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Effect of iron oxide and aluminium oxide nanoparticles on biochemical parameters in Wistar rats

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ABSTRACT: The current research aimed to investigate the changes in biochemical characteristics induced by nano-iron oxide and nano-aluminium oxide particles in Wistar rats during a 90-day repeated dose study. A total of 35 Wistar rats were randomly divided into two groups: control (with 20 animals) and test (with 15 animals). Throughout the duration of 90 days of the experiment, the control group received standard feed and purified reverse osmosis water. The treatment group was administered with a mixture of nano-iron oxide and nano-aluminium oxide in distilled water at a dose 15mg/kg body weight for nano-iron oxide and 3mg/kg body weight for nano-aluminium oxide, along with standard feed and RO water. Blood samples were collected from both G1 and G2 on 30th, 60th and 90th DPT for biochemical studies. There was significant increase in aspartate aminotransferase (AST),alanine aminotransferase (ALT),creatine and BUN whereas there was decrease in serum total protein,serum albumin,serum globulin and serum gamma globulin in treated group as compared to the control group. Based on the above findings, it concluded that combination of iron and aluminium nano particles at the rate of half of their NOAEL dose causes ill effects on the health status of Wistar Rats. It induces alteration in various biochemical parameters and thus causes health hazards.

Key words: Aluminium oxide,biochemical, Iron oxide,nanoparticles,Wistar rats

A nanomaterial is defined as a particle that possesses at least one dimension within the nanometer scale, typically ranging from one to a few hundred nanometers (Cui and Gao, 2003). The primary goal of nanotechnology is to gain a profound understanding of nanoscale phenomena and materials. The term "nanotechnology" originates from the Greek word "nanos," which means "dwarf. " It mainly focuses on creating structures, devices, and systems with unique properties and functions attributed to their incredibly small size. There is a need for a more thorough examination into the exposure concerns posed by engineered nanomaterials (ENMs) to people and other living things because of the rapid increase in nanotechnology application in consumer goods.

Iron is a crucial metal ion in the body, playing essential roles in various physiological processes, such as DNA synthesis, mitochondrial respiration, and oxygen transportation (Mounsey and Teismann, 2012). The body relies on iron for synthesizing oxygen transport proteins, particularly hemoglobin and myoglobin, as well as for forming heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reduction reactions (McDowell, 2003). Iron nanoparticles find application as food additives in Fe-fortified beverages and cereals for human consumption (Fidler *et al.,* 2004). Excessive intake of iron can harm the intestinal mucosa and increase its permeability (Nurmi *et al.,* 2005). Notably, nano-sized Zero-valent iron (FeO) exhibits higher reactivity compared to micro-sized FeO particles (Nchito *et al*., 2006). Additionally, Fe metal serves as an essential micronutrient vital for numerous crucial biological processes and ranks as the most abundant transition metal in the body. However, it can induce oxidative stress in aqueous solutions through the generation of reactive oxygen species (ROS) (Puntarulo, 2005).

Aluminium (Al) is the world's third most abundant element and is widely recognized as a neurotoxin in the environment. Aluminium-based nanoparticles (NPs) have diverse applications in fields like fuel

cells, polymers, paints, coatings, textiles, and biomaterials. However, studies have indicated the toxic effects of aluminium oxide nanoparticles (Al_2O_3-NPs) on cell viability, mitochondrial function, oxidative stress, and the expression of tight junction proteins in the blood-brain barrier (BBB) (Chen *et al.,* 2008).

The environmental and health implications of Al_2O_3-NPs have garnered significant interest due to their common use in abrasive, wear-resistant coatings, solid rocket fuel, and drug delivery systems (Tyner *et al.,* 2004). With the increasing utilization of these nanoparticles, human and animal exposure is rising through various entry routes like ingestion, inhalation, and dermal penetration, prompting extensive research on their health effects.

MATERIALS AND METHODS

The study was conducted over a 90-day period using 35 Wistar rats, both male and female, aged 18 weeks. These rats were randomly divided into two groups: a control group consisting of 20 rats and a test group with 15 rats. Iron oxide and Aluminium Oxide (Boehmite) nano dispersion were used as the nanoparticles in this research.

The iron oxide and aluminium oxide (Boehmite) nanodispersion, procured from Sisco Research Laboratories Pvt. Ltd, was orally administered to the rats at a dose of 15mg/kg body weight and 3mg/kg body weight respectively per day, which is half of the NOAEL dose. The rats were provided with standard ration from the beginning of the experiment and had access to RO drinking water ad-libitum until the last day of the 90-day study.

Before commencing the experiment, all the rats were given a period of 7 days to acclimate to the experimental animal house.

Serum Biochemistry

Biochemical tests were performed using standard protocol given along with kits supplied by ERBA India limited (Koller, 1980).

Total Serum Protein

In serum of test and control rats, total serum protein was measured using standard protocol of Biurate method using Erba total protein kit. For this 1000µl of the reagent provided in the kit was taken in three eppendorf and marked them control, standard and test.20µl of DW, standard mentioned in the kit and test serum sample was added in the control, standard and test eppendorfs, respectively. These eppendorfs were then incubated at 37°C for 10min and then absorbance of the standard and test was taken against the control at 546 nm.

Serum Albumin

In tests and control rats, serum albumin was measured using standard protocol of bromocresol green dye method using Erba albumin kit. For this 1000µl of the reagent provided in the kit was taken in three eppendorf's and marked them control, standard and test.10ul of each i. e. DW, standard given in kit and test serum sample was added in the control, standard and test eppendorfs, respectively. These eppendorfs then incubated at 37°C for 1 min and then absorbance of the standard and test was taken against the control at 630 nm.

Serum Globulin

Concentration of the serum globulin was calculated by subtracting concentration of serum albumin form from concentration of total serum protein.

Serum Gamma Globulin

Serum gamma globulin concentration was determined by mixing 5.7ml ammonium sulfate (19.5%) and sodium chloride (2.03%) solution and 0.3ml serum sample. After mixing, the solution was kept in ice bath for overnight and then it was centrifuged at 1250g for 10 min to separate the precipitate. Precipitate obtained was then dissolved in the 0.2 ml NSS and process of obtaining precipitate was repeated. Precipitate obtained was then dissolved in 2ml of NSS and mixed with 5ml of biurate regent. Mixture was then incubated at room temperature for

10 minutes and optical density was read at 555 nm (Jager and Nickerson, 1948; Chauhan, 1998).

Serum Creatinine

In tests and control rats, serum creatinine was estimated using standard protocol of Jaffe's method using Erba creatinine kit. For this 1000µl of the reagent provided in the kit was taken in two eppendorf and marked them standard and test.100 µl of each i. e., standard given in kit and test serum sample was added and mixed in the standard and test eppendorf's, respectively. Initial and final absorbance of the standard and test was taken 20sec and 80sec after mixing, respectively at 505 nm. The difference of both the reading was presented as delta absorbance.

Serum Blood Urea Nitrogen (BUN)

Serum blood urea nitrogen was determined by GLDH- Urease method, using a kit from Erba Diagnostics Mannheim Ltd. Baddi, Dist. Solan (HP), India. The standard and test were prepared as per the standard procedure given in the kit and the absorbance of the standard and each of the test were read at 340 nm at 20 (A1) and 80 (A2) seconds after mixing using UV-Vis spectrophotometer. The results were expressed as milligram per deciliter (mg/dl).

Aspartate Aminotransferase (AST)

In tests and control rats, serum AST was measured using standard protocol recommended by International Federation of Clinical Chemistry using Erba AST kit. For this 1000µl of the reagent provided in the kit was taken and mixed with 100µl of the test serum sample. Initial and final absorbance of the mixture was taken at lag time of 60 sec at 340nm.

Alanine Aminotransferase (ALT)

In tests and control rats, serum ALT was measured using standard protocol recommended by International Federation of Clinical Chemistry using Erba ALT kit for this 1000ul of the reagent provided in the kit was taken and mixed with 100ul of the test serum sample. Initial and final absorbance of the mixture was taken at lag time of 60 sec at 340nm.

RESULTS AND DISCUSSION

Total Serum Protein

The mean values of total serum protein in rats of control and treated group weredetermined at every 30-day interval during the course of experiment. The data of mean values of total serum protein are expressed in g/dl and are presented in Table 1 and Figure 1. The mean values of total serum protein in rats of control group were 5.42+0.22, 5.72±0.30, 6.08+0.25 and 6.64±0.17 g/dl and in rats of treated group were to 5.42+0.22, 5.55+0.23, 5.57+0.21 and 5.60±0.25 g/dl at day 0, 30, 60 and 90 of the experiment, respectively. Total serum protein for treated group was recorded decreased at day 30, 60 and 90 by 2.97%, 8.38% and 15.6%, respectively when compared with control group. Results of mean values of total serum protein showed significant difference between test and control group at 90 DPT. Significant difference in mean values of total serum protein level was observed in control group between 0-90 and 30-90 DPT and in treated group there was no statistically significant difference observed.

Serum Albumin

The values of mean serum albumin in rats of control and treated group were determined at every 30-day interval during the course of experiment. The data of mean serum albumin are expressed in g/dl and are presented in Table 2 and figure 2. The values of mean serum albumin in rats of control group were2.68+0.18, 2.74+0.26.2.73±0.11 and 2.78+0.15 g/dl and in rats of treated group were 2.68+0.18, 2.91+0.18, 2.61±0.21 and 2.55+0.17 g/dl at day 0, 30, 60 and 90 of the experiment, respectively. Mean serum albumin for treated group was recorded to increase at day 30 by 6.56% and then decreased at day 60 and 90 by 4.39% and 8.27%, respectively when compared with control group. There was no statistically significant difference recorded between the groups and within the groups.

Table 1: Total serum protein (g/dl)of experimental rats at different time intervals

Day Post-Treatment	Total Serum Protein (Mean ± SE)		% increase or decrease than control
	Group I (Control)	Group II (Fe_2O_3+Al_2O_3NP)	
0	5.42±0.22^{A}	5.42±1.22	(0%)
30	5.72±0.30^{A}	5.55±0.23	(-2.97%)
60	6.08±0.25^{AB}	5.57±0.21	(-8.38%)
90	6.64±0.17^{aB}	5.60±0.25^{b}	(-15.6%)

Alphabetical letters (a and b) indicate significant (P<0.05) difference between group at a particular DPT whereas different alphabetical letters (A and B) indicate significant (P<0.05) difference within days in a particular group

Table 2: Serum Albumin (g/dl) of experimental rats at different time intervals

Day Post- Treatment	Serum Albumin Value (g/dl) (Mean ± SE)		% increase or decrease than control
	Group I (Control)	Group II (Fe_2O_3+Al_2O_3NP)	
0	2.68±0.18	2.68±0.18	(0%)
30	2.74±0.26	2.91±0.18	(6.56%)
60	2.73±0.11	2.61±0.21	(-4.39%)
90	2.78±0.15	2.55±0.17	(-8.27%)

Table 3: Serum Globulin (g/dl) of experimental rats at different time intervals

Day Post- Treatment	Serum Globulin (g/dl) (Mean ± SE)		% increase or decrease than control
	Group I (Control)	Group II (Fe_2O_3+Al_2O_3NP)	
0	2.95±0.06^{A}	2.95±0.06^{AB}	(0%)
30	2.60±0.14^{A}	2.94±0.08^{AB}	(43.75%)
60	4.52±0.31^{B}	3.48±0.18^{B}	(-15.78%)
90	5.01±0.11^{aC}	2.54±0.10^{aB}	(-36.36%)

Alphabetical letters (a & b) indicate significant (P<0.05) difference between groups at a particular DPT whereas different alphabetical letters (A, B and C) indicate significant (P<0.05) difference within days in a particular group

Table 4: Serum Gamma Globulin (g/dl) of experimental rats at different timeintervals

Day Post- Treatment	Serum Gamma Globulin in (g/dl) (Mean ± SE)		% increase or decrease than control
	Group I (Control)	Group II (Fe_2O_3+Al_2O_3NP)	
0	0.15±0.02	0.15±0.02^{AB}	(0%)
30	0.16±0.03	0.23±0.02^{B}	(43.75%)
60	0.19±0.01	0.16±0.03^{AB}	(-15.78%)
90	0.22±0.01^{a}	0.14±0.02^{bA}	(-36.36%)

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

Table 5: Serum Creatinine (mg/dl) of experimental rats at different time intervals of the experimental period

Day Post- Treatment	Serum Creatinine (mg/dl) (Mean ± SE)		% increase or decrease than control
	Group I (Control)	Group II (Fe_2O_3+Al_2O_3NP)	
0	0.42±0.03^{A}	0.42±0.03^{A}	(0%)
30	0.47±0.05^{AB}	0.58±0.07^{A}	(23.4%)
60	0.55±0.08^{AB}	0.75±0.02^{A}	(36.36%)
90	0.67±0.11^{aB}	1.19±0.07^{bB}	(77.6%)

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

Table 6: Serum BUN (mg/dl) of experimental rats at different time intervals of the experimental period

Day Post- Treatment	Serum BUN Value (mg/dl) (Mean ± SE)		% increase or decrease than control
	Group I (Control)	Group II (Fe_2O_3+Al_2O_3NP)	
0	20.97±1.39	20.97±1.39A	(0%)
30	21.35±1.39	23.32±0.07^{A}	(9.22%)
60	22.92±0.79^{a}	27.1±0.85bB	(18.25%)
90	22.62±0.85^{a}	31.61±0.60^{bC}	(39.74%)

*Alphabetical letters (a &b) indicate significant (P<0.05) difference between groups at a particular DPT whereas different alphabetical letters (A, B and C) indicate significant (P<0.05) difference within days in a particular group

Table 7: Mean Aspartate Aminotransferase (IU/L) of experimental rats at different time intervals

Day Post- Treatment	AST (IU/L) (Mean ± SE)		% increase or decrease than control
	Group I (Control)	Group II (Fe_2O_3+Al_2O_3NP)	
0	39.98±1.44^{A}	39.98±1.44^{A}	(0%)
30	41.72±1.39^{A}	42.46±1.20^{A}	(1.79%)
60	44.76±1.76^{aAB}	57.81±2.11^{bB}	(29.12%)
90	47.93±1.47^{aB}	66.44±2.43^{bC}	(38.61%)

*Alphabetical letters (a & b) indicate significant (P<0.05) difference between groups at a particular DPT whereas different alphabetical letters (A, B and C) indicate significant (P<0.05) difference within days in a particular group

Table 8: Alanine Aminotransferase (IU/L) of experimental rats at different time intervals

Day Post- Treatment	ALT (IU/L) (Mean ± SE)		% increase or decrease than control
	Group I (Control)	Group II (Fe_2O_3+Al_2O_3NP)	
0	24.43±2.24^{A}	24.43±2.24^{A}	(0%)
30	28.72±1.74^{AB}	31.62±0.94^{B}	(11.06%)
60	30.75±1.17^{aB}	37.75±1.32^{bC}	(22.76%)
90	33.16±1.20^{aB}	47.54±2.28^{bD}	(43.36%)

*Alphabetical letters (a & b) indicate significant (P<0.05) difference between groups at a particular DPT whereas different alphabetical letters (A, B and C) indicate significant (P<0.05) difference within days in a particular group

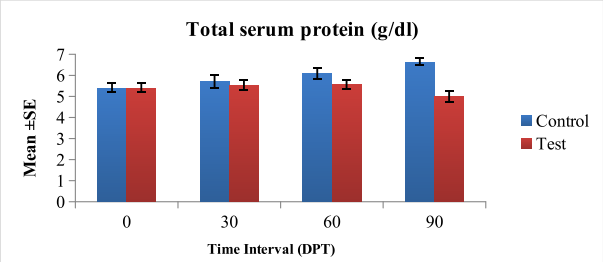

Fig.1: Total Serum protein (g/dl) ofexperimental rats

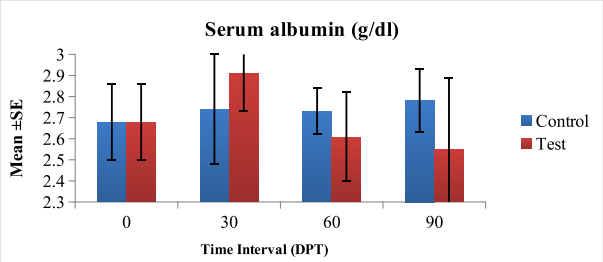

Fig.2:Serum albumin in g/dl of experimental rats at different time intervals

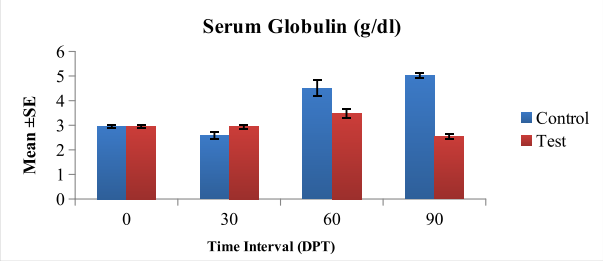

Fig.3: Serum globulin in g/dl of experimental rats at different time intervals

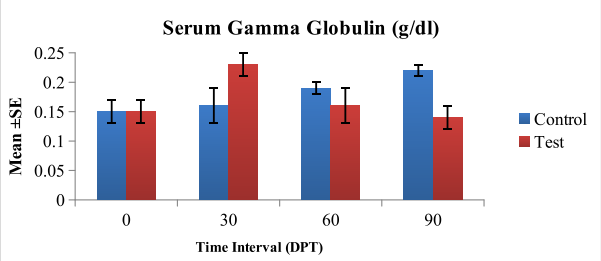

Fig.4:Serum gamma globulin (g/dl) of experimental rats at different time intervals

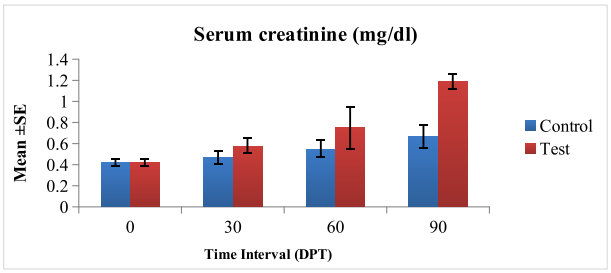

Fig.5:Serum creatinine (mg/dl) of experimental rats at different time intervals

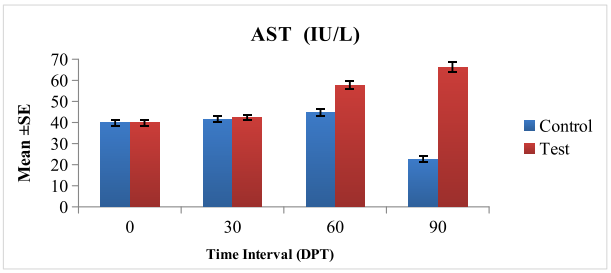

Fig.6:Serum BUN (mg/dl) of experimental rats at different time intervals

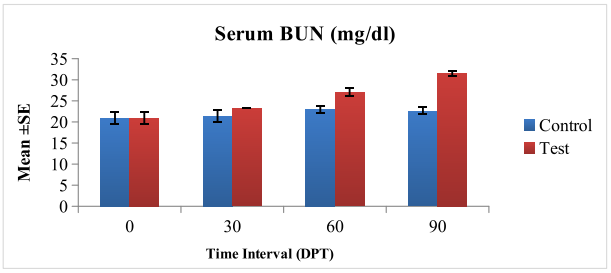

Fig.7:Aspartate Aminotransferase in IU/L of experimental rats at different time intervals

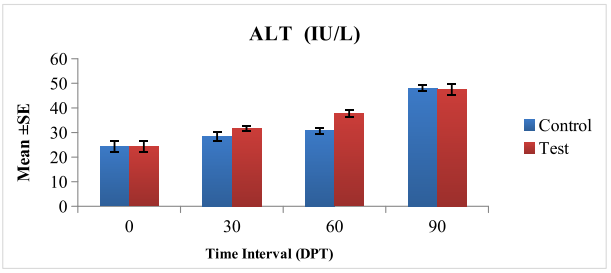

Fig.8:Alanine Aminotransferase in IU/L of experimental rats at different time intervals

Serum Globulin

The mean values of serum globulin in rats of control and treated groups were determined at every 30-day interval during the course of experiment. The data mean values of serum globulin are expressed in g/dl and are presented in Table 3 and figure 3. Mean serum globulin determined in rats of control group were 2.95±0.06.2.60±0.14, 4.52±0.19 and 5.01+0.11 g/dl and in rats of treated group were 2.95±0.06, 2.94 0.08, 3.48±0.18 and 2.54±0.10 g/dl at day 0, 30, 60 and 90 of the experiment, respectively. Mean serum globulin for treated group was recorded to increase at day 30 by 13.07% and then decreased at day 60 and 90 by 23% and 49.3%, respectively when compared with control group. Results showed significant difference between mean values of serum globulin in the test and control group at 90 DPT. Significant difference in serum globulin level was also observed in control group between 0-60, 0-90, 30-60, 30-90 and 60-90.

Serum Gamma Globulin

The values of mean serum gamma globulin in rats of control and treated group were determined at every 30 day interval during the course of experiment. The data obtained are expressed in g/dl and are presented in table 4 and figure 4. Mean serum gamma globulin obtained in rats of control group were 0.15±0.02, 0.16 0.03, 0.19±0.01 and 0.22±0.01 g/dl and in rats of treated group were 0.15±0.02, 0.23 ±0.02, 0.16 0.03 and 0.14±0.02 g/dl at 0, 30, 60 and 90 day of the experiment, respectively. Mean serum gamma globulin in treated group were recorded increased at day 30 by 43.75% and was recorded decreased at day 60 and 90 by 15.78% and 36.36%. respectively when compared with control group. Results showed significant difference between mean values of serum gamma globulin in the test and control group at 90 DPT. Significant difference in serum gamma globulin level was also observed in treated group between 30-90 DPT.

Serum Creatinine

The values of mean serum creatinine in rats of control and treated group were determined at every 30 day interval during the course of experiment. The values of mean serum creatininein rats are expressed

in mg/dl and are presented in Table 5 and figure 5. Mean serum creatinine in rats of control group were 0.42+0.03, 0.47±0.06, 0.55±0.08 and 0.67±0.11mg/dl and in rats of treated group were 0.42±0.03, 0.58±0.07, 0.75±0.21 and 1.19±0.07 mg/dl at 0, 30, 60 and 90 day of the experiment, respectively. Mean serum creatinine values in treated group were recorded increased at day 30, 60 and 90 by 23.4%, 36.36% and 77.6%, respectively when compared with control group. Results showed significant difference between test and control group at 90DPT. The Significant difference in mean serum creatinine levels were observed in control group between 0-90DPT and in treated group between 0-90, 30-90 and 60-90.

Serum BUN

The values of mean serum BUN in rats of control and treated group were determined at every 30 day interval during the course of experiment. The values of mean serum BUN in rats are expressed in mg/dl and are presented in table 6 and figure 6. Mean serum BUN in rats of control group were 20.97+1.39, 21.35+1.39, 22.92+0.79 and 22.62+0.85mg/dl and in rats of treated group were 20.97±1.39, 23.32+0.07, 27.1±0.85 and 31.61±0.60 mg/dl at 0, 30, 60 and 90 day of the experiment, respectively. Mean serum BUN values in treated group were recorded increased at day 30, 60 and 90 by 9.22%, 18.25% and 39.74%, respectively when compared with control group. Results showed significant difference between test and control group at 60 and 90 DPT. Significant difference in mean serum BUN levels were observed in treated group between 0-60, 0-90, 30-60, 30-90 and 60-90 DPT.

Serum Aspartate Aminotransferase (AST)

Mean AST values in rats of control and treated group were determined at every 30-day interval during the course of experiment. The data obtained are expressed in IU/L and are presented in Table 7 and figure 7. Mean AST values in rats of control group were 39.98+1.44, 41.72+1.39, 44.76±1.76 and 47.93±1.47 IU/L and in rats of treated group were 39.98±1.44, 42.46±1.20, 57.81+2.11 and 66.44+2.43 IU/L at 0, 30, 60 and 90 day of the experiment, respectively. Mean serum AST values in treated group were recorded increased at 30, 60 and 90 day by 1.79%, 29.12% and 38.61%, respectively when compared with control group. Results showed significant increase between mean serum AST values of test and control group at 60 and 90 DPT. Significant difference in serum AST was observed in control group between 0-90 and 30-90 DPT and in treated group significant difference was observed in between 0-60, 0- 90, 30-60, 30-90 and 60-90 DPT.

Serum Alanine Transaminase (ALT)

The mean ALT values in rats of control and treated group were determined at every 30-day interval, expressed in IU/L and are presented in Table 8 and figure 8. The values of mean ALT in rats of control group were 24.43+2.24, 28.47±1.74, 30.75+1.17 and 33.16±1.20 IU/L and in rats of treated group were 24.43+2.24, 31.62+0.94, 37.75+1.32 and 47.54±2.28 IU/L at 0, 30, 60 and 90 day of the experiment, respectively. Mean ALT values in rats of treated group were recorded increased at 30, 60 and 90day by 11.06%, 22.76% and 43.36%, respectively when compared with control group. Results showed significant increase between the values of mean serum ALT in test and control group at 60 and 90DPT. Significant difference in serum ALT was observed in control group between 0-60 and 0-90 and treated group between 0-30, 0-60, 0-90, 30-60, 30-90 and 60-90 DPT.

Decrease in serum protein, serum albumin, serum globulin and serum gamma globulin was observed in the treated rats when compared with the control rats. Albumin as an antioxidant and function as scavenger for free radicals to protect blood cells against oxidative damage (Tolia *et al.,* 2013). The free radicals produced by the nano iron and nano aluminium may lead to excessive utilization of albumin which may result itno decreased level of serum albumin and also decrease in total serum protein. In addition, this decrease in total serum protein can be attributed to the damage of rough endoplasmic reticulum caused by the ROS (Kutlubay *et al.,* 2007).

There was an elevated serum level of liver function enzyme i. e., AST and ALT in nano iron and nano aluminium treated group as compared to that of control during the course of experiment. Alanine transaminase (ALT) and aspartate aminotransferase (AST) are two of the most reliable markers of hepatocellular injury or necrosis. Liver is an organ of the reticuloendothelial system that is highly sensitive to oxidative stress due to its high blood flow (Nel *et al.,* 2006). NPs are primarily readily taken up by hepatocytes and Kupffer cells specialized macrophages located in the liver (Novotna *et al.,* 2012). So, this might be due to increase in production of free radicals and beginning of reactive oxygen species (ROS) reactions, that causes damage to hepatocytes in liver and increase the level of liver enzymes due to tissue destruction and releasing these enzymes into the blood stream. As both nano-iron and nano-aluminium enhance the production of ROS and thus damage the hepatocytes (Sadegh *et al.,* 2015; Morsy *et al.,* 2016). This will lead to the necrosis as seen in our histopathological examination. Damage to the liver membrane is the reason for the release of liver enzymes in blood. This result is also in confirmation with the study by Wang *et al.,* (2010) and Sadeghi *et al.,* (2015), on Wistar rats given IONPs, study by (Morsy *et al*., 2016) on nanoalumina fed rats, study by Shodhan, (2019) on Wistar rats admimistered nano-aluminum oxide and study by Babadi *et al*., (2012) on rats given IONPSs**.**

Increase in the concentration of serum BUN and Creatinine level was observed in the experiment. The increase in the level of BUN and serum creatinine is a significant indicator of renal dysfunction (El-Demerdash, 2004). Significant reduction in total serum protein as seen in our experiment may suggest enhanced protein catabolism which may be also responsible for increased BUN level in blood stream (Mahieu *et al.,* 2009). Increase in the serum creatinine may be attributed to relation between serum creatinine and glomerulus filtration rate shows parabolic reaction and level of serum creatinine rises only after 50% loss in the renal function (Hosten, 1990) and it may be due to the abnormal glomerular filtration rate (Aziz and Zabut, 2011). The results are confirmed by the abnormal dilation of vascular glomeruli as observed under histopathological examination of the kidneys, in the present experiment. Also previous data presented that, in vitro studies, iron and aluminium NPs are able to disrupt the renal system by exerting cytotoxic effects on glomerular and tubular cell lines, while the in vivo studies showed that NPs might alter the normal structure of the nephron thus affecting the physiological functions of the kidney (Iavicoli, 2016).

CONCLUSION

Based on the above findings, it can be concluded that combination of iron and aluminium NPs at the rate of half of their NOAEL dose i. e., 15mg/kg BW and 3mg/kg BW confirm toxic effects on various parts of the body leading to hepatopathy andnephropathy that may result into the down regulation of immunity. The characteristics of these nanoparticles such as smaller particle size, higher surface reactivity and higher relative surface area along with the native characteristics of iron and aluminium can be attributed to the above changes in the body parameters. Further studies, should be carried out in different animal models using varied doses and increased duration of iron and aluminium nanoparticles to exactly find out the immunosuppressive effect of these particles. As immunosuppression is not an acute toxicity, which can be easily monitored; it affects function of the immune system, and assessing functional changes involves long-term, systematic, multi- parameter evaluating various aspects of immunity. Also it can be suggested that, defining safety limits should be set for the usage of these nanoparticles in biomedical applications.

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