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Effect of Nano-Nickel Particles on Immune System of Wistar Rats

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ABSTRACT

Nanoparticles that are targeting the immune system is an highly active area of study. Lots of new techniques for immunosuppression depends on nanoparticles as carriers for the delivery of immunosuppressive compounds in small molecules. Efforts have been made to understand mechanisms by which nanoparticles interact directly with the immune system. Nanoparticles immunological activity depends on the physiochemical properties of the nanoparticles and its subsequent internalization inside the body. As the underlying factors for certain reactions are explained, there could be more nanoparticles established and evaluated to cause immunosuppression and to complement immunosuppressive drugs. The current trends and advances in understanding how nanoparticles cause immunosuppressive reactions is still requires more research work in field of nanotechnology.

HIGHLIGHTS

- Effect of mitogens (CON-A and PHA-M) are well defined on cellular immunity of Wistar Rats.
- Immuno-suppressive effect of nano-nickel was observed by using various immunological test.

Keywords: Nanoparticles, immunosuppression, internalization

Immunosuppression is the result of a drug or another substance reduces immune system function or efficacy. Nano particle activates the immune system of the body by following the different types of pathways (Andersen *et al*., 2013). Increment in both humoral and cell mediated immune response is observed after oral intake of nanoalumina at the dose of 200 ppm by the chickens (Kuntal, 2017). The innate immune response of the body depends upon the reliability of pathogen associated molecular pattern which belongs to the family of toll like receptor. Nanoparticles also have very important role on adaptive immunity. They can be used to deliver different types of vaccine antigens through phagocytosis, endocytosis and pinocytosis (Hubbell *et al*., 2009). The antigen presenting cells phagocytise these antigens present on histocompatibility complex (Gregory *et al*., 2013). These antigens also activate the T helper cells and these cells help in proliferation of eosinophils and differentiation of B cells to produce immunoglobulins thereby helping to promote the humoral immunity (Elsabahy and Wooley, 2013). Nano particles such as nickel stimulate the T-cell response and also play a very important role in homeostasis, these nanoparticles invoke inflammatory cytokines and aid in the maturation of dendritic cells. Immune system of the body is very sensitive to acute toxicity of nickel nanoparticles (Graham *et al*., 1975). Exposure to nickel nanoparticle resulted in suppression of immune system of body which leads to development of various respiratory infections such as streptococcal infection. Nano nickel

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particles interact with the immune system and recognized as foreign body by the immune system thereby stimulating it (Klippstein *et al*., 2010). Nano particle causes various types of immunological disturbance in the body which produces different types of severe diseases in the body (Glaser and Kiecoltglaser, 2005). Nano particle interaction with immune system leads to production of severe types of auto immune diseases (Ambwani *et al*., 2015; Zolnik *et al*., 2010). When the nanoparticles are used for drug delivery then they are very crucial for the immune system of the body (Moyano *et al*., 2012). Immuno modulatory effects of nanoparticles are very significant in animals which are susceptible to infection and cancer (Zolnik *et al*., 2010).

MATERIALS AND METHODS

Humoral immunity

Humoral immune response was assessed by using hemagglutination (HA) and hemagglutination inhibition (HI) assays, ELISA and B lymphocyte blastogenesis assay.

Hemagglutination and Hemagglutination inhibition tests

Hemagglutination and Hemagglutination inhibition assay was performed on serum separated from blood of 5 rats from each group at 30th, 60th and 90th DPT by using standard procedure (Allan and Gough, 1974).

Hemagglutination test procedure

Briefly, 50 microlitre of phosphate buffer saline was taken in all columns of a U shaped microtitre plate. Then 50 microlitre of virus antigen was placed and mixed in wells of first columns and 50 microlitre from first well was transferred to the next then onwards to make the two fold dilution of viral antigen viz. 1:2, 1:4, 1:8, 1:16, 1:32 and so on. From the eleventh well 50 microlitre mixture was discarded. 0.5% chicken erythrocyte suspension was prepared in phosphate buffer saline. For preparation of this suspension, Alsever's solution was taken and chicken blood was collected in it. Then the suspension was centrifuged and washed 3 times with this solution. 0.1 ml of RBC suspension was placed in each well from 1 to 12 well and was mixed by rotating the plate. The plate was placed in an incubator at 37°C for 1 hr and examined after 15 minutes.

Hemagglutination inhibition procedure

In this, persistent concentrations of virus and diluted serum sample were used. A 50 µl of serum sample was placed from every sample into the respective wells of U-bottom 96 well micro titre plate. Then two fold dilution of the serum was prepared in normal saline solution, starting from 1:2 to 1:1024. Then 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 temperature for 1 hour duration. In the last, 50 µl of 0.5% chicken erythrocytes was added to each well and the plate was kept at 40°C for the settlement of RBCs. The results were observed after 1 hour of incubation. The HI titre of the serum was calculated by the following formula:

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

Enzyme Linked Immunosorbant Assay

In this, Ranikhet disease R2B strain was used as antigen. Antigen was diluted in coating buffer and U bottom wells of the polystyrene microplate were coated with 100 µl of antigen diluted in 0.05 M carbonate bicarbonate buffer pH 9.6 (dilution 1:24) and plates were kept at 4°C overnight. Next day, the plates were washed thrice with washing solution (0.2 M PBS containing 0.05% tween-20 solution) followed by tapping against a towel to get rid of any unadsorbed material in the wells. Immobilization of the remaining unsaturated sites of polystyrene plates was done using by 5% skim milk powder and the suspension was kept 37°C for one hr. Plates were again washed using washing solution and tapped thrice. A 100 µl of diluted serum (1:1000) was added in duplicate wells and the plates were kept at 37°C for 2 hours. Washing with PBS-tween and tapping thrice was done. Thereafter, 100 µl of diluted anti IgG conjugate (1:1000) was applied in all wells and kept at 37°C for 2 hours incubation. Washing and tapping thrice was done again. Substrate to be used i.e. Orthophenylene diamine dihydrochloride (OPD) was prepared as 8 mg in 15 ml of 0.1 M citric acid phosphate

buffer containing 5 µl 30% hydrogen peroxide. Again after washing of plate, 100 µl of the OPD was added in each well and after 30 minutes, reaction was stopped with stopping reagent containing 1M H_2SO_4 and absorbance was be read at 492 nm (Joshi and Chauhan, 2012).

Calculation for ELISA values was done by the following formula:

$$\text{ELISA value} = \frac{\text{OD of test}}{\text{OD of control}}$$

B-lymphocyte blastogenesis assay

This test was performed on lymphocytes collected from spleen from 5 rats from each groups at 30th, 60th and 90th DPT using lipopolysaccharide as mitogen (Chauhan, 1998). Spleen was collected just after slaughter in ice cube packed box. Washing and separation of lymphocyte was done by using lymphocyte separation media. The lymphocyte were collected and resuspended in small amount of RPMI-1640 media. Trypan blue dye at the concentration of 0.5% was used for checking viability of cells in the Neubauer's counting chamber. In this, the live lymphocyte did not take any strain while dead cells are stained black. Finally, the live cell count is adjusted to 1×10^7 cells per ml in RPMI-1640 media containing 10% fetal calf serum. The cell suspension was made in a 96 well, U bottom cultural plates. A 100 microlitre cell suspension was taken in triplicate wells. A set of three wells were kept as control and 50 microlitre of RPMI 1640 media was added to them. A second set of three wells were loaded with 50 microlitre of lipopolysaccharide. The plate was then covered by aluminium foil and placed in carbon dioxide incubator for 68 hr at 37°C at 5% CO2 tension. After the incubation, 50 microlitre of MTT dye (4 mg/ml) was added to each well and incubated for 4 hr at 37°C in CO_2 incubator. Thereafter the supernatant was discarded and acid isopropanol (0.04 N HCL in isopropanol) was added to each well and mixed well with the help of micro pipette. Then absorbance of plate was measured by spectrophotometer at wavelength of 570 nm. The optical density of triplicate well was averaged and the mean optical density of mitogen stimulated culture was obtained by subtracting the mean optical density of control well from mean optical density of wells with mitogen and presented as delta optical density. The delta optical density of mitogen lipopolysaccharide was plotted on separated graph for calculating the index stimulation of B-lymphocyte blastogenesis assay.

Cell mediated immunity

Cell mediated immune response was studied by using T-lymphocyte blastogenesis assay, Macrophage Function Test and Delayed type hypersensitivity reaction.

T-lymphocyte blastogenesis assay

This test was performed on lymphocytes collected from spleen of 5 rats from each groups at 30th, 60th and 90th DPT using Con-A or PHA-M as mitogen as per the protocol described by Chauhan (1998). Spleen was collected just after slaughter of the rats in ice cube packed box. After that, washing and separation of lymphocyte was done by using lymphocyte separation media. The lymphocyte were collected and resuspended in small amount of RPMI-1640 media. To know the viability of lymphocytes, 0.5% trypan blue dye was used and the viability of cells was checked in the neubauer counting chamber. The live lymphocytes did not take any stain while dead cells are stained black. The live cell count was adjusted to 1×10^7 cells per ml in RPMI-1640 media containing 10% fetal calf serum. This cell suspension was prepared in a 96 well flat bottom. A 100 microlitre of this cell suspension was poured in a set of triplicate wells. A set of three wells was kept as control and 50 microlitre of RPMI-1640 media was further added to it. A second set of three well was added with mitogen Con-A and the third set of triplicate well was added with 50 microlitre of PHA-M. The plate was then covered by aluminium foil and placed in carbon dioxide incubator for 68 hr at 37°C at 5% CO2 tension. After incubation, 50 microlitre of MTT dye (4 mg/ml) is added to each well and incubated again for 4 hr at 37°C in CO_2 incubator. There after the supernatant was discarded and acid isopropanol (0.04 N HCL in isopropanol) was added in each well, mixed properly with the help of micro pipette to stop the reaction. The OD of the triplicate well was averaged and the mean OD of the control well was subtracted from the mean OD of the test well with mitogen was done to get the mean Δ OD of the cell culture stimulated by mitogen (Joshi and Chauhan, 2012).

Macrophage function test

This test was performed using heparinized blood from 5 rats from each group at 30th, 60th and 90th DPT as per the method using nitro blue tetrazolium (NBT) reduction assay (Chauhan, 1998; Joshi and Chauhan, 2012). Peripheral blood (1 ml) was collected in heparin coated vials and added equal amount of RPMI-1640. A volume of 5 ml of Histopaque-1119 (Himedia-LSM 1119) was taken in another centrifuge tube and the blood-media mixture was gradually overlaid without mixing them. These tubes were centrifuged at 400 g, for about 10-15 minutes and the upper opaque layer formed after centrifugation contained most of the phagocytic cells. This layer was then screened for NBT. A mixture of 0.3 ml of 0.2 per cent NBT in PBS, 0.2 ml of cell suspension which contains 10^7 cells/ml, and 0.1 ml of activated plasma (activated plasma was prepared by combining 1 ml of plasma + 15 µl LPS) was prepared. The mixture was then incubated in water bath at 37°c for 30 minutes. Then, the reaction was stopped by adding cold PBS. The cell suspension was then centrifuged at 500g for 5 minutes and supernatant was discarded. The cells were resuspended in 0.5 ml phosphate buffer saline. A drop of cell suspension was put on clean dry glass slide and smear was made. The smear was air dried and fixed with methanol for 2 minutes. The cells were counter-stained with Safranin dye (0.5%) for 30 sec. The slides were then air dried and viewed under oil immersion at 100x. The NBT positive cells were counted. Positive cells appeared black in colour due to blue-black colour of NBT dye (Chauhan, 1998).

Delayed type hypersensitivity reaction

For delayed type hypersensitivity reaction, 1 cm^2 area of skin was shaved, cleaned and applied 70% ethanol as antiseptic. The area was sensitized with 25 µl of 1% DNFB. 2 weeks later rats were challenged again with 25 µl of 0.1% DNFB at the same area used for previous exposure. Skin thickness was measured at 0, 12, 24, 36, 48 and 60 days post exposure (Joshi and Chauhan, 2012).

RESULTS AND DISCUSSION

Humoral immune response

Hemagglutinin inhibition test (HI)

Mean HI titer of experimental rats in different groups expressed as $\log_2$ and are given in Table 1. Mean values of group I rats, were 9.1 ± 0.12, 8.31 ± 0.37 and 9.4 ± 0.40 at 30th, 60th and 90th DPT, respectively. In group II rats, these values were 8±0.31, 7.2±0.37 and 7.9±0.29 at 30th, 60th and 90th DPT, respectively. There were decrease in mean HI titre by 12.08%, 13.25% and 15.95% at 30th, 60th and 90th DPT, in group II rats as compared to group I rats. There were significant differences in mean HI titre at 30th, 60th and 90th DPT in group II rats as compared to group I rats. When these values were compared with in the same group at different time intervals, there was no significant difference in group I and group II rats.

Table 1: Hemagglutination inhibition titre in $\log_2$ of experimental rats at different time intervals of the experimental period (Mean ± SE)

Day Post Treatment	HI titre in $\log_2$ (Mean ± SE)	
	Group I (Control)	Group II (Treated)
30	9.1 ± 0.12^{aA}	8 ± 0.31^{aB} (-12.08%)
60	8.3 ± 0.31^{aA}	7.2 ± 0.37^{aB} (-13.25%)
90	9.4 ± 0.40^{aA}	7.9 ± 0.29^{aB} (-15.95%)

*Alphabetical letters (A and B) indicate significant (P<0.05) difference between groups at a particular DPT (DPT= Day Post-Treatment) whereas different alphabetical letters (a and b) indicate significant (P<0.05) difference within days in a particular group.

Enzyme Linked Immunosorbent Assay (ELISA)

Mean ELISA values of experimental rats in both the groups are given in Table 2.

Table 2: ELISA values of experimental rats at different time intervals of the experimental period (Mean ± SE)

Day Post Treatment	ELISA value (Mean ± SE)	
	Group I (Control)	Group II (Treated)
30	0.76 ± 0.03^{bA}	0.64 ± 0.08^{cB} (-18.75%)
60	1.39 ± 0.06^{aA}	1.18 ± 0.15^{aB} (-17.79%)
90	0.71 ± 0.08^{cA}	0.69 ± 0.06^{bB} (-2.89%)

*Alphabetical letters (A and B) indicate significant (P<0.05) difference between groups at a particular DPT (DPT= Day Post-Treatment) whereas different alphabetical letters (a, b and c) indicate significant (P<0.05) difference within days in a particular group.

Mean values in group I rats were 0.76±0.03, 1.39±0.06 and 0.71±0.08 at 30th, 60th and 90th DPT, respectively. In group II rats, these values were 0.64±0.08, 1.18 ±0.15 and

0.69± 0.06 at 30th, 60th and 90th DPT, respectively. There were decrease in mean ELISA values by 18.75%, 17.79% and 2.89% at 30th, 60th and 90th DPT in group II rats as compared to group I rats. There were significant difference between control and treated rats at 30th, 60th and 90th DPT. When these values were compared with in the same group at different time intervals, there was significant difference in group I and II rats at 30th, 60th and 90th DPT.

B- Lymphocyte Blastogenesis assay using lipopolysaccharide (LPS)

The mean values of delta optical density of LST using LPS as mitogen in both the groups of experimental rats at various time intervals are presented in Table 3. Mean values in group I rats were 0.92±0.12, 1.43±0.08 and 1.01±0.1 at 30th, 60th and 90th DPT, respectively. In group II rats, these values were 0.82±.09, 1.03±0.08 and 0.93±0.25 at 30th, 60th and 90th DPT, respectively. There were increase in values by 10.87%, 36.36% and 7.92% at 30th, 60th and 90th DPT in group II rats as compared to group I rats. There were significant differences between control and treated rats at 30th, 60th and 90th DPT. When these values were compared with in the same group at different time intervals, there was significant difference in group I rats at 30th, 60th and 90th DPT and in group II rats at 30th, 60th and 90th DPT.

Table 3: Delta optical density of experimental rats in B-lymphocyte blastogenesis assay using (LPS) (Mean ± SE)

Day Post Treatment	Delta OD of LST (LPS) (Mean ± SE)	
	Group I (Control)	Group II (Treated)
30	0.92 ± 0.12^{cA}	0.82 ± 0.09^{cB} (-10.87%)
60	1.43 ± 0.08^{aA}	1.03 ± 0.08^{bB} (-27.97%)
90	1.01 ± 0.1^{bA}	0.93 ± 0.25^{aB} (-7.92%)

*Alphabetical letters (A and B) indicate significant (P<0.05) difference between groups at a particular DPT (DPT= Day Post-Treatment) whereas different alphabetical letters (a and b) indicate significant (P<0.05) difference within days in a particular group.

Cell mediated immunity

T-Lymphocyte Blastogenesis using Concanavalin A (CON – A)

The mean values of delta optical density of LST using Con-A as mitogen in different groups of experimental rats at different time intervals are presented in Table 4. Mean values for group I rats were 0.76± 0.09, 0.77±0.09 and 0.94±0.23 at 30th, 60th and 90th DPT, respectively. In group Π rats, these values were 0.65±.02, 0.69±.02 and 0.76±0.01 at 30th, 60th and 90th DPT, respectively. There were decrease in mean Con-A values by 14.47%, 10.39 and 23.68% at 30th, 60th and 90th DPT in group II rats as compared to group I rats. There were significant difference between control and treated rats at 30th, 60th and 90th DPT. When these values were compared with in the same group at different time interval, there was significant difference in group II rats at 30th, 60th and 90th DPT. While in group II values, there was no significant change throughout the period of experimentation.

Table 4: Delta optical density of experimental rats in T-lymphocyte blastogenesis assay using Con-A at different time intervals of the experimental period (Mean ± SE)

Day Post Treatment	Delta optical density (Mean ± SE)	
	Group I (Control)	Group II (Treated)
30	0.76 ± 0.09^{aA}	0.65 ± 0.02^{cB} (-14.47%)
60	0.77 ± 0.09^{aA}	0.69 ± 0.02^{bB} (-10.39%)
90	0.94 ± 0.23^{aA}	0.76 ± 0.01^{aB} (-23.68%)

*Alphabetical letters (A and B) indicate significant (P<0.05) difference between groups at a particular DPT (DPT= Day Post-Treatment) whereas different alphabetical letters (a, b and c) indicate significant (P<0.05) difference within days in a particular group.

T lymphocytes blastogenesis assay using phytohem agglutinin-M (PHA- M)

The mean values of delta optical density of LST using PHA –M as mitogen in different groups of experimental rats at various time intervals are presented in Table 5. Mean values for group I rats were 0.66±0.03, 0.91±0.2 and 0.95±0.06 at 30th, 60th and 90th DPT, respectively. In group II rats, these values were 0.51±0.10, 0.86±0.06 and 0.86±0.04 at 30th, 60th and 90th DPT, respectively. There were decrease in values by 29.41%, 5.81% and 10.46% at 30th, 60th and 90th DPT in group II rats as compared to group I rats. There were significant difference between control and treated rats at 30th, 60th and 90th DPT, respectively. When these values were compared with in the same group at different time intervals, there was significant difference

in group I rats at 30th, 60th and 90th, DPT and in group II rats at 30th DPT.

Table 5: Delta optical density of experimental rats in T-lymphocyte blastogenesis assay using PHA-M at different time intervals of experimental period (Mean ± SE)

Day Post Treatment	Delta optical density (PHA- M) (Mean ± SE)	
	Group I (Control)	Group II (Treated)
30	0.66 ± 0.03^{cA}	0.51 ± 0.10^{bB} (-29.41%)
60	0.91 ± 0.2^{bA}	0.86 ± 0.06^{aB} (-5.81%)
90	0.95 ± 0.06^{aA}	0.86 ± 0.04^{aB} (-10.46%)

*Alphabetical letters (A and B) indicate significant (P<0.05) difference between groups at a particular DPT (DPT= Day Post-Treatment) whereas different alphabetical letters (a, b and c) indicate significant (P<0.05) difference within days in a particular group.

Macrophage function test

NBT positive cells in macrophage function test were counted, expressed in percent and presented in Table 6. Mean values of NBT positive cells in case of group I rats were 32.20±2.26, 36.60±3.61 and 45.80±2.74 at 30th, 60th and 90th DPT, respectively. In group II rats, these values were 42.8±2.00, 51.4±3.69 and 52.4±2.83 at 30th, 60th and 90th DPT, respectively. There were increase in mean values of NBT positive cells by 24.77%, 40.43% and 14.4% at 30th, 60th and 90th DPT, respectively in group II rats as compared to group I rats. There were significant difference between control and treated rats at 30th and 60th and 90th DPT.

When these values were compared with in the same group at different time intervals, there was significant difference in group I rats at 90th DPT and in group II rats at 30th DPT.

Table 6: NBT positive cells (%) in Macrophage Function Test

Day Post Treatment	NBT positive cells in (%) (Mean ± SE)	
	Group I (Control)	Group II (Treated)
30	32.20 ± 2.26^{bB}	42.8 ± 2.00^{bA} (24.77%)
60	36.60 ± 3.61^{bB}	51.4 ± 3.69^{aA} (40.43%)
90	45.80 ± 2.74^{aB}	52.4 ± 2.83^{aA} (14.4%)

*Alphabetical letters (A and B) indicate significant (P<0.05) difference between groups at a particular DPT (DPT= Day Post-Treatment) whereas different alphabetical letters (a and b) indicate significant (P<0.05) difference within days in a particular group.

Delayed type hypersensitivity reaction using dinitrofluorobenzene (DNFB)

Delayed type hypersensitivity in rats was studied by measuring DNFB applied skin thickness is expressed in cm and shown in Table 7. Mean skin thickness of group I rats were 0.17±0.004, 0.18±0.04, 0.52±0.0032, 0.72±0.005 and 0.89±0.004 cm at 0, 12, 24, 48 and 72 hours post-challenge (HPC), respectively. Mean skin thickness of group II rats were 0.11±0.004, 0.21±0.0037, 0.32±0.0032, 0.47±0.04 and 0.71±0.004 cm at 0, 12, 24, 48 and 72 HPC, respectively. There were decrease in mean skin thickness by 35.29%, 20%, 53.19%, 20.22% at 0, 24, 48, and 72 HPC while increase in mean skin thickness by 16.67% at 12 HPC in group II rats as compared to group I rats. There were significant differences in mean skin thickness of group II rats at 48 and 72 HPC as compared to group I rats. When these values were compared with in the same group at different time intervals, there was significant difference in group I and group II rats at 24, 48 and 72 HPC.

Table 7: Thickness of the skin measured (cm) in delayed type hypersensitivity reaction by DNFB (Mean ± SE)

Hours post-challenge	Thickness of skin in cm (Mean ± SE)	
	Group I (Control)	Group II (Treated)
0	0.17 ± 0.004^{dA}	0.11 ± 0.004^{dA} (-35.29%)
12	0.18 ± 0.04^{dA}	0.21 ± 0.0037^{dA} (16.67%)
24	0.52 ± 0.0032^{cA}	0.32 ± 0.0032^{cA} (-20%)
48	0.72 ± 0.005^{bA}	0.47 ± 0.04^{bB} (-53.19%)
72	0.89 ± 0.004^{aA}	0.71 ± 0.004^{aB} (-20.22%)

*Alphabetical letters (A and B) indicate significant (P<0.05) difference between groups at a particular DPT (DPT= Day Post-Treatment) whereas different alphabetical letters (a, b, c and d) indicate significant (P<0.05) difference within days in a particular group.

Nickel is a naturally occurring silvery metal found in the earth's crust in the form of several nickel minerals. Nickel consists of the 0.009% of the earth's crust. Nickel and its compound present in all components of the environment including plant and animal, air, drinking water, river, lakes, oceans and soil. Some household sources like kitchen utensils, pipes, jewelries, button and zippers, beverages containers are the common source of exposure of nanonickel to the human and animal. These days, nanoparticles have gained a great deal of attention

not only because of their widespread applications but also because of their adverse effects on the environment and human health. Nanonickel has fascinated greater attention due to their different flexible properties (Singh *et al*., 2016; Jahromi *et al*., 2015) and increasingly used in various day-to-day applications such as catalyst, gas sensor, alkaline battery cathode, electro-chrome film, magnetic and fuel cell (Rao and Sunandana, 2008; Rani *et al*., 2010; Mu *et al*., 2011). Nickel nanoparticles are also found in roots, almonds, vegetables, coco, nuts, cereals, chocolates, oatmeal, soybean (Samal and Mishra, 2011). Nickel nanoparticles have wide role in various application and industries, despite the many applications, nickel nanoparticles have limited information on nanonickel toxicity at cell and molecular level. Due to the ever growing exploitation of these nanoparticles, toxicological studies on these nanoparticles are very rare and sporadic. The studies in particular concerning the release and the accumulation of potentially noxious by products are very limited. The present experiment was therefore conducted to study the pathological effects of nanonickel along with study of its accumulation in rats for a period of 90 days.

In this experiment, five week old, 35 Wistar rats of either sex were administered nanonickel at NOAEL dose (5 mg/kg/day) for a period of 90 days. These rats were randomly divided into two groups, the control group (group I) with 20 rats and treated group (group II) with 15 rats. The effect of nanonickel on behaviour, growth feed intake and health of the rats were studied. Various hematological, biochemical, immunological and pathological alteration were studied. There was no mortality seen in any rat among any group.

There were significant increase in ALT at 60^{th} and 90^{th} DPT and significant increase in AST at 90^{th} DPT values indicates tissue damage in liver (Yang *et al*., 2008). The increased accumulation of nanonickel content in the liver tissue might be due to the increased metabolic activity of these organs and their function in xenobiotic detoxification (Nwokocha *et al*., 2011).

High nanonickel accumulation in the liver tissue might be related to filtration and the presence of mononuclear phagocytic cells in the liver. These phagocytic cells form a part of the reticuloendothelial system, which participates in nanoparticles sequestration. The elements of the reticuloendothelial system in the liver are Kupffer cells, which are located in specific sinusoidal walls so that impurities can be efficiently removed from the blood. Increase in target biochemical enzymes might be attributed to increase in cell membrane permeability, tissue necrosis and animal adaptive mechanism attributed to nanonickel stress.

In hemagglutination inhibition (HI) assay, a significant decrease in antibody titer was observed in group II rats at all DPT as compared to group I rats. ELISA values also revealed a significant decrease in antibody titer in group II rats as compared to group I rats at all DPT. It confirms the immunotoxic effect of nanonickel leading to immunodeficiency which results in the vaccine failure (Banga *et al*., 2005; Chauhan *et al*., 2018).

Williams *et al*. (1997) studied that ELISA was 10 times more sensitive than HI in detecting low level of antibodies to new castle disease virus (NDV) in ostrich. ELISA has more advantage over HI as it uses untreated serum in a single dilution as compared to HI for which pretreated serum in titration is used. Moreover, there has been development of nanoparticles- antibody conjugates to enhance the sensitivity of ELISA (Day *et al*., 2017).

Lymphocyte stimulation test done using con-A and PHA-M mitogen for T- cell blastogenesis showed decrease in the T-cell proliferation. Con- A exposure results in significant decrease in T- cell population at all DPT in nanonickel treated group in comparison with the control. PHA-M also resulted in significant decrease in T-cell proliferation at all DPT. Lymphocytes stimulation test done using LPS mitogen for B- cell blastogenesis showed significant decrease in B-cell proliferation at all DPTs in nanonickel treated group in comparison with the control. This result is in confirmation with that of Obone *et al*. (1999). They also reported alterations in T-cell and B-cell subpopulations in rats when rats were exposed to the nickel nanoparticle. CON- A, PHA-M and LPS are the different types of mitogen were used in the lymphocyte stimulation test to influence the activity of immature T- lymphocytes, mature T- lymphocytes and for B-lymphocyte. PHA-M increases the activity of T- lymphocyte by increasing the blastogenesis activity in cell cycle in Go to G_1 stage.

There was significant increase in macrophage phagocytic activity in rats of group II throughout the experiment, Phagocytosis plays a crucial role in initiating macrophage derived inflammatory responses. Nanonickel induce a dose-dependent reactive oxygen production which leads to

activation of NLRP3 inflammasome results in stimulation of macrophages (Morishige *et al*., 2010).

CONCLUSION

Immunopathological studies revealed that significant variation between control and treated groups. T-lymphocyte blastogenesis assay by using CON-A, PHA-M and LPS shows significant decrease in values at all DPT, while macrophage function test showed significant increase at all DPT. Delayed type hypersensitivity test showed significant variation at 48 and 72 hr post challenges between control and treated rats. In case of humoral immunity, HI titre and ELISA were showing significant variation between control and treated rats.

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