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STUDY OF SYNTHESIS AND EFFECTS OF SILVER NANOPARTICLES INDUCTION ON BLOOD HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS: A STUDY IN RATS

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ABSTRACT

The aim of this study was the synthesis of silver nanoparticles by the most common chemical reduction method and to investigate their effects on blood hematology and biochemical parameters in Albino Wistar female rats. For this purpose, sixteen female rats were randomly distributed in two groups (n=8). Group I was control and Group II served as test. The test group received silver-nanoparticles (500mg/kg) intraperitoneally twice a week for four weeks. After synthesis, silver nano-particles were characterized by Scanning Electron Microscopy. Significant dose related changes in body weights, in tissue weights, in hematology and biochemical parameters of female rats were noticed. Decrease in body weight and in tissue weight especially in liver and heart was observed. Decrease in white blood cells, granulocytes, lymphocytes, monocytes, Mean corpuscular Hemoglobin Concentration , Platelet Distribution Width, Peripheral lymphocytes count, Mean Platelet Volume and Red width Cell distribution were observed while increase in hemoglobin, Red Blood Cell, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin , Hematocrit, Platelet and Platelet Crit were noticed. Similarly, reduced concentrations of glucose, cholesterol, HDL, LDL and triglycerides were found in the test group. Thus, administration of silver nano-particles at a dose of 500mg/kg body weight resulted in induction of toxicity in female rats.

Key words: Silver nanoparticles, blood hematology parameters, glucose, cholesterol, triglycerides

INTRODUCTION

Nanotechnology is a remarkable field of research, and it is widely regarded as one of the most important progressive times, innovations in recent times, due to its numerous beneficial applications in fields such as medicine, electronics, food, textiles, information communication technologies, automobiles, and construction. The use of nanoparticles in drug delivery mechanisms has been studied for more than two decades, resulting in the development of new dosage types with improved therapeutic outcomes and physicochemical characteristics (Patra *et al*., 2018). AgNPs can cause tumour cells to die by inactivating proteins and controlling signaling pathways, or they can stop tumor cells from spreading by inhibiting angiogenesis within the lesion, may be used in a variety of medical procedures, including bone repair and wound healing, in addition to antimicrobial and anticancer properties and may also be used as a dental substance additive or adjuvant (Paladini and Pollini., 2019). Many types of NPs and their derivatives have sparked significant interest due to their antimicrobial properties. After immersion in gels, nano-sized silver particles are used as edible films that are coated on vegetables and fruits to protect them from excessive water loss and increase consumer acceptability. Silver nanoparticles have bactericidal properties and are used in toothpaste, shampoo, soap, wipes, and bone cement, as well as sanitizers and water filters (Sodha *et al*., 2015).

A variety of techniques have been used to characterize various physicochemical properties of nanoparticles. X-ray diffraction (XRD), x-ray photoelectron spectroscopy (XPS), infrared (IR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Brunaver – Emmett-Teller (BET), and particle size analysis are some of the techniques used (Natsuki *et al.*, 2015).

Excessive use of nanoparticles can harm the body's immune system. Previous studies found evidence of substantial toxic effects of silver nanoparticles due to incidental skin contact, inhalation, and ingestion, and that this exposure resulted in blood nanotoxicity. They can also be consumed in the body of an individual in lymph nodes, spleen, adrenal, bone marrow, liver, and kidneys, among other tissues. When NPs have a strong effect on a cell, DNA damage and oxidative damage can occur, have a high affinity for accumulating in the liver and even absorb in the bloodstream (El-Ansary and Faddah, 2016).

An inflammatory reaction to nanoparticles in the blood was identified. The interaction of nanoparticles causes damage to blood components (platelets, RBCs, WBCs), plasma protein, complement proteins, and vascular endothelium (Cruz *et al.,* 2018). AgNPs have recently drawn a lot of attention due to their disinfectant properties,

distinct chemical and physical properties, with sizes varying from 1 to 150 nm, can be stored in the body and are difficult to extract, can be excreted by urine, saliva, and hair (Prabhu *et al*., 2012).

The toxicity of AgNPs is strongly influenced by a few characteristics of the particles, including their scale, shape, composition, concentration, and solubility. Smaller particles have a larger surface area, making them more harmful. The toxicity of small particles is higher. Previous studies found evidence of substantial toxic effects of silver nanoparticles due to incidental skin contact, inhalation, and ingestion, and that this exposure resulted in blood nanotoxicity (Yun *et al*., 2015).

The aim of this study was to use a chemical reduction method to synthesize silver nanoparticles and to investigate the effects of silver nanoparticles induction on blood components.

MATERIALS AND METHODS

Sixteen female Albino Wistar rats weighing 180-200g were purchased from animal house of Dow University of Health Sciences, Karachi (Pakistan).Animals were acclimatized to the laboratory conditions before the start of experiment and caged in a quiet temperature-controlled animal room. Rats had free access to water and a standard rat diet.

ETHICAL GUIDELINES

The experiments were conducted according to ethical guidelines of the Ethical review board (ERB) and international principles for laboratory use and care of animals in research (Health research extension Act 1985).

STUDY DESIGN

Synthesis of silver Nanoparticles

For the synthesis of AgNps (silver nanoparticles) suitable concentration (2.0 Mm PER 100 mL) of silver nitrate ($AgNO_3$) countered with (1.0Mm/100 mL) citric acid ($C_6H_8O_7$). $AgNO_3$ behaved as a metal precursor and citric acid as reducing agent to give the stability to the reaction. Hydrazine Hydrate ($N_2H_4.H_20$) 6% (v/v) 10 micro liter added in the reaction. Sodium Dodecyl Sulfate (SDS) was added drop by drop (2.0Mm per 100 ml) with continuous stirring at 1500 rpm at room temperature till the colorless solution turned into yellow and reddish-brown color. The appearance of color indicates the formation of silver nanoparticles. The purification was done by cold centrifugation at (10 ^{0}C on 15000 rpm). Supernatant discarded after centrifugation and remaining material washed by distilled water. These particles were kept in autoclave at 250 ^{0}C which gave powdered form of the silver nanoparticles (Sodha *et al*.,2015).

Administration of silver nanoparticles

The experimental rats were randomly distributed into two groups (n = 8). Study period consisted of four weeks. Group I was considered as control which remained untreated and group II served as test received silver nanoparticles (IP) twice a week at a dose of 500 mg/kg body weight. At the end of the treatment after 24h of the last dose rats were sacrificed, blood samples were collected in sterile EDTA tubes and used for estimation of blood hematology and biochemical parameters.

Estimation of blood hematology parameters

Complete blood counts were performed on the samples of whole blood of control and treated animals, using an automated Hematology analyzer-Medonic M32M (Japan). This analyzer consistently differentiates normal Red blood cells (RBCs), white blood cells (WBCs), Hemoglobin (HGB), Mean corpuscular volume (MCV), Platelets (plts) etc. from abnormal populations, thereby decreasing the chances of error.

Estimations of plasma glucose, plasma cholesterol and plasma triglycerides were carried out by using commercially prepared reagent kits.

STATISTICAL ANALYSIS

The values were presented as means ± SD of different groups. Differences between the mean values were estimated using student t-test. The results were considered statistically significant when $p < 0.05$.

RESULTS

Characterization of Silver nanoparticles

The reddish brown or yellowish-brown color appeared to confirm the formation of nano-particles (Fig. 1). Scanning Electron Microscopy (SEM) was used to determine the morphology and size of AgNPs that confirmed the synthesis of needle shaped silver nanoparticles ranging 82.2 nm to 156 nm in size (Fig. 2).

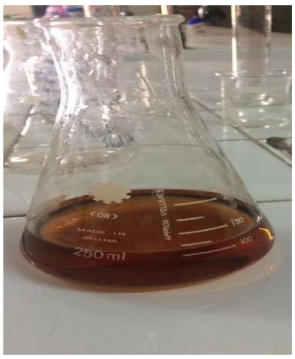

Fig 1.Synthesis of silver nanoparticles in solution form by chemical reduction method.

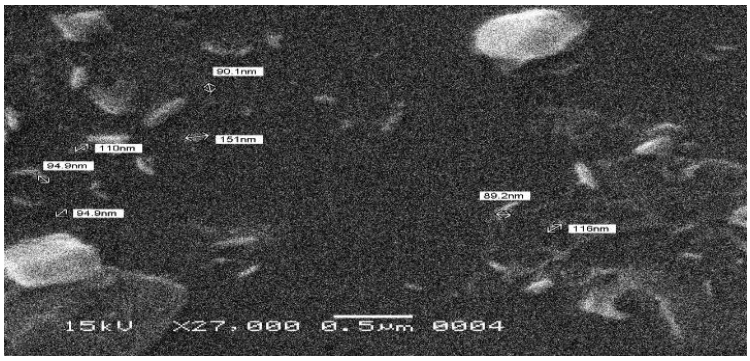

Fig 2. SEM images of Silver nanoparticles, 82nm to 156nm detected in aggregated form.

Effect of silver nanoparticles induction on body weights of experimental rats

A significant decrease in body weight was observed in test group (179.8 ± 9.9, $p < 0.05$) as compared to that of the control group (184.8 ± 5.25) after four weeks administration of silver nano-particles (Table 1).

Table 1. Effect of silver nanoparticles induction on body weights of experimental rats.

Groups	Control	Test	Effect
Body Weights Mean(g)	184.8 ± 5.25	179.8 ± 9.9*	↓

All data are expressed as mean ± standard deviation.*p – Value < 0.05 were considered as statistically significant

Effect of silver nano-particles induction on tissue's weight of experimental rats

Four weeks silver nano-particles induction resulted in a significant decrease in liver's weight in test group (5.9 ± 0.66, $p < 0.05$) as compared to that of the control group (6.5 ± 0.7). In the same way a significant decrease in heart's weight was also found in test group (0.8 ± 0.24, $p < 0.05$) as compare to that of control group (0.9 ± 0.2) but kidney weights were found similar in both control and test groups (Table 2).

Table 2**.** Effect of silver nano-particles induction on tissue's weight of experimental rats.

Tissues	Liver	Heart	Kidney
Control	6.5 ± 0.7	0.9 ± 0.2	0.5
Test	5.9 ± 0.66*	0.8 ± 0.24*	0.5

All data are expressed as mean ± standard deviation.*p – Value < 0.05 were considered to be statistically significant

Effect of silver nano-particles induction on hemoglobin level in experimental rats

Four weeks silver nano-particles induction resulted in significantly increased hemoglobin level in test group (13.28 ± 1.172, $p < 0.05$) as compared to that of the control group (12.72 ± 1.97) (Table 3).

Effect of silver nano-particles induction on red blood cell concentration in experimental rats

Red blood cell concentration was found increased in test group (6.498 ± 0.666, $p < 0.05$) as compared to that of the control group (6.332 ± 1.92) after four weeks administration of silver nanoparticles (Table 3).

Effect of silver nano-particles induction on mean corpuscular volume (MCV)in experimental rats

Silver nano-particles induction was resulted in markedly increased mean corpuscular volume in test group (54.22 ± 1.749, $p < 0.05$) as compared to that of the control group (52.66 ± 2.28) (Table 3).

Effect of silver nano-particles induction on hematocrit value in experimental rats

Hematocrit value was found significantly increased in test group (35.2 ± 3.448, $p < 0.05$) as compared to that of the control group (33.4 ± 3.43) after four weeks induction of silver nano-particle (Table 3).

Effect of silver nano-particles induction on platelets count and platelet crit value in experimental rats

Administration of silver nano-particles resulted in increased platelet count in test group (711.2 ± 139.5, $p < 005$) as compared to that of the control group (632.2 ± 145.6). In the same way, Platelet crit value was also found higher in test group (0.422 ± 0.207, $p < 0.05$) than that of the control group (0.384 ± 0.048) (Table 3).

Effect of silver nano-particles induction on mean corpuscular hemoglobin in experimental rats

Mean corpuscular hemoglobin (MCH) was found slightly increased in test group (20.5 ± 0.609, $p < 0.05$) as compared to that of the control group (20.1 ± 0.64) after four weeks of silver nano-particles induction (Table 3).

Table 3. Effect of silver nano-particles induction on blood hematological parameters in experimental rats.

Parameters	Control	Test	Effects
Hemoglobin (HGB)	12.72 ± 1.97	13.28 ± 1.172*	↑
Red Blood Cell (RBC)	6.332 ± 1.92	6.498 ± 0.666*	↑
Mean Corpuscular Volume (MCV)	52.66 ± 2.28	54.22 ± 1.749*	↑
Hematocrit (HCT)	33.4 ± 3.43	35.2 ± 3.448*	↑
Platelet (PLT)	632.2 ± 145.6	711.2 ± 139.5*	↑
Platelet Crit (PCT)	0.384 ± 0.048	0.42 ± 0.207*	↑
Mean Corpuscular Hemoglobin (MCH)	20.1 ± 0.64	20.5 ± 0.609*	↑

All data are expressed as mean ± standard deviation.*p – Value < 0.05 were considered as statistically significant

Effect of silver nano-particles induction on white blood cells countin experimental rats

Four weeks induction of silver nano-particles resulted in markedly decreased concentration of white blood cells in test group (6.54 ± 0.372, $p < 0.05$) as compared to that of the control group (7.32 ± 1.92) (Table 4).

Effect of silver nano-particles induction on lymphocytes concentration in experimental rats

Lymphocyte concentration was significantly reduced in test group (4.76 ± 0.659, $p<0.05$) as compared to that of the control group (5.12 ± 5.02) (Table 4).

Effect of silver nano-particles induction on monocytes concentration in experimental rats

Monocytes concentration was slightly decreased in test group (1.1 ± 0.303, $p < 0.05$) as compared to that of the control group (1.28 ± 0.39) as a result of silver nano-particles induction (Table 4).

Effect of silver nano-particles induction on granulocytes concentrationin experimental rats

Administration of silver nano-particles resulted in significantly reduced granulocyte concentration in test group (0.68 ± 0.324, $p < 0.05$) as compared to that of the control group (0.92 ± 0.53) (Table 4).

Effect of silver nano-particles induction on mean corpuscular hemoglobin concentration (MCHC)in experimental rats

Mean corpuscular hemoglobin concentration (MCHC) was decreased in test group (37.84 ± 0.682, $p < 0.05$) as compared to that of the control group (38.16 ± 0.52) after four weeks of silver nano-particles induction (Table 4).

Effect of silver nano-particles induction on Red width Cell distribution (RDW) in experimental rats

Administration of silver nano-particles resulted in decreased RDW in test group (13.3 ± 0.416, $p < 0.05$) as compared to that of the control group (13.86 ± 1.03) (Table 4).

Effect of silver nano-particles induction on Peripheral lymphocytes count (P-LC) in experimental rats

Peripheral lymphocytes count (P-LC) was significantly reduced in test group (4.28 ± 0.577, $p < 0.05$) as compared to that of the control group (5.36 ± 2.360) (Table 4).

Effect of silver nano-particles induction on Platelet Distribution Width (PDW) in experimental rats

Platelet Distribution Width (PDW) was decreased in test group (38.16 ± 0.463, $p < 0.05$) as compared to that of the control group (38.5 ± 0.918) (Table 4).

Effect of silver nano-particles induction on Mean Platelet Volume (MPV) in experimental rats

Mean platelet volume was significantly decreased in test group **(**5.98 ± 0.146, $p < 0.05$) as compared to that of the control group (6.18 ± 0.573) (Table 4).

Table 4. Effect of silver nano-particles induction on blood hematological parameters in experimental rats.

Parameters	CONTROL	TEST	
White Blood Cell (WBC)	7.32 ± 1.92	6.54 ± 0.372*	↓
Lymphocytes (LYM)	5.12 ± 5.02	4.76 ± 0.659*	↓
Monocytes (MID)	1.28 ± 0.39	1.1 ± 0.303*	↓
Granulocytes (GRA)	0.92 ± 0.53	0.68 ± 0.324*	↓
Mean corpuscular Hemoglobin Concentration (MCHC)	38.16 ± 0.52	37.00 ± 0.382*	↓
Red width Cell distribution (RDW)	13.96 ± 1.03	13.02 ± 0.416*	↓
Peripheral lymphocytes count (P-LC)	5.36 ± 2.360	4.28 ± 0.577*	↓
Platelet Distribution Width (PDW)	38.5 ± 0.918	37.16 ± 0.463*	↓
Mean Platelet Volume (MPV)	6.18 ±0.573	4.98 ± 0.146*	↓

All data are expressed as mean ± standard deviation.*p – Value < 0.05 were considered as statistically significant

Effect of silver nano-particles induction on Plasma glucose level in experimental rats

Four weeks silver nano-particle induction in female rats resulted in significantly decreased level of plasma glucose in test group (113.2 ± 31.82, $P<0.05$) as compared to that of the control group (138.25 ± 35.80) (Table 5).

Effect of silver nano-particles induction on Plasma Cholesterol, HDL and LDL level in experimental rats

Plasma cholesterol level was markedly increased in test group (53.8 ± 16.78, $p < 0.05$) as compared to that of the control group **(**36 ± 4.72). HDL was also found increased in test group (16.14 ± 5.020, $p < 0.05$) as compared to that of the control group(10.8 ± 1.341). In the same way, LDL was also significantly increased in test group (37.66 ± 11.71, $p < 0.05$) as compared to that of the control group (25.2 ± 3.29) (Table 5).

Table 5. Effect of silver nano-particles induction on biochemical parameters in experimental rats.

NO	Parameters	CONTROL	Test
1-	**Glucose**	138.25 ± 35.80	113.2 ± 31.82*
2-	**Cholesterol**	36 ± 4.72	53.8 ± 16.78*
3-	**HDL**	10.8 ± 1.341	16.14 ± 5.020*
4-	**LDL**	25.2 ± 3.29	37.66 ± 11.71*
5-	**TG**	34.4 ± 2.939	45.4 ± 22.99*

All data are expressed as mean ± standard deviation.*p – Value < 0.05 were considered as statistically significant

Effect of silver nano-particles induction on plasma Triglycerides level in experimental rats

Silver nano-particles induction resulted in significantly increased plasma triglyceride level in test group (45.4 ± 22.99, $p < 0.05$) as compared to that of control group (34.4 ± 2.939) (Table 5).

DISCUSSION

SEM (scanning electron microscopy) was used to confirm the form and size of silver nanoparticles which ranged from 80.3 to 156nm (Fig. 2). A previous study found that due to differences in cellular absorption mechanisms, different sized silver nanoparticles less than and more than 100 nm could generate varied effects. AgNPs with diameters ranging from 5 to 100 nanometers were found to have cytotoxic effects in human cell lines, whereas AgNPs with diameters ranging from 20 to 50 nanometers were found to be involved in ROS formation and cell cycle blockage (Fehaid *et al*., 2019).

In vivo studies have shown that various forms of MNPs, such as nano-metal monomers and nano-metal oxides, appear to deposit in the liver, causing significant toxicity, causes liver dysfunction, which leads to structural changes in the liver, induced inflammation, which could lead to changes in the coefficients (Yao *et al*.,2019).

In our study loss of liver and heart weights were observed in AgNPs treated rats and kidneys weight were unchanged (Table 2).Body weight loss and insufficient diet consumption were seen in the test group in the current investigation (Table 1). In silver nanoparticles treated Wistar rats (Zayerzadeh *et al*., 2018) observed a substantial drop in body weight, physical activity, and diet consumption.

HGB, HCT, RBCs, MCV, and MCH concentrations were found to be elevated in the present study (Table 3). In a previous study, Espinosa-Critobal *et al*.(2013) found that AgNps induced poorer oxygenation from the lungs and absences of oxygen in the blood, resulting in higher concentrations of RBCs and HGB in the blood of the test group(Espinosa-Critobal *et al*., 2013).Cations on red blood cell membranes, on either other, interact with negatively charged silver nanoparticles and trigger hemolysis. Erythrocytes' defensive mechanism, which contains glutathione and other antioxidants including peroxiredoxin and catalase, prevents fast damage. Surfactants, on the other hand, can cause ions hemolysis.Long-term hemolysis caused deadly illnesses to develop (Cruz *et al*., 2017). The formation of red blood cells and other cells could be influenced by AgNps exposure, according to (Osama *et al*; 2014). Aside from the benefits, AgNps have been linked to cytotoxicity and oxidative stress in living organisms.

WBCs, LYM, MID, GRA, MCHC, RDW, PDW, and P-LC, MPV all decreased significantly in this study (Table- 4). Previous research has suggested that nanocrystalline silver is involved in the inhibition of inflammatory cytokines via inducing apoptosis in inflammatory cells. Increased PLT and PCT values were also observed in our study (Table 4). Previous research has found that silver nanoparticles caused platelet aggregation by increasing intracellular calcium levels. The activation of GPIIb/IIIa in platelets causes an increase in intracellular calcium, which raises platelet activity. In female rats, the treatment of AgNPs at a dose of (1000 mg/kg) resulted in a reduction in the active Partial thromboplastin time (Kim *et al*., 2008). The toxic dose may have caused blood coagulation. AgNPs have been found to have proadherent, prohaemolytic, and procoagulant effects, all of which increase the risk of thrombosis (Laloy *et al*., 2014)

Silver nanoparticles interact with platelets in the bloodstream, activating cascade processes and causing clot formation. Platelets are engaged in homeostasis and prevent hemorrhages, and platelet aggregations disturb the normal coagulation process, inducing strokes. Previous studies reported that there are few metallic NPs on haemoglobin content and erythrocyte count. The most common index is MCV. By dividing the hematocrit by RBC, it determines the average amount of red blood cells. (Svoboda *et al*., 2001) observed changes in MCV, MCH, and MCHC values in Cyprinuscarpio exposed to pesticides at 60 and 120 g/L concentrations. In certain diseases, the number of white blood cells may dramatically increase or decrease (Banaee *et al.,* 2008).There was a major difference among three types of white blood cells (lymphocytes, neutrophile and eosinophile). Changes in white blood cell count may indicate haematological tissue dysfunction (spleen and kidney) or infection.

Haemolysis is caused when cations on the RBC membrane interact with negatively charged silver nanoparticles. The protection mechanism of erythrocytes, which includes glutathione and antioxidants including peroxiredoxin and catalase, prevents rapid harm. Surfactants, on the other hand, may cause ions haemolysis. Long-term haemolysis can result in fatal illnesses (Cruz *et al*., 2017).

RBC and other cell production could be impaired by AgNps exposure (Osama *et al.,* 2014). Increased cholesterol, triglycerides, LDL, HDL, and hypoglycemia were found in present study (Table5). According to previous research, a strong interaction between positively charged silver nanoparticles (AgNps) and negatively charged lipids causes hyperlipidemia because nanoparticles form tight complexes with lipid molecules, causing lipids to accumulate in the plasma (Grinceviciute *et al*., 2015).

Hypoglycemia was seen in the treated group in present investigation. In a previous study, blood insulin levels in diabetic groups treated with AgNps and ZnONPs were found to be higher (Alkalany *et al*., 2010)

The upregulation of glycogenolysis and lipid synthesis/storage in the liver of treated rats was suggested by the observed reduction in glucose with an increase in total lipids and cholesterol (Almansour *et al*., 2016). Because silver is an oxidative component in liver tissue that causes strong oxidative stress, it promotes lipid beta oxidation (Lotifi *et al*., 2017).

Conclusion

The present study confirms that administration of silver nan-oparticles at a dose of 500 mg/kg body weight twice a week induces the toxicity in female Albino wistar rats.

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