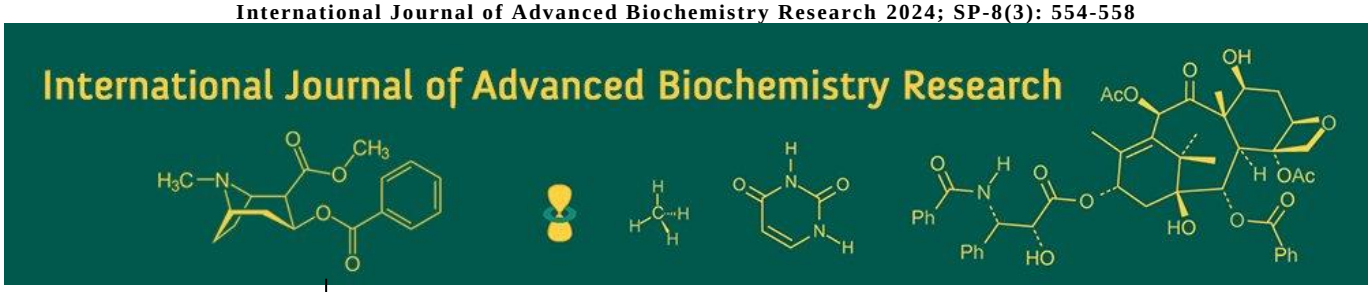

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Effects of nanosilver administration on biochemical parameters in wistar rats

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Abstract
The present study was conducted to know the effects of nanosilver (AgNPs) administration at NOAEL dose on biochemical parameters in Wistar rats for a period of 90 days. Thirty-five, 6-weeks-old, Wistar rats of both the sexes were divided randomly in two groups. Group 1 was kept as control and comprising of 20 rats. Group II was comprising of 15 rats and were orally administered silver nanoparticle mixed in distilled water orally at NOAEL dose rate of 30 mg/kg body weight/day from 0 day of experiment till 90th days post treatment (DPT). Blood was collected from 5 rats from each group at 0 (only from group I), 30th, 60th and 90th DPT and serum was used for biochemical studies. In group I rats, there was no significant change in any biochemical parameter at any DPT. Group II rats revealed significance increase in value was observed in ALT, AST, and cholesterol at 30th, 60th and 90th DPT as compared to group I rats. While total serum protein, serum albumin, serum gamma globulin and serum globulin levels were significantly decreased at 90th DPT in treated group as compared to control group. Serum glucose levels were also decreased at 30th, 60th and 90th DPT in treated group as compared to control. There was no significance difference in the serum calcium and serum phosphorus level in group I & II rats at any time interval. It can be concluded from the present studies that nanosilver exerted adverse effects on biochemical parameters of Wistar rats at NOAEL dose for a period of 90 days.

Keywords: Biochemical parameters, nanosilver, wistar rats, NOAEL dose

Introduction
Nanotechnology has provided us with solutions for early disease detection and treatment of metabolic disorders (Sanhai *et al*., 2008) [26]. Nanoparticles have great potential in regenerative medicine, such as tissue cultures. They display unique, physical, and chemical properties and represent an increasingly important material in the development of novel drugs and medicines (Zhang and Saltzman, 2013) [37]. The popularity of nanotechnology is increasing due to its positive benefits, but there must be serious concerns about its possible health risks (Ema *et al*., 2010) [13]. Toxicities linked with NPs and other nanoparticles are not limited to industrial or medicinal uses (Bahadar *et al*., 2016) [5]. 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 and animal and plant cell toxicity studies have been conducted. 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) [21]. Silver is used in medical applications such as wound dressings, urinary catheters, and other medical instruments due to its ability to prevent the growth of bacteria and fungi. The antimicrobial action of ionic silver stems from its ability to produce reactive oxygen species (ROS) and inactivate microbial enzymes has been known for centuries. Silver (particularly in the form of soluble silver compounds) has toxic effects in both animals and humans, in addition to its antibacterial properties.

Studies indicate that over exposure of silver ion in humans led to damage to various organs (Skalska and Strużyńska, 2015) [28] and (Drake and Hazelwood, 2005) [12]. The toxic effects are particularly more in people working in the silver mining, manufacturing, or packaging industries (Al Gurabi, *et al*., 2015) [2].

Keeping in view the above facts, the present study was planned to know the effects of nanosilver administration on biochemical parameters in Wistar rats at NOAEL dose for a period of 90 days.

Materials and Methods

Apparently healthy, six weeks old, 35 Wistar Rats of both the sexes were procured from Indian Veterinary Research Institute, Izatnagar, Bareilly, India. The rats were maintained in experimental animal house, under standard managemental conditions. The project was approved by Institutional Animal Ethics Committee. 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. The nanoparticles were suspended in distilled water and working samples of recommended dose formulation was prepared on daily basis during the entire period of study. The silver nanoparticles, as silver nitrate, were 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 in group II (Kim *et al*., 2010) [16]. The rats were vaccinated with 0.1 ml of R2B strain of Newcastle Disease vaccine intraperitoneally at 0 DPT and 30th DPT.

The blood samples were collected from 5 rats from each group (control and treated) at 0, 30th, 60th and 90th DPT and serum was used to study the biochemical parameters. The biochemical parameters studied were serum alanine transaminase (ALT) (Bradley *et al*., 1972) [7], serum aspartate aminotransferase (AST) (Wolf *et al*., 1972) [34], total serum protein (Flack and Wollen, 1984) [14], serum albumin, (Doumas *et al*., 1972) [11], serum globulin, serum gamma globulin (Chauhan, 1998) [9], serum glucose (Pennock *et al*., 1973) [23], serum total cholesterol (Allain *et al*., 1974) [4], serum calcium (Cossar and Fitzpatrick, 1974) [10] and serum phosphorus (Luthra, 2008) [19] were studied using commercial kits with trade name Erba. These kits were manufactured by Transasia Bio–medicals Pvt. Ltd., Solan, India. The data generated during the course of experiment was subjected to statistical analysis by using standard statistical protocol and procedures (Snedecor and Cochran, 1994) [29].

Table 1: Mean body weight and haematological parameters in different groups of rats at different time interval of the experiment

Parameters studied	Days Post-Treatment							
	0 Day		30th Day		60th Day		90th Day	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
ALT (IU/L)	29.78±0.62^{Aa}	29.78±0.62^{Aa}	31.39±0.71^{Ba}	34.15±1.54^{Cb}	33.30±0.86^{Da}	45.75±1.59^{Ec}	31.74±0.87^{Fa}	52.68±1.16^{Gd}
AST (IU/L)	72.37±2.04^{Aa}	72.37±2.04^{Aa}	72.65±1.62^{Ba}	79.33±1.04^{Cb}	73.47±2.04^{Da}	87.94±1.81^{Ec}	73.77±1.66^{Fa}	95.26±0.94^{Gd}
Serum protein (g/dl)	5.32±0.12^{Aa}	5.32±0.12^{Aa}	5.43±0.23^{Ba}	5.44±0.11^{Ba}	5.71±0.23^{Ca}	4.91±0.19^{Ca}	6.35±0.19^{Da}	4.33±0.20^{Ea}
Albumin (g/dl)	3.68±0.17^{Aa}	3.68±0.17^{Aa}	4.11±0.24^{Ba}	3.65±0.18^{Ba}	4.18±0.23^{Ca}	3.77±0.22^{Ca}	4.29±0.21^{Da}	3.28±0.16^{Ea}
Globulin (g/dl)	2.13±0.08^{Aa}	2.13±0.08^{Aa}	2.35±0.07^{Ba}	2.28±0.08^{Ba}	2.55±0.04^{Ca}	2.18±0.04^{Ca}	2.92±0.09^{Da}	1.95±0.18^{Eb}
Gamma Globulin (g/dl)	0.45±0.06^{Aa}	0.45±0.06^{Aa}	0.47±0.05^{Ba}	0.46±0.03^{Ba}	0.42±0.04^{Ca}	0.38±0.04^{Db}	0.44±0.04^{Ec}	0.34±0.03^{Fc}
Glucose (mg/dl)	93.63±6.67^{Aa}	93.63±6.67^{Aa}	95.08±6.37^{Ba}	94.36±3.35^{Ba}	96.56±5.93^{Ca}	90.67±1.98^{Db}	95.91±8.20^{Ea}	88.53±4.44^{Fc}
Cholesterol (mg/dl)	45.72±2.55^{Aa}	45.72±2.55^{Aa}	47.38±1.27^{Ba}	48.58±0.24^{Ba}	48.25±0.24^{Ca}	50.37±0.18^{Dc}	46.65±1.14^{Eb}	53.05±1.42^{Fd}
Calcium (mg/dl)	8.96±0.13^{Aa}	8.96±0.13^{Aa}	9.01±0.35^{Ba}	8.62±0.18^{Ba}	8.88±0.23^{Ca}	8.75±0.32^{Ca}	9.21±0.08^{Da}	8.08±0.07^{Da}
Phosphorus (mg/dl)	2.81±0.20^{Aa}	2.81±0.20^{Aa}	3.73±0.24^{Ba}	3.58±0.13^{Ba}	3.48±0.09^{Ca}	3.33±0.13^{Ca}	4.08±0.16^{Da}	3.98±0.16^{Da}

*Alphabetical letters (A, B, C, D, E, F and G) indicate significant (p<0.05) difference between groups at a particular DPT (Day Post-Treatment), whereas different alphabetical letters (a, b and c) indicate significant (p<0.05) difference within day in a particular group.

Results

Blood Serum concentration of ALT and AST

The data pertaining to the mean concentration of ALT (IU/L) and AST (IU/L) in blood serum of experimental rats presented in the Table 1. Analysis of variance indicated a significant increase in mean serum ALT and AST values at 30th, 60th and 90th DPT in silver nanoparticle treated rats as compared to group I rats. When these values were compared within the same group at different time intervals, there was no significant difference in group I rats at any time interval.

Total serum protein, albumin and Globulin

Mean total serum protein values of experimental rats in different groups at different time intervals, expressed in g/dl are presented in Table. 1. There was a significance decrease in mean total serum protein, albumin and globulin value at 90th DPT in group II rats as compared to group I rats. When these values were compared within the same group at different time intervals, there was no significant difference in both groups I and II at any time interval throughout experiment.

Serum gamma globulin

Mean serum gamma globulin values of experimental rats in both the groups at different time intervals expressed in g/dl are presented in Table 1. There was significant decrease in mean serum gamma globulin values at 60th and 90th DPT, respectively in group II rats as compared to group I rats.

Serum glucose

Mean serum glucose values expressed in mg/dl are presented in Table 1. There was significance decrease in mean serum glucose value at 60th and 90th DPT, respectively in silver nanoparticle treated group as compared to control.

Serum cholesterol

There was significance increase in mean serum cholesterol values at 60th and 90th DPT in group II rats as compared to group I rats. When these values were compared within the same group at different time intervals, there was a significant increase in mean serum cholesterol in group I rats at 90th DPT.

Calcium and Phosphorus

Mean serum calcium and phosphorus values of experimental rats in different groups at different time intervals expressed in mg/dl are presented in Table 1. There was no significant difference in mean calcium values in group II rats as compared to group I rats at any time interval. When these values were compared within the same group at different time intervals, there were no significant differences in the values of groups I and II rats between any time intervals.

Mean serum phosphorus values in different groups at different time intervals expressed in mg/dl are presented in Table 1. There was no significance difference in mean total serum phosphorus values in group II rats as compared to group I rats at any time interval.

Discussion

Exposed AgNPs in group II rats caused significant changes in serum biochemical values including serum alanine amino transaminase, serum aspartate aminotransferase, serum protein, serum albumin, serum globulin, serum gamma globulin, serum glucose, serum cholesterol, serum calcium and serum phosphorous. In the present study, there was a significant increase in mean serum ALT values at 30th, 60th and 90th DPT in group II rats as compared to group I rats. When these values were compared within the same group at different time intervals, there was a significant increase in group II rats at 30th, 60th and 90th DPT. These results are in accordance with that of Lee *et al*. (2018) [8] and Monfared *et al*. (2013) [20]. There was significant increase in mean serum AST values at 30th, 60th and 90th DPT in group II rats as compared to group I. When these values were compared within the same group at different time intervals, there was no significant difference AST value in group I rats at any time interval. These results are accordance with Parang and Moghadamnia (2018) [22] and Lee *et al*. (2018) [8]. Additionally, systemic exposure to silver nanoparticles was demonstrated to cause liver damage and NLRP3-dependent inflammation with increase concentrations of AST and ALT in research by Ramadi *et al*., (2016) [24]. ALT and AST are generally found in the cells of numerous organs, including the liver. They are also considered as a significant sign when it comes to determining the state of liver. The liver is the major organ for MNPs detoxification (Yao *et al*., 2019) [36]. This makes liver a major organ for intoxication of AgNPs. Increase in ALT and AST values indicates damage to liver (Yang *et al*., 2008; Verma *et al.*, 2022) [35, 33]. Liver dysfunction caused by AgNPs leads to structural changes in the liver. AgNPs cause inflammation, which may lead to changes in liver coefficients and increase in ALT and AST values indicated liver injury (Recordati *et al*., 2015) [25]. The damage to liver after nanosilver exposure was also evident from the histopathological examination of liver of the rats of treated group in the present study (Kumar, 2021) [17]. There was a significance decrease in mean total serum protein values at 90th DPT in group II rats as compared to group I. The results of present study are in corroboration with that of Monfared *et al*. (2013) [20]. The decrease in the total serum protein might be due to liver damage due to toxicity and lymphocytotoxic effects of AgNP. Total serum proteins were reduced in treated groups. The decreased levels of total protein suggest that the protein might be used as an alternative source of energy, due to high energy demand induced by nanosilver intoxication. This has also been shown in previous study (Hori *et al*., 2006) [15]. It might also lead to the significant decrease in albumin and globulin level. There was significant decrease in serum albumin at 90th DPT in group II as compared to group 1. These results are in confirmation with the study by Sulaiman *et al*., (2015) [30] on silver nanoparticles in Wistar rats. Following exposure, AgNPs can accumulate in kidneys (Tang *et al*., 2009) [31] and can cause kidney damage. The decrease in serum albumin level can be due to the leakage of albumin from the kidney tubules due to any dysfunction in the kidney or degeneration of kidney tubular epithelium due to the exposure of nanosilver. This is also evident from the histopathological studies of kidneys of treated group. There was damage to glomeruli in the treated group rats (Kumar, 2021) [17]. Albumin is the major protein in the liver that acts as an antioxidant and protects tissues and cells from damage to free radicals. The alteration in serum albumin levels may denote stress on the liver imposed by the nanoparticles. The albumin is synthesized in the liver and is useful indicator of the synthetic functions of the liver (Adeyemi *et al*., 2012) [1]. Histopathological studies in the present experiment also revealed liver damage in the treated group (Kumar, 2021) [17]. There was significant decrease in mean serum globulin values at 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 a significant decrease in serum globulin level in group II rats at 90th DPT. These results also correspond with that of Vali *et al*., (2020) [32] in Ag-NPs exposed silver carp fish. The serum globulin (GLO) has important immunological and nutritional implications. The liver produces and stores certain globulins; any decrease in the globulin levels might indicate liver dysfunction (Bunglavan *et al*., 2014) [8]. Any condition where there is damage to liver can lead to decrease in levels of serum globulins. This has already been discussed above. Silver nanoparticles might reduce serum globulin in a variety of ways, including using protein as a source of energy under stressful situations, causing injury to intestine and kidney cells and hence protein discharge, disrupting amino acid absorption, and lowering liver protein synthesis. There was significant decrease in serum gamma globulin values at 60th and 90th DPT, respectively 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 a significant decrease in serum gamma globulin level in group II rats at 60th and 90th DPT. Immunoglobulins are mainly excreted by B cells and these neutralizes the pathogens and toxins (Vali *et al*., 2020) [32]. The significant decrease in serum gamma globulin in AgNPs treated rats can be because nanopartcles can bind to immune proteins or enzymes and lower their activities. AgNPs can exhaust immune cells through continuous stimulation at lower concentrations and degenerate them through oxidative damage at higher concentrations. Similar observations were made by Vali *et al*. (2020) [32] in AgNPs exposed common carp. The increase in gamma globulin in both groups might be due to production of antibody at 30th day due to vaccine administration given in the present study. There was significant decrease in mean serum glucose level at 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 significance decrease in serum glucose level in treated group at 60th and 90th DPT. Similar results have also been reported by Birudu *et al*., (2015) [6] in Wistar rats. Silver nanoparticles may cause a

decrease in hexokinase and glucose-6-phosphatase, resulting in a decrease in serum glucose levels in treated rats (Birudu *et al*., 2015) [6]. AgNPs have also been linked to a decrease in alpha glucosidase (Birudu *et al*., 2015) [6], which can prolong the process of digestion and absorption of the energy source, carbohydrates, and hence lower blood glucose levels (Birudu *et al*., 2015) [6]. There was significant increase in mean serum cholesterol values at 60th and 90th DPT in group II rats as compared to group I. When these values were compared within the same group at different time intervals, there was significant increase in mean serum cholesterol in group II rats at 60th and 90th DPT. Similar results have also been reported by Sulaiman *et al*., (2015) [30] in Wistar rats. An increase in serum cholesterol indicates hepatic damage (Shannahan *et al*., 2015) [27] which was also seen in the present study. Liver function complications can hinder the ability of organ to produce or clear cholesterol. The AgNPs showed a capability to alter the lipid profile by elevating the serum cholesterol. In the present study, no significance difference in mean total serum calcium values in group II rats as compared to group I rats at any time interval. When these values were compared within the same group at different time intervals, there were also no significant difference in both group I and group II rats. However, Ali *et al*., (2022) [3] also reported non-significant changes in serum calcium levels in rats administered nanosilver at a dose rate of 680 ppm. In the present study, no significance difference in mean total serum phosphorus values in group II rats as compared to group I. When these values were compared with in the same group at different time intervals, there were no significant differences in both group I and group II rats. Lee *et al*. (2018) [8] also reported no significance change in serum calcium and inorganic phosphorus levels in six-week-old male specific-pathogen-free (SPF) Sprague-Dawley rats at different doses. This indicates nanosilver has no effect on the calcium and phosphorous metabolism of the experimental rats.

It can be concluded from the present study that nanosilver has adverse effects on these biochemical parameters in Wistar rats at NOAEL dose for a period of 90 days.

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