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Effects of nanosilver administration on immune responses in Wistar Rats

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ABSTRACT: The present study was conducted to know the effects of nanosilver administration at NOAEL dose on immune responses in Wistar rats for a period of 90 days. Thirty-five, 6-week-old, Wistar rats of both the sexes were divided randomly in two groups. Group 1 with 20 rats served as control. Group II comprising of 15 rats were administered silver nanoparticle through distilled water *per os* at NOAEL dose rate of 30 mg/kg body weight/day from 0 day of experiment till 90th days post treatment (DPT). Five rats from each group (I and II) were slaughtered at 0th (5 rats from group I only, was slaughtered), 30th, 60th and 90th DPT and blood and spleen were collected at these intervals to study the effect of nano-silver on both HIR and CMI. In group II, reduced titre of HI and ELISA test values, significant decrease in serum gamma globulin values at 90th DPT showed the deleterious effect of nanosilver on the humoral immune response. Decreased thickness of the DNFB applied skin in delayed type hypersensitivity reaction and NBT positive cells in macrophage function test in the test rats showed harmful effects of the nano-silver on the cell mediated immunity. Significant decrease in ΔOD during the lymphocytes timulation test performed on splenocytes of the test rats using Con-A,PHA-M and LPS mitogens also showed lymphocyto toxic effect against both mature and immature T lymphocytes and B lymphocytes. It was concluded from the present studies that nanosilver exerted adverse effects on immune responses in Wistar rats at NOAEL dose for a period of 90 days.

Key words: Immune responses, nanosilver, NOAEL dose, Wistar rats

There is a surge of interest in the emerging fields of nanoscience and nanotechnology at the turn of the century as nanotechnology has provided us with solutions for early disease detection and treatment of metabolic disorders (Sanhai *et al.,* 2008). Nanoparticles (NPs) display unique, physical, and chemical properties and represent an increasingly important material in the development of novel drugs and medicines (Zhang and Saltzman, 2013). The popularity of nanotechnology is increasing due to its beneficial effects, but there must be serious concerns about its possible health risks (Ema *et al.,* 2010). Although humans have been exposed to nanoparticles throughout history, it increased dramatically during the industrial revolution. Water, soil, and air are all pathways through which NPs infiltrate the environment when humans engage in various activities. Animals and human beings are knowingly/unknowingly exposed to various synthetic or natural nanoparticles present in our micro-environment. In addition, studies have shown that NPs can enter organisms by ingestion or inhalation and can travel throughout the body to numerous organs and tissues where they can exert their reactive toxicological effects. Toxicological effects of metallic nanoparticles (MNPs) on both animal and plant cells are currently under investigations. Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties (Natsuki *et al.,* 2015). Unfortunately, most of the interactions of the silver nanoparticles with the human body are still unclear; as a consequence, the most desirable characteristics for the silver nanoparticles do not seem to be well established. Silver (particularly in the form of soluble silver compounds) has toxic effects in both animals and humans, in addition to its antibacterial properties. The toxic effects are particularly more in people working in the silver mining, manufacturing, or packaging industries (Al Gurabi *et al.,* 2015). Keeping in view the above facts, the present study was conducted to investigate the effects of nanosilver administration on immune responses in Wistar rats

at a NOAEL dose of 30mg/kg body weight per day (Kim *et al.,* 2010) for a period of 90 days.

MATERIALS AND METHODS

Apparently healthy, six weeks old, 35 Wistar rats of both the sexes were procured from Laboratory Animal Resources, Indian Veterinary Research Institute, Izatnagar, Bareilly, India. The rats were maintained in Experimental Animal House of Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pantnagar under standard managemental conditions. The project was approved by Institutional Animal Ethics Committee vide letter no. IAEC/CVASc/VPP/420 dated 12-12-2020. All the rats were acclimatized for a period of seven days prior to experiment. Rats were randomly divided in two groups, group I with 20 rats as control group and group II with 15 rats as treatment group. The silver nitrite nanoparticles used in this study were having aerodynamic particular size of less than 90 nm and molecular weight is 107.87 was purchased from Sisco Research Laboratories Pvt. Ltd., India. It was suspended in distilled water and working samples of recommended dose formulation was prepared on daily basis during the entire period of study. It was homogenized by sonication just prior to administration and were gavaged once daily for 90 days, at the NOAEL dose of 30mg/kg body weight/day (Kim *et al*, 2010). The rats were vaccinated with 0.1 ml of R2B strain of Newcastle Disease vaccine intraperitoneally at 0thdays post treatment (DPT) and 30th DPT. The experimental design in tabular form is given in Table 1.

Five rats from each group (I and II) were slaughtered at 0th (5 rats from group I only, was slaughtered), 30th, 60th and 90th DPT as shown in the Table 1. Blood and spleen were collected. Various immunological parameters for studying both humoral and cell mediated immune responses are as under.

Table 1: Experimental design

Days post treatment	Control group (GI) (Normal feed and water)	Treatment group (GII) (AgNP+ Normal feed and water)
0	5 Rats	-
30th	5 Rats	5 Rats
60th	5 Rats	5 Rats
90th	5 Rats	5 Rats
Total	20 Rats	15 Rats

Humoral Immunity

Humoral immunity was studied by determination of serum gamma globulins, serum antibody titres by haemagglutination inhibition test (HA and HI tests), ELISA and B-lymphocyte blastogenesis assay applied on splenocytes, at the above said intervals.

Determination of serum gamma globulins

Serum gamma globulin concentration was estimated by the protocol mentioned by Chauhan, 1998. Briefly, serum was mixed with the ammonium sulfate and sodium chloride solution. It was kept in ice bath for overnight and then it was centrifuged to separate the precipitate. Precipitate obtained was then dissolved in the NSS, mixed with biuret reagent and then optical density was read against 555 nm.

Determination of antibody tire

Antibody titre was assessed in serum by employing the haemagglutination inhibition test (HA and HI tests) and ELISA at the above said intervals.

Haemagglutination Inhibition Test

Humoral immune response was assessed by using HA and HI tests on the serum separated by using protocol as mentioned by Allan and Gough, 1974. Briefly, 50 µl of phosphate buffer saline was added in all columns of the round bottom microtitre plate. In first well of each column, 50 µl of virus antigen (New Castle Disease Vaccine R2B strain) was added and mixed thoroughly 2 fold dilution. From the eleventh well, 50 µl content was discarded. Then, 1% chicken erythrocyte suspension was prepared in phosphate buffer saline. For preparation of this suspension, chicken blood was collected in Alsever's solution and centrifuged. After centrifugation it was washed 3 times with phosphate buffer saline. A 0.1 ml of RBC suspension was added in each well from

1st to 12th column and mixed by rotating the plate. It was kept inside the incubator at 37°C for 1 hr and was examined after 15 minutes past incubation for better results.

Haemagglutination Inhibition test procedure

In this, persistent virus concentrations and diluted serum sample was used. A 50 µl of serum sample was placed from each sample into the respective wells of round bottom 96 wells microtitre plate. Then two-fold dilution of the serum was made in normal saline solution, starting from 1:2 to 1:1024. 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 temperature for 1 hour duration. Finally, 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 duration.

The HI titre was calculated by the following formula:
HI titre = Reciprocal of the end point showing complete inhibition of haemagglutination is directly expressed as HI titre of the serum.

Determination of antibody tire by Enzyme Linked ImmunosorbantAssay

This was done as per the protocol of Joshi and Chauhan, 2012. In this, round bottom wells of microplates were coated with 100 µl antigen diluted in coating buffer (0.05 M carbonate bicarbonate buffer pH 9.6, dilution 1:24). 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. Blocking of the remaining unsaturated sites of polystyrene plates was done using by 5% skim milk powder and the suspension was kept 37°C for 1 hr. Plates was 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 was done thrice each. Thereafter, 100 µl of diluted anti IgG conjugate (1:1000) was added in all wells and incubated at 37°C for 2 hours. Washing and tapping was done three times each. Substrate to be used i. e., Ortho phenylenediaminedihydrochloride (OPD) was prepared (8 mg in 15 ml of 0.1 M citric acid phosphate buffer containing 5 µl 30% hydrogen peroxide). After washing of plate again, 100 µl of the OPD was added in each well and after 30 minute reaction was stopped with stopping reagent containing 1M H_2SO_4 and absorbance was read at 492 nm.
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 was done using protocol of Joshi and Chauhan, 2012. Briefly, single cell suspension of the lymphocytes was made by the grinding of the spleen and then separation of the cell is done by using density gradient centrifuge. Harvested lymphocytes were then cultured in RPMI-1640 medium. Survivability of cells was checked using the technique of Trypan blue dye exclusion (Azadmehr *et al.,* 2016). By using 100 µl cell cultures, 3 sets of triplicate wells were prepared. First set of 3 wells was used as control by putting 100 µl of RPMI-1640 in each well. In second set of 3 wells, 50 µl of LPS along with 50 µl of RPMI-1640 was added, respectively. After proper sealing of the plate, it was incubated at 37 °C with 5% CO_2. After 68 hrs of incubation, 50 µl of MTT dye was added in wells and the plate was again incubated for 4 hrs at 37°C with 5% CO_2. After 4 hours of incubation, the clear fluid was discarded and 100 µl of acid isopropanol was added in the wells and mixed properly. The absorbance was taken at 570 nm using ELISA reader. For calculating the B lymphocyte stimulation index, graph was plotted for OD of LPS mitogen.

Cell mediated immunity

Cell mediated immunity was studied by using T-lymphocyte blastogenesis assay, Macrophage function

test at the above said intervals and the delayed type hypersensitivity reaction (DTH).

T-lymphocyte blastogenesis assay

This was done using protocol of Joshi and Chauhan, 2012 as described above in the B-lymphocyte blastogenesis assay except Con A or PHA-M was used in place of LPS and for calculating the T lymphocyte stimulation index, graph was plotted for OD of Con-A or PHA-M mitogen.

Macrophage Function Test

This test was performed using heparinized blood using NBT reduction assay as per the protocol of Joshi and Chauhan (2012). Briefly, peripheral blood (1 ml) was collected in heparin coated vials and equal amount of RPMI-1640 was added. A 5 ml of Histopaque-1119 (Himedia-LSM 1119) was taken in another centrifuge tube and the blood-media mixture was gradually overlaid without mixing the materials. The tube was centrifuged at 400 g, for 10-15 minutes and the upper opaque layer was formed containing most of the phagocytic cells. 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 PBS. A drop of cell suspension was placed on clean dry glass slide and smear was made. The smear was air dried and fixed in methanol for 2 minutes. The cells were counter-stained with Safranin (0.5%) for 30 sec. The slides were air dried and observed under oil immersion. NBT positive cells were counted. Positive cells appeared black in color due to blue-black color of NBT dye.

Delayed Type Hypersensitivity Test

For delayed type hypersensitivity reaction, an area of $1cm^2$ of skin was shaved, cleaned and sterilized and then sensitized with 25 µl of 1% DNFB at 72th DPT. Rats were again challenged with 25 µl of 0.1%DNFB at 87th DPT. Skin thickness was measured at 0, 12, 24, 36,48 and 72 hours post challenge (HPC) (Curzytek *et al.,* 2013).

The data generated during the course of experiment was subjected to statistical analysis by using two way ANOVA (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The results pertaining to immune responses, both humoral and cell mediated, measured by various parameters are as under:

Humoral Immunity
Serum gamma globulin

Mean serum gamma globulin values of experimental rats in both the groups at different time intervals expressed in g/dl and are presented in Table 2. Mean values in group I rats were 0.45±0.06, 0.47±0.05, 0.42±0.04 and 0.44±0.04g/dl at 0, 30th, 60th and 90th DPT, respectively. In group II rats, these values were 0.45±0.07, 0.46±0.03, 0.38±0.04 and 0.34±0.03g/dl at 0, 30th, 60th and 90th DPT, respectively. Mean serum gamma globulin value of group II rats was found to decrease by 2.3%, 9.82% and 22.5% at 30th, 60th and 90thDPT, respectively when compared with group I rats. There was significant decrease in serum gamma globulin at 90th DPT when compared with the control group rats. When these values were compared within the same group at different time intervals, there was no significant difference in the values of group I and II rats at any time interval.

Haemagglutination Inhibition Test

Mean HI titer determined in experimental rats of both the groups at various time intervals of the experiment expressed in $\log_2$ value and are presented in Table 3. Mean values of HI titer observed in group I rats were 8.4±0.36, 8.4±0.27, 8.6±0.35 and 8.8±0.34 at 0th, 30th, 60th and 90th DPT, respectively. In group II rats, mean values were 8.4±0.38, 7.2±0.21, 7.40.28 and 7.20.39 at 0th, 30th, 60th and 90th DPT, respec-

tively. The values of mean HI titer in group II were decreased by 14.28%, 13.95% and 18.18 % at 30th, 60th and 90th DPT, respectively when compared with the control group rats. Significant decrease in the HI titer was observed in treated rats at 90th DPT when compared with the control group rats. When these values were compared within the same group at different time intervals, there was no significant difference in the values of group I and II rats at any time interval.

Enzyme Linked Immuno-Sorbent Assay (ELISA)

Mean ELISA values of experimental rats in both the groups at different time intervals are presented in Table 4. Mean ELISA values in group I rats were 0.82±0.04, 0.85±0.03, 0.99±0.05 and 1.21±0.06 and in group II rats, mean values were 0.82±0.06, 0.81±0.05, 0.89±0.04 and 0.93±0.03 at 0, 30th, 60th and 90th DPT, respectively. Mean ELISA values of group II rats were found to decrease by 4.7%, 10.24% and 23.14% at 30th, 60th and 90th DPT of the experiment, respectively when compared with group I rats. There was significant decrease in mean ELISA values at 90th DPT when compared with the control group rats. When these values were compared within the same group at different time intervals, there was no significant difference in the values of group I and II rats at any time interval.

B-Lymphocyte Blastogenesis Assay

The values of mean Δ optical density (ΔOD) in rats of both groups at different time intervals of the experiment for B-lymphocyte blastogenesis assay using LPS mitogen are presented in Table 5. Mean ΔOD values in group I rats were 0.70± 0.08, 0.76± 0.05, 0.92±0.05 and 1.37±0.14 at 0, 30th, 60th and 90th DPT, respectively. In group II rats, mean ΔOD values were 0.70± 0.08, 0.71±0.09,0.78±0.07 and 0.98±0.07 at 0, 30th, 60th and 90th DPT, respectively. The values of mean ΔOD in group II rats decreased by 6.57 %, 15.21 % and 28.46 % at 30th, 60th and 90th DPT when compared with the values of control group. Results of mean Δ OD in rats for B-lymphocyte blastogenesis assay using LPS mitogen showed significant difference between test and control group at 90th DPT. When these values were compared within the same group at different time intervals, there was a significant increase in the values of group I at 60th and 90th DPT compared to 0 DPT and a significant increase in the values of II rats at 90th DPT compared to 0th DPT.

Table 2: Mean (Mean± SE) serum gamma globulin (g/dl) in different groups of experimental rats at different time intervals of the experiment

Day Post	Mean (Mean± SE) serum gamma globulin (g/dl)*	
Treatment	Group I (Control)	Group II (Treated)
0	0.45 ± 0.06^{aA}	0.45 ± 0.07^{aA} (0%)
30th	0.47 ± 0.05^{aA}	0.46 ± 0.03^{aA} (-2.3%)
60th	0.42 ± 0.04^{aA}	0.38 ± 0.04^{aA} (-9.82%)
90th	0.44 ± 0.07^{aA}	0.34 ± 0.03^{bA} (-22.5%)

* Different small alphabetic letters (a and b) indicate significant (P<0.05) difference between groups on particular DPT, whereas different capital alphabets (A and B) indicate significant (P<0.05) difference within days in a particular group (DPT= Days post treatment).

Table 3: Mean (Mean± SE) haemagglutination inhibition titer (log_2) of different groups of experimental rats at different time intervals of the experiment

Day Post	HI (Mean± SE) titer in log_2*	
Treatment	Group I (Control)	Group II (Treated)
0	8.4 ± 0.36^{aA}	8.4 ± 0.38^{aA} (0%)
30th	8.4 ± 0.27^{aA}	7.2 ± 0.21^{aA} (-14.28%)
60th	8.6 ± 0.35^{aA}	7.4 ± 0.28^{aA} (-13.95%)
90th	8.8 ± 0.34^{aA}	7.2 ± 0.39^{bA} (-18.18%)

Different small alphabetic letters (a and b) indicate significant (P<0.05) difference between groups on particular DPT, whereas different capital alphabets (A and B) indicate significant (P<0.05) difference within days in a particular group (DPT= Days post treatment).

Table 4: Mean (Mean ± SE) antibody titer in serum of different groups of experimental rats at different time intervals of the experiment measured by ELISA

Day Post	ELISA values (Mean ± SE)*	
Treatment	Group I (Control)	Group II (Treated)
0	0.82 ± 0.04^{aA}	0.82 ± 0.06^{aA} (0%)
30th	0.85 ± 0.03^{aA}	0.81 ± 0.05^{aA} (-4.70%)
60th	0.99 ± 0.05^{aA}	0.89 ± 0.04^{aA} (-10.24%)
90th	1.21 ± 0.06^{aB}	0.93 ± 0.03^{bA} (-23.14%)

*Different small alphabetic letters (a and b) indicate significant (P<0.05) difference between groups on particular DPT, whereas different capital alphabets (A and B) indicate significant (P<0.05) difference within days in a particular group (DPT= Days post treatment).

Cell Mediated Immunity

Cell mediated immune response was studied by using T-lymphocyte blastogenesis assay for both mature and immature T lymphocytes, Macrophage Function Test, and delayed type hypersensitivity reaction.

Table 5: Mean (Mean ± SE) delta optical density values in B-lymphocyte blastogenesis assay using LPS in different groups of experimental rats at different time intervals of the experiment

Day Post	Delta optical density (Mean ± SE)*	
Treatment	Group I (Control)	Group II (Treated)
0	0.70±0.08^{aA}	0.70±0.08^{aA} (0%)
30th	0.76±0.05^{aA}	0.71±0.09^{aA} (-6.57%)
60th	0.92±0.05^{aB}	0.78±0.07^{aA} (-15.21%)
90th	1.37±0.14^{aBC}	0.98±0.07^{bB} (-28.46%)

*Different small alphabetic letters (a and b) indicate significant (P<0.05) difference between groups on particular DPT, whereas different capital alphabets (A and B) indicate significant (P<0.05) difference within days in a particular group (DPT= Days post treatment).

Table 6:Mean (Mean ± SE) delta optical density values in T-lymphocyte blastogenesis assay using Con-A indifferent groups of experimental rats at different time intervals of the experiment

Day Post	Delta optical density (Mean ± SE)*	
Treatment	Group I (Control)	Group II (Treated)
0	0.73±0.03^{aA}	0.73±0.03^{aA} (0%)
30th	0.75±0.07^{aA}	0.69±0.05^{aA} (-8%)
60th	0.81±0.06^{aA}	0.74±0.08^{aA} (-8.64%)
90th	1.4±0.07^{aB}	0.85±0.08^{bA} (-39.28%)

*Different small alphabetic letters (a and b) indicate significant (P<0.05) difference between groups on particular DPT, whereas different capital alphabets (A and B) indicate significant (P<0.05) difference within days in a particular group (DPT= Days post treatment).

Table 7: Mean (Mean ± SE) delta optical density values in T-lymphocyte blastogenesis assay using PHA-M in different groups of rats at different time intervals of experiment

Ds		
0	0.61±0.04^{aA}	0.61±0.04^{aA}(0%)
30th	0.65 ± 0.07^{aA}	0.62±0.09^{aA}(-4.61%)
60th	0.89± 0.09^{aB}	0.78±0.15^{aB} (-12.35%)
90th	1.2±0.12^{aBC}	0.88±0.07^{bB} (-26.66%)

*Different small alphabetic letters (a and b) indicate significant (P<0.05) difference between groups on particular DPT, whereas different capital alphabets (A and B) indicate significant (P<0.05) difference within days in a particular group (DPT= Days post treatment).

T- Cell blastogenesis assay using Concanavalin A

The values of mean Δ optical density (ΔOD) in both the groups of experimental rats at different time intervals are presented in Table 6. Mean ΔOD values in group I rats were 0.73± 0.03, 0.75± 0.07, 0.81±0.06 and 1.4±0.07 at 0, 30th, 60th and 90th DPT, respectively. In group II rats, mean ΔOD values were 0.73±0.03, 0.69±0.05, 0.74±0.08 and 0.85±0.08 at 0, 30th, 60th and 90th DPT, respectively. The values of mean ΔOD in group II rats were decreased by 8%, 8.64% and 39.28% at 30th, 60th and 90th DPT when compared with the values of control group. The decrease in group II values was significant as compared to group I at 90th DPT. When these values were compared within the same group at different time inter-

Table 8: Mean (Mean ± SE) NBT positive cells in macrophage function test in different groups of rats at different time intervals of experiment

Day Post	NBT positive cells in % (Mean ± SE)*	
Treatment	Group I (Control)	Group II (Treated)
0	48±1.84^{aA}	48±1.82^{aA} (0%)
30th	48.6±1.47^{aA}	46±1.35^{aA} (-5.34%)
60th	50.2±1.98^{aA}	42.4±2.64^{aA}(-15.53%)
90th	49.8±2.52^{aA}	37.5±3.48^{bA}(-24.69%)

* Different small alphabetic letters (a and b) indicate significant (P<0.05) difference between groups on particular DPT, whereas different capital alphabets (A and B) indicate significant (P<0.05) difference within days in a particular group (DPT= Days post treatment).

Table 9: Mean (Mean ± SE) skin thickness measured during delayed type hypersensitivity reaction in different groups of rats at different time intervals post antigen challenge

Hours post-	Thickness of skin in cm (Mean ± SE)*	
challenge	Group I (Control)	Group II (Treated)
0	0.18±0.03^{aA}	0.13±0.02^{bA} (-27.77 %)
12th	0.25±0.02^{aB}	0.19±0.05^{bB} (-24%)
24th	0.62±0.04^{aBC}	0.55±0.03^{aBC} (-11.29%)
48th	0.76±0.03^{aBCD}	0.68±0.02^{aBCD} (-10.52%)
72nd	0.71±0.02^{aBCE}	0.64±0.04^{aBCD} (-9.85%)

* Different small alphabetic letters (a and b) indicate significant (P<0.05) difference between groups on particular DPT, whereas different capital alphabets (A, B, C, D and E) indicate significant (P<0.05) difference within days in a particular group (DPT= Days post treatment).

vals, there was a significant increase in the values of group I at 90th DPT compared to 0 DPT but there was no significant difference in the values of group II rats throughout the experiment.

T- Cell blastogenesis assay using Phytohaemagglutinin- M

The values of mean Δ optical density (ΔOD) in both the groups of experimental rats at different time intervals are presented in Table 7. Mean ΔOD values in group I rats were 0.61± 0.04, 0.65± 0.07, 0.89±0.09 and 1.2±0.12 at 0, 30th, 60th and 90th DPT, respectively. In group II rats, mean ΔOD values were 0.61±0.04, 0.62±0.09, 0.78±0.15 and 0.88±0.07 at 0, 30th, 60th and 90th DPT, respectively. The values of mean ΔOD in group II rats were decreased by 4.61%, 12.35% and 26.66% at 30th, 60th and 90th DPT when compared with the values of control group. There is a significant decrease in mean Δ OD of rats of group II rats when compared with that of group I rats at 90th DPT. When these values were compared within the same group at different time intervals, there was a significant increase in these values of group I and II rats at 60th and 90th DPT compared to 0 DPT.

Macrophage Function Test

The mean values of NBT positive cells i. e. cells containing blue/black granules, of both the groups of rats at different time intervals,expressed in % and are presented in Table 8. The values of mean NBT positive cells in rats of control group were 48±1.84, 48.6±1.47, 50.2±1.98 and 49.8±2.52 and in rats of test group were 48±1.82, 46±1.35, 42.4±2.64 and 37.5±3.48 at day 0th, 30th, 60th and 90th of the experiment, respectively. Mean NBT positive cells in test group were recorded decreased at day 30th, 60th and 90th by 5.34%, 15.53% and 24.69%, respectively when compared with control group. Significant decrease in mean NBT positive cells of group II rats was observed at 90th DPT when compared with that of group I rats. When these values were compared within the same group at different time intervals, there was no significant difference in the values of group I and II rats at any time interval.

Delayed Type Hypersensitivity Reaction

Mean skin thickness of experimental rats in both the groups at different time intervals expressed in cm and are presented in Table 9. The values of mean thickness of the skin in control group were 0.18±0.03, 0.25±0.02, 0.62±0.04, 0.76±0.03 and 0.710.02 and in treated group were 0.13±0.02, 0.19±0.05, 0.55±0.03, 0.68±0.02 and 0.640.04 at 0, 12th, 24th, 48th and 72thhour post challenge (HPC), respectively. The values of mean thickness of skin in group II rats decreased by 27.77 %, 24 %, 11.29 %, 10.52 % and 9.85 % at 0, 12th, 24th, 48th and 72th HPC respectively. Results of mean skin thickness showed significant decrease between group II and group I at 0 and 12 HPC. Significant increase in mean skin thickness was observed in group I between 0-12, 0-24, 0-48, 0-72, 12-24, 12-48, 12-72 and 24-48 HPC and in group II significant increase in mean skin thickness was observed between 0-12, 0-24, 0-48 and 0-72HPC.

There was significant decrease in serum gamma globulin values at 90th DPT in group II rats compared to group I rats. Immunoglobulins that are mainly produced by B cells, play role in neutralization of antigen and toxin (Vali *et al.,* 2020). The significant decrease in serum gamma globulin in silver nanoparticles treated rats might be due to the fact that nanoparticles can bind to immune proteins or enzymes in systemic circulation, and lower their activities. Silver nanoparticles can exhaust immune cells through continuous stimulation at lower concentrations and degenerate them through oxidative damage at higher concentrations. Similar results were obtained by Vali *et al.,* 2020 in silver nanoparticles exposed common carp.

Decreased antibody concentration was recorded in serum of the group II rats in comparison to the controls as measured by HI and ELISA. The HI titer represents the specific antibodies present in the serum of rats due to the response of Ranikhet disease vaccine including both IgM and IgG immunoglobulins. That indicates the immunotoxic effect of nanoparticles on the synthesis of immunoglobulins leading to decrease in their titer in serum. ELISA was used to measure the level of serum IgG concen-

tration in groups I and II of rats; results of which also suggest a decrease in the concentration of IgG antibodies. It further confirms the immunotoxic effect of the nanoparticles on antibodies which might be exhibited with a serious outcome of immunodeficiency leading to vaccine failure and increased susceptibility of the individuals resulting in occurrence of new diseases and increased incidence of cancer (Banga *et al.,* 2005).

Decrease B-lymphocyte activity measured as mean delta optical density in B lymphocytes blastogenesis assay using LPS as mitogen was observed in splenic cell cultures of group II rats compared to the controls. Significant decrease in values was observed at 90th DPT, which was considered as an important parameter to indicate decrease in antibody synthesis. It further confirmed the deleterious toxic effect on antibodies synthesis and lympho-cytotoxic effect on B lymphocytes due to silver nanoparticles. These results are in accordance with that of Ghosh *et al.,* 2012; Greulich *et al.,* 2011; Huang *et al.,* 2016; Shin *et al.,* 2007. In these studies, it was revealed that AgNPs causes cytotoxicity and inhibition of proliferation in B-lymphocytes. Li and associates in 2018 also demonstrated a time- and dose-dependent cytotoxicity in cPBMCs involving apoptosis at high concentrations of citrate-silver nanoparticles (10 and 50 μg/ml). Even at sub-lethal doses of 0.1 and 1 μg/ml, citrate-silver nanoparticles negatively affected the proliferative activity of PBMCs (Li *et al.,* 2018).
Decreased percentage of NBT positive cells in nanoparticles treated group II rats in comparison to group I rats in macrophage function test is an indication of down regulation of macrophage functions leading to reduced engulfment and digestion power of the phagocytic cells. This deleterious effect of nanoparticles on phagocytic cells might further manifested in the form of down regulation of B lymphocytes resulting in decreased antibody titer because macrophages do participate in the process of antigen presentation and processing to furthering the immune process and remain in contact with other cells through a cell-to-cell communication. Under the circumstances of down regulation of phagocytic cells, the cell-to-cell communication also get disturbed due to the lack or inefficient production and / or activity of cytokines particularly the interleukins leading to down regulated immune functions (Chauhan and Tripathi, 2002). Similar changes were also observed in various studies, where cytotoxicity and inhibition of proliferation of macrophages were revealed on long term exposure to silver nanoparticles (Ghosh *et al.,* 2012; Greulich *et al.,* 2011; Huang *et al.,* 2016; Shin *et al.,* 2007).

Decreased mean thickness of the skin in group II rats, in which DNFB was applied to assess the delayed type hypersensitivity (DTH) reaction, was also observed when compared with the group I rats. Although the results were not statistically significant but these tests suggest deteriorating effect of silver nanoparticles on the cell mediated immunity. It may be due to the cytotoxic effect of the nanoparticles on the immune cells. Results are also confirmed by decreased absolute lymphocyte count and monocyte count in the group II test rats (Kumar, 2021). Silver nanoparticles are shown to elicit an immunosuppressive response (Ngobili and Daniele, 2016). T- Lymphocyte blastogenesis assay was also performed to assess the effect of nanoparticles on the blastogenesis activity of the T lymphocytes. Decrease in mean delta optical density in T lymphocytes blastogenesis assay using Con-A and PHA-M was observed in group II rats at 30th, 60thand 90th DPT as compared to the group I rats. Results were found to be significant at 90th DPT when Con-A as well as PHA-M was used as mitogen. This might because of the lympho-cytotoxic effect of the silver nanoparticles. It is a known fact that the mitogen Con-A acts on immature or newly recruited lymphocytes while PHA-M acts on fully differentiated and mature lymphoid cells; down regulation in the activity of both the types of cells is indicative of immuno-suppression particularly of cell mediated immunity. Various studies revealed cytotoxicity and inhibition of lymphocyte proliferation on long term exposure to silver nanoparticles (Ghosh *et al.,* 2012; Greulich *et al.,* 2011; Huang *et al.,* 2016; Shin *et al.,* 2007). In the present study, the T lymphocytes were first sensitized due to vaccination of the rats with Newcastle Disease vaccine and then on challenge by DNFB such sensitized cells get accumulated in skin where the mitogen application was done. Mean skin thickness was less in the nanosilver

treated group due to decreased accumulation of these cells as a result of deleterious effect of nanoparticles on the activity of the participating cells including down regulation of cell-to-cell communication, reduced cell migration at the site of challenge and reduced activity of leucocyte migration inhibition factor that leads to decreased DTH reaction. All this in conjunction, down regulation of T lymphocyte blastogenesis and DTH reaction are indicative of decreased cell mediated immunity due to the deleterious immunotoxic effects of silver nanoparticles. These results are supported by decrease in absolute lymphocyte count and total leucocyte count (Kumar *et al.,* 2024) along with depletion and necrosis observed in white pulp area of spleen in the test rats during the experiment (Kumar, 2021).

CONCLUSION

It is concluded from the present study that nanosilver has adverse effects on immune system leading to decrease in both humoral as well as cell mediated immune responses in Wistar rats at NOAEL dose for a period of 90 days.

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REFERENCES

Al Gurabi, M. A., Ali, D., Alkahtani, S. and Alarifi, S. (2015). *In vivo* DNA damaging and apoptotic potential of silver nanoparticles in Swiss albino mice. *Onco Targets Ther.,* 8: 295-302.

Allan, W. H. and Gough, R. E. (1974). A standard haemagglutination inhibition test for new castle disease. A comparison of macro and micro methods. *Vet. Rec.*, 95 (6):120-123.

Azadmehr, A., Hajiaghaee, R., Molla Hassan, A. T., Jafari Vesiehsari, M. and Oladnabidozin, M. (2016). Splenocyte proliferation, NK cell activation and cytokines production by extract of *Scrophularia variegata*; an *in vitro* study on micespleen cells. *Pharmacogn. Res.,* 3 (4): 9-15.

Banga, R. K., Chauhan, R. S. and Telang, A. G. (2005). Immunodeficiency disease of livestock and poultry: A review. *Ind. J. Ani. Sci.,*75 (4): 445-455.

Chauhan, R. S. (1998). *Laboratory Manual of Immunopathology*, G. B. Pant University of Agriculture & Technology. Pantnagar, India, Pp.19-52.

Chauhan, R. S. and Tripathi, B. N. (2002). *Veterinary Immunopathology: Theory and practice,* 1st edition. International Book Dist. Co. Lucknow. Pp221.

Curzytek, K., Kubera, M., Majewska- Szczepanik, M., Szczepanik, M., Marciñska, K., Ptak,W., Duda,W., Leœkiewicz, M., Basta-Kaim, A., Budziszewska, B. and Lasoñ, W. (2013). Inhibition of 2,4-dinitrofluorobenzene-induced contacthy persensitivity reaction by antidepressant drugs. *Pharmacol. Rep.,* 65 (5):1237-1246.

Ema, M., Kobayashi, N., Naya, M., Hanai, S. and Nakanishi, J. (2010). Reproductive and developmental toxicity studies of manufactured nanomaterials. *J. Reprod. Toxicol.,* 30 (3): 343–352.

Ghosh, M., Manivannan, J., Sinha, S., Chakraborty, A., Mallick, S. K., Bandyopadhyay, M. and Mukherjee, A. (2012). *In vitro* and *in vivo* geno toxicity of silver nano particles. *Mutat. Res. Genet. Toxicol. Environ. Mutagen,*749 (1-2): 60-69.

Greulich, C., Diendorf, J., Simon, T., Eggeler, G., Epple, M. and Köller, M. (2011). Uptake and intra cellular distribution of silver nano particles in human mesenchymal stem cells. *Acta Biomater*, 7 (1):347-354.

Huang, H., Lai, W., Cui, M., Liang, L., Lin, Y., Fang, Q., Liu, Y. and Xie, L. (2016). An evaluation of blood compatibility of silver nano particles. *Sci. Rep.,*6 (1): 1-15.

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

Kim, Y. S., Song, M. Y., Park, J. D., Song, K. S., Ryu, H. R., Chung, Y. H., Chang,H. K., Lee, J. H., Oh, K. H., Kelman, B. J. and Hwang, I. K. (2010). Subchronicoral toxicity of silver nanoparticles. *Part. Fibre Toxicol*., 7 (1):1-11.

Kumar, N. (2021). Clinicopathological studies on nanosilver administered Wistar rats. MVSc Thesis. G.B. Pant University of Agriculture and Technology, Pantnagar.

Kumar, N., Batra, M., Verma, M. K., Singh, J. and Chauhan, R. S. (2024). Clinico-hematological effects of nanosilver administration in Wistar Rats. *Journal of Advances in Biology and Biotechnology*, 27 (3): 171-177.

Li,W. T.,Chang, H. W., Yang, W. C., Lo, C., Wang, L. Y., Pang, V. F., Chen, M. H. and Jeng,C. R. (2018). Immunotoxicity of silver nano particles (Silver nano particles) on the leukocytes of common bottlenose dolphins (*Tursiop struncatus*). *Sci. rep.,* 8 (1): 1-12.

Natsuki, J., Natsuki, T. and Hashimoto, Y. (2015). A review of silver nanoparticles: synthesis methods, properties, and applications. *Int. J. Mater. Sci. Appl.,* 4 (5): 325-332.

Ngobili, T. A. and Daniele, M. A. (2016). Nano particles and direct immuno suppression. *Experimental Biology and Medicine*, 241 (10):1064-1073.

Sanhai, W. R., Sakamoto, J. H., Canady, R. and Ferrari, M. (2008). Seven challenges for nanomedicine. *J. Nat. Nanotechnol.,* 3 (5): 242–244.

Shin, S. H., Ye, M. K., Kim, H. S. and Kang, H. S. (2007). The effects of nano-silver onthe proliferation and cytokine expression by peripheral blood mononuclearcells. *Int. Immunopharmacol.,* 7 (13): 1813-1818.

Snedecor, G. W. and Cochran, W. G. (1994). Statistical Methods, 8th edition. Ames Iowa State University Press, USA. 507

Vali, S., Mohammadi, G., Tavabe, K. R., Moghadas, F. and Naserabad, S. S. (2020). The effects of silver nanoparticles (Ag-NPs) sublethal concentrations on common carp (*Cyprinus carpio*): Bioaccumulation, hematology, serum biochemistry and immunology, antioxidant enzymes, and skin mucosal responses. *Ecotoxicol. Environ. Saf.,* 194: 110353.

Zhang, J. and Saltzman, M. (2013). Engineering biodegradable nanoparticles for drug and gene delivery. *Chem. Eng. Prog*., 109: 25–30.

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