengenheister, L., Kucki, M., Wick, P. and Buerki-Thurnherr, T. (2016). Nanoparticle transport across the placental barrier: pushing the field forward! *Nanomedicine,* 11: 941–957.

Nel, A., Xia, T., Madler, L. and Li, N. (2006). Toxic potential of materials at the nanolevel. *Science*, 311: 622–627

Oberdorster, G., Oberdorster, E. and Oberdorster, J. (2005). Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environmental Health Perspectives,* 113: 823–839.

Perez, G., Garbossa, G., Sassetti, B., Di Risio, C. and Nesse, A. (1999). Interference of aluminium on iron metabolism in erythroleukaemia K562 cells. *Journal of Inorganic Biochemistry,* 76:105–12.

Park, E.J., Sim, J., Kim, Y., Han, B.S., Yoon, C., Lee, S., Cho, M.H., Lee, B.S. and Kim, J.H. (2015). A 13-week repeated-dose oral toxicity and bioaccumulation ofaluminum oxide nanoparticles in mice. *Archives of Toxicology,* 89(3): 371-379.

Pathak, Abhivyakti. (2018). Pathology of Zinc nano Particles in Wistar Rats.Thesis, MVSc. G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Snedecor, G.W. and Cochran, W.G. (1994). *Statistical methods (eighth edition)* Calcutta, India: Oxford and IBH Publishing Co.

Yildirim, A., Ozgur, E. and Bayindir, M. (2013). Impact of mesoporous silica nanoparticle surface functionality on hemolytic activity, thrombogenicity andnon-specific protein adsorption. *Journal of Materials Chemistry B,* 1: 1909-1920.

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.

Zoroddu, M.A., Medici, S., Ledda, A., Nurchi, V.M., Lachowicz, J.I. and Peana, M. (2014). Toxicity of nanoparticles. *Current Medicinal Chemistry,* 21: 3837–3853.

Received: August 14, 2023
Accepted: August 22, 2023