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ASSESSMENT OF SIZE BASED ORAL TOXICITY OF SILVER NANOPARTICLES ON SERUM LIPID PROFILE, LIVER AND KIDNEY FUNCTION OF MALE WISTAR RATS

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ABSTRACT

Nanotechnology has received unprecedented growth and height which has revolutionized the consumer products, medical and industrial applications, thereby making it more compact, feasible and appropriate. But uncurbed use of nanoparticles and nanomaterials has led to its release into the environment and its exposure to human beings. Present study aimed to quest the repercussion of PVP coated silver nanoparticles (20 nm and 40 nm) administered orally at a dose level 1 and 2 mg/kgb.wt./day for 45 days on male albino rat. On 46th day body weight was measured and autopsies were performed to collect serum for anaylsis. Organ like liver and kidney were used for histopathologic examination. Group recevied high dose showed highly significant decline. Liver weight was found to be affected. The mild to severe alteration in serum profile was obtained indicating liver damage whereas kidney damage was clear from blood urea and creatinine level in serum. These results were supported by histopathology observations of liver and kidney. Thus it can be concluded from data that the oral administration of AgNPs (20 nm and 40 nm) drive to hepatic and renal toxicity in experimental groups at such a low dose under *in vivo* condition. Catastrophic effects shown by AgNPs (20 nm) are more compared to AgNPs (40 nm).

KEYWORDS: AgNPs, serum lipid profile, liver, kidney, histopathology.

INTRODUCTION

Growth and advancement in nanotechnology influences each and every sector of industries and society. But this expansion and magnification of artificial nanomaterials product exposure also augmented the risk of various health hazards. Risk associated with these nanomaterial has been documented in research work.[1-4] Silver is paramount nanoparticles in consumer product inventories, listing 313 products (55.40% of all nano-products).[5] like bedding, washers, water purification systems, tooth paste, shampoo, fabrics, deodorants, filters, paints, kitchen utensils, toys, textiles. With the rise in silver nanoparticles containing consumer product, the risk of its release into the environment also increases (6). Most of these commercially available silver nanoparticles containing products are synthesized chemically or/and physically.[7]

Data defect related to exposure of silver nanoparticles released from consumer product into the environment and at workplace makes urgent need to understand its toxic effects as these nanoparticles enter into the body through inhalation or oral route. Present study aimed to determine the whether and to what extent the given silver nanoparticles pose hazard to rat.

MATERIALS AND METHODS

Silver Nanoparticles

Silver nanoparticles (CAS No. 7440-22-4) were purchased from Nano Beach (New Delhi, India), and were at least 99.98% pure. Average mean particles size of nanoparticles is 20 nm and 40 nm.

Animal model

Twenty four healthy adult male rats (*Rattus norvegicus*) weighing 150-200 gms were selected for experimentation. Rats were housed in polypropylene cages at room temperature with natural light and dark cycles (12 h dark, 12 h light) and relative humidity 55±5 %. They were fed on standard commercial pallet feed procured from Ashirwad food industries ltd., Chandigarh, India and water *ad libitum*. All experiments on animals were performed as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental Design

Rats were randomly divided into four groups having six animals in each group.

Group I served as control, received only the vehicle (water)

Group II received sonicated silver nanoparticles (size 20 nm) at a dose level 1 mg/kg b.wt./day for 45 days

Group III received sonicated silver nanoparticles (size 20 nm) at a dose level 2 mg/kg b.wt./day for 45 days

Group IV received sonicated silver nanoparticles (size 40 nm) at a dose level 1 mg/kg b.wt./day for 45 days.

Group V received sonicated silver nanoparticles (size 40 nm) at a dose level 2 mg/kg b.wt./day for 45 days

All the doses were given orally by pearl point needle. On 46th day treated animals along with control were weighed and sacrificed under light ether anaesthesia. Blood was collected by cardiac puncture and serum was then separated by centrifugation at 4000 rpm.

Parameters studied

Body Weight and Organ Weight

Lipid Profile: Serum separated from clotted blood was used to total cholesterol.[8] total protein.[9] phospholipid.[10] triglyceride.[11] HDL-cholesterol.[12] LDL-cholesterol.[13] VLDL-cholesterol.[13]

Liver function test: SGOT.[14] SGPT.[14] acid phosphatase.[15] alkaline phosphatase.[15] and bilirubin.[16]

Kidney Function: Urea.[17] and creatinine.[18]

Histopathologic Observation: Liver and Kidney

Statistical analysis: Statistical analysis was performed with SPSS (Version10). Statistical evaluation was performed by one way ANOVA following multiple comparison tests tukeys method. The level of statistical significance was set at $P<0.05$ and highly significant ($P<0.01$).

RESULTS

Nanoparticles interact with biological medium such as serum and alter their aggregation state, surface chemistry and shape of biological molecules.[19] Nanoparticles have numerous applications in different sectors due to its fascinating physciochemical properties like small

size, shape, large surface area, high local charge density.[6] Although these propitious associated with nanoparticles is just one side of a coin. It interacts with bio-molecules in their biological fluids.[20] Research has shown that AgNPs may be toxic even at low doses.[21,22]

Body Weight and Organ Weight

Pre and post body weight of control and treated groups male albino rats were assessed to determine effects of oral administration of AgNPs for 45 days on health status. Body weight was found to be decreased in treated groups but the changes are non-significant. However group IV has shown highly significant ($P<0.01$) decrease in body weight (table no. 1). Organ like liver weight was decreased highly significant ($P<0.01$) in group II, III and group V compared to control showing ill effects of silver nanoparticles on liver, whereas change in kidney weight was found to be non significant in treated group compared to control (table no. 1).

Lipid Profile

Table no. 2 shows serum total protein levels were significantly higher in group II, III, IV and group V compared to group I group with $P<.05$, whereas phospholipid level reduced highly significantly compared to control in all the treatment groups at both the size and dose level in dose dependent manner. Triglycerides was found to be increased significantly and highly significantly ($P<0.05$ and $P<0.01$) after administration of AgNPs (20 nm) in dose dependent manner, whereas in case of AgNPs (40 nm) significant ($P<0.05$) increase was obtained at 1 mg/Kg and highly significant ($P<0.01$) increase was observed at 2 mg/kg dose level as compared to control. Significantly ($P<0.05$) and highly significant ($P<0.01$) increase in cholesterol level was observed in both size of silver nanoparticles in dose dependent manner as compared to control. HDL level reduced highly significant ($P<0.01$) at both dose and size of AgNPs, however greater reduction was observed at 40 nm compared to control. Treatment with AgNPs showed highly significant ($P<0.01$) decrease in the LDL and VLDL level at both the size and dose in comparison to control.

Liver function Analysis

Liver functioning is represented in form of graph in figure 1 (A-D). There were highly significant ($P<0.01$) increment in alanine amino transferase level was observed between the treatment groups, however among the different treatment groups, group II and III showed more increase compared to group IV and V ($P<0.01$). Similar results were obtained with aspartate amino transferase test showing highly significant ($P<0.01$) increase in values in

dose dependent manner compared to control. Although treatment group II and III of AgNPs (20 nm) showed more pronounced effect compared to AgNPs (40 nm). Alkaline Phosphatase level compared to control was found to be highly significant ($P<0.01$) increased in all treatment groups. However toxic effects of AgNPs at both size and dose level are almost similar. A significant ($P<0.05$) increase in group II and highly significant ($P<0.01$) increase were observed in group III, IV and V in the level of acid phosphatase compared to control. Also significant higher ($P<0.01$) in bilirubin level in case of treatment group II whereas group III, IV and V showed highly significant ($P<0.01$) increase compared to control.

Kidney function test

Creatinine level was found to be increased highly significant ($P<0.01$) in all the treatment groups as compared to control (figure 1E). Further, highly significant ($P<0.01$) increase was also observed blood urea level compared to control (figure 1F).

Histoarchitecture of Tissue

The treated animals showed distinct morphological changes in the kidney and liver on microscopic observation when compared with the control group, indicating unhealthy cells.

Liver

Normal histoarchitecture was observed in control liver tissue (Figure 1), while damage in hepatocytes and narrowing of the sinusoidal lumen semithin sections of treated liver tissue was observed (Figure 2). Neutrophil infiltration was clearly visible in the section which may be due inflammation.

Kidney

Figure 3 shows the normal histoarchitecture of renal tubules in kidney of control group. However, treatment group shows swollen epithelium in the renal cortex and number of membranous vacuoles in the cytoplasm with some hypertrophied nucleoli in the nucleus. Another treatment group shows tubular epithelium swelling, vacuolization of cytoplasm, increased cellularity in glomeruli and obliteration of Bowman's space. Atrophy of glomerular tuft and dysplastic renal tubules were also observed.

Table No.1: Comparison of silver nanoparticles effects on body weight and organ weight of treated and control male albino rats

Treatment	Body weight		Liver	Kidney
	Initial	Final		
Group I	184.16 ± 5.968	194.83 ± 5.804	6.938 ± 0.550	1.35 ± 0.085
Group II	187.16 ± 4.20	171.66 ± 4.216 ^{ns}	5.911 ±0.286**	1.15 ± 0.039 ^{ns}
Group III	177.83 ± 4.261	133.83 ± 5.319 ^{ns}	5.996 ±0.333**	1.16 ± 0.041^{ns}
Group IV	180 ± 6.952	160.33 ± 7.490^{ns}	7.081 ± 0.312 ^{ns}	1.48 ± 0.082 ^{ns}
Group V	181.66 ± 8.232	133.83 ± 5.319**	5.903 ± 0.270**	1.21 ± 0.042 ^{ns}

(Mean ±SEM of 6 Animals) Group II, III, IV and V compared with group I

ns = non-significant

* = significant (P<0.05)

** = highly significant (P<0.01)

Table No. 2: Analysis of silver nanoparticles effects on serum biochemical assay

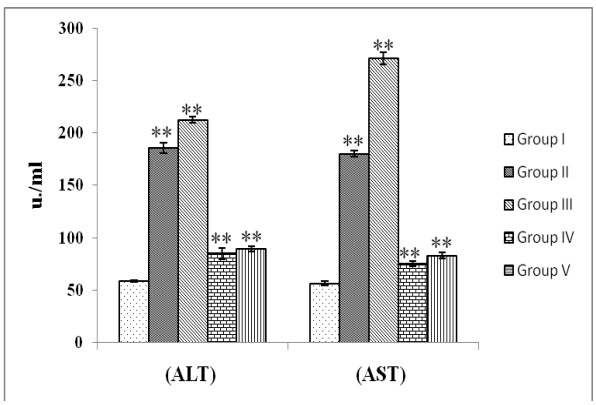
Groups	Total Protein	Phospholipid	Triglyceride	Total-Cholesterol	HDL	LDL	VLDL
	mg/dl						
Group I	6.28 ± 0.270	181.16 ± 10.081	74.50 ± 10.828	76 ± 3.151	27 ± 1.879	12.5 ± 1.231	11 ± 1.211
Group II	9.53 ± 0.230**	123.33 ± 3.574**	104 ± 3.890*	87.58 ± 0.840*	19.50± 0.428**	28.33 ± 0.954**	27.50 ± 0.885**
Group III	9.98 ± 0.087**	87.66 ± 2.764**	145.16 ± 1.661**	93.16 ± 1.661**	17.50 ± 3.149**	34.5 ± 2.012**	28.33 ± 1.115**
Group IV	7.66 ± 0.108**	121.66 ± 3.018**	100.33 ± 3.303*	87.33 ± 6.075*	15.66± 1.054 **	31.5 ± 1.607**	23.83 ± 2.562**
Group V	9.36 ±0.306**	116 ± 7.120**	111.16 ± 6.544**	88.66 ± 1.475**	13.33 ± 0.954**	36.5 ± 4.303**	19.5 ± 1.056**

(Mean ±SEM of 6 Animals) Group II, III, IV and V compared with group I

ns = non-significant

* = significant (P<0.05)

** = highly significant (P<0.01)



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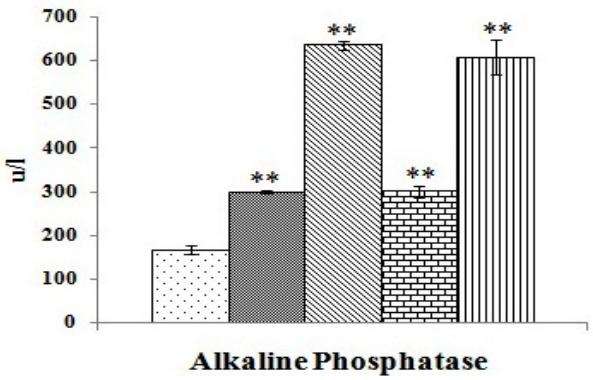
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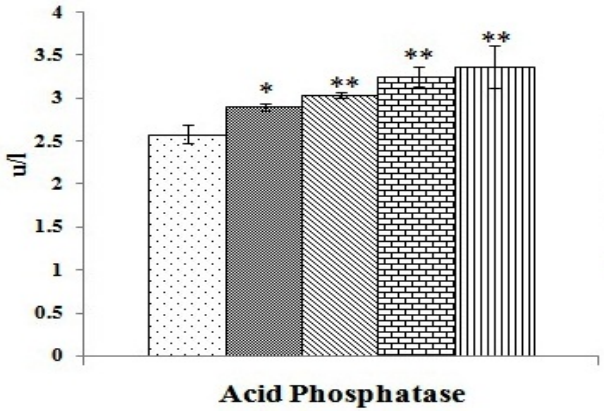
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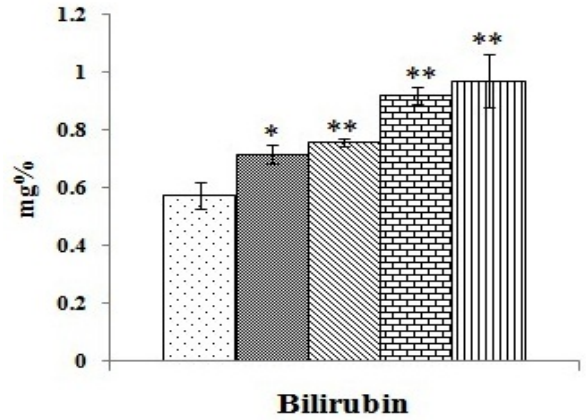
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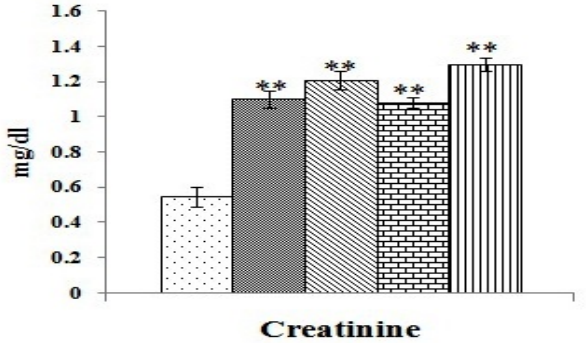
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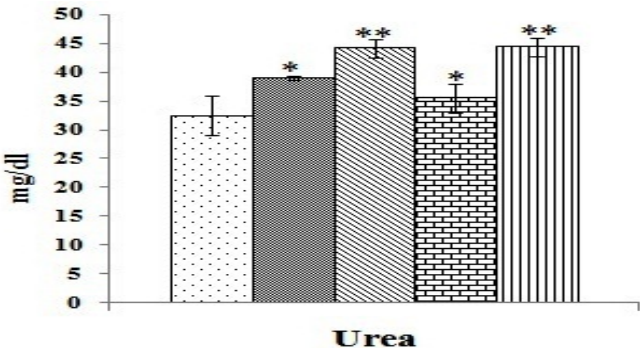
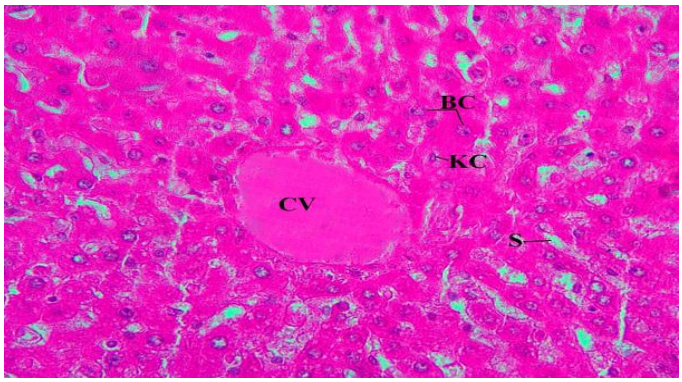


F

Figure 1 (A-F): Effects of AgNPs on ALT and AST, Alkaline phosphatase, Acid phosphatase, Bilirubin, creatinine and Urea levels of Wistar rats.

Data were expressed as the mean ± SEM (n= 6). Group II, III, IV and V compared with group I and expressed as P<0.05 significant P<0.01 highly significant compared with control group.



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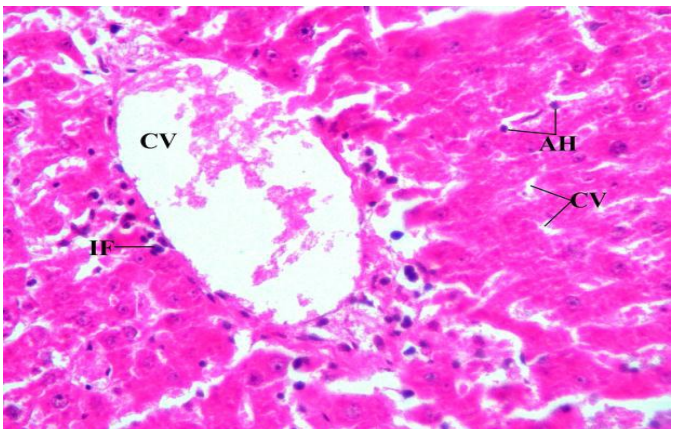

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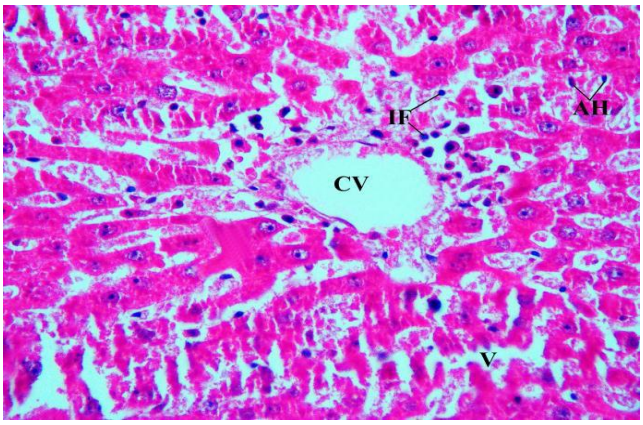

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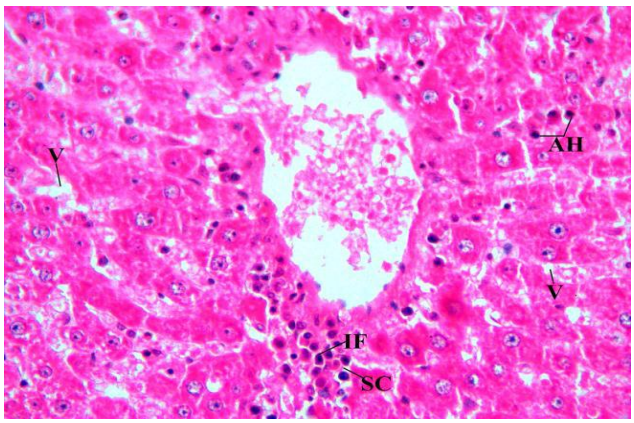

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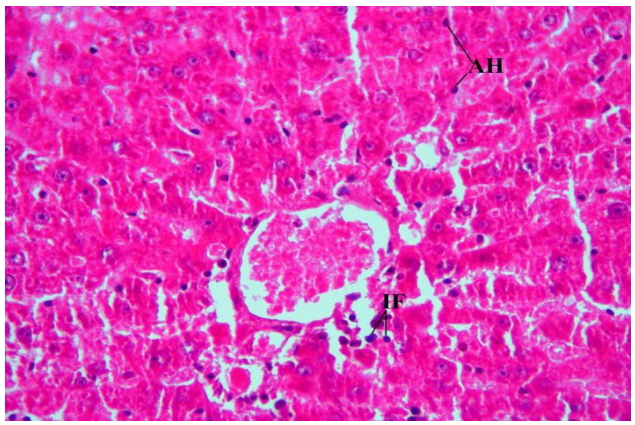


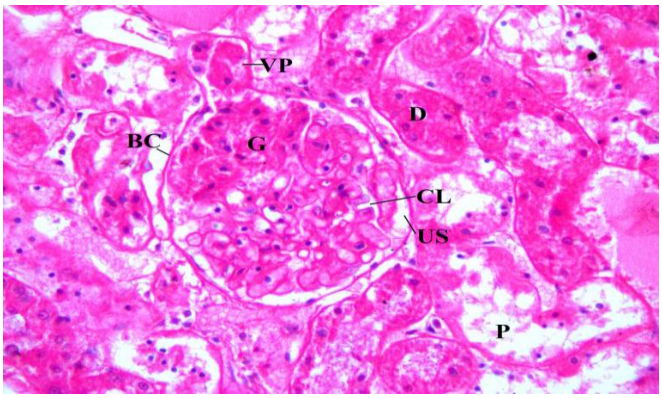
E

BN- Binucleate cells; K- Kupffer cells; IF- Inflammatory cells infiltration; CV/V- Cytoplasmic vacuolization; AH- Apoptotic hepatocytes

Figure 2: Liver microphotograph at 400X: A. control liver showing normal histoarchitecture of hepatic lobules with central vein (CV) lined with endothelium. CV is completely filled with lumen. Endothelial cells with small nuclei in sinusoids are also visible. Binucleate cells, kupffer cells are seen.

In **B and C** central vein distorted and almost become empty & completely empty in group II and III (AgNP 20 nm) respectively. Inflammatory cells and apoptotic hepatocytes are present in both the groups. Degenerative changes including disorganized hepatocytes and cytoplasmic vacuolization was also apparent group V.

D and E represent group IV and group V AgNPs (40 nm) showing mild dilation of sinusoids space. Inflammatory cells and apoptotic hepatocytes are visible proving damaged condition of liver. Hypertrophy and disorganization of hepatocytes are prominent in group III. Sinusoid congestion is clearly visible in group III.



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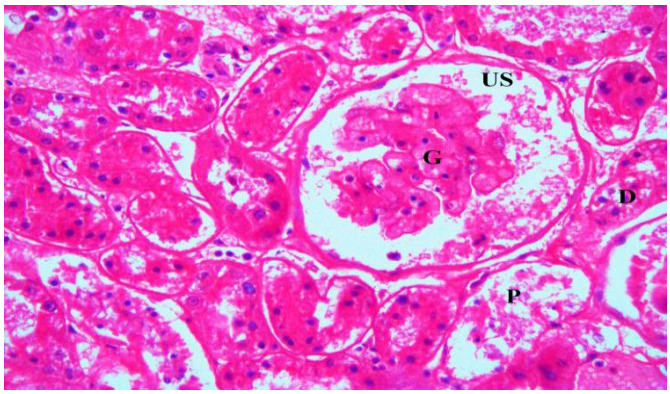

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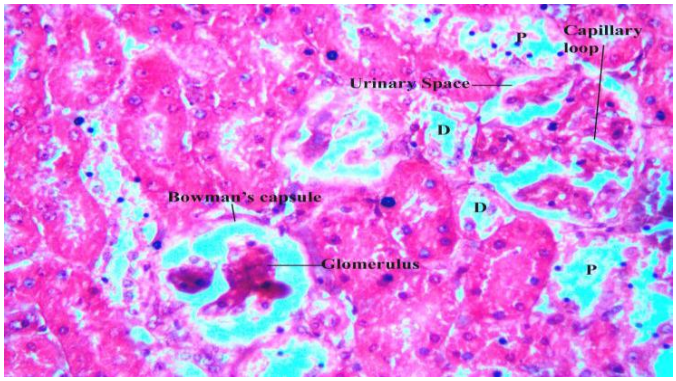

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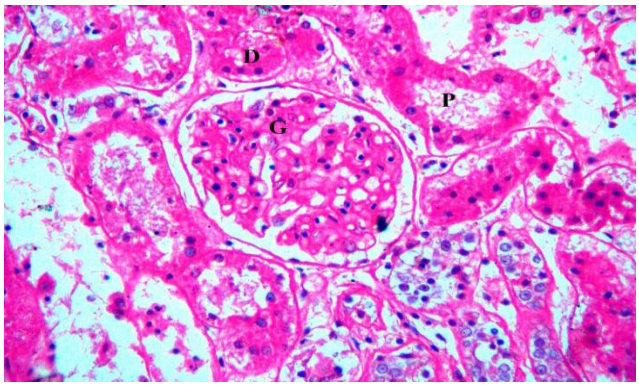

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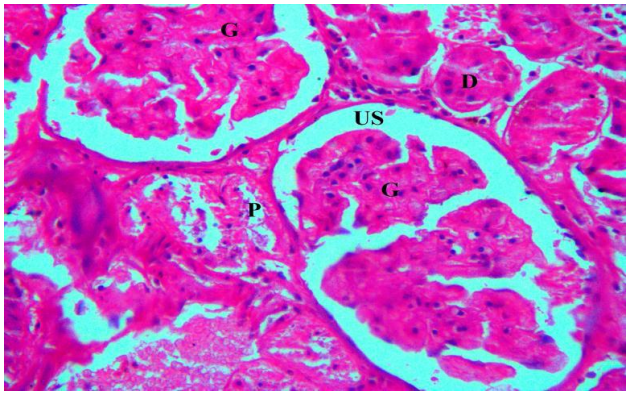

E

US – Urinary Space; BC – Bowman's capsule; G – Glomerulus; D – Distal convulated tubule; P – Proximal convulated tubule; GC – Glomerular capillaries; AA – Afferent arteriole; EA - Efferent arteriole; CL – Capillary loop; VP-Vascular pole

Figure 3: Liver microphotograph at 400X: A control kidney showing normal histoarchitecture of glomeruli, renal tubules and collecting tubules. Afferent and efferent arterioles are also apparent.

B and C represent group II and III showing reduction in size of glomeruli, vacuolization and increase in urinary space. Proximal and distal convoluted tubules also damaged.

D and E are microphotograph of group IV and V exhibiting degenerative changes in glomeruli and change in shape of bowman's capsule. Vacuolization and increase in urinary space can be clearly seen.

DISCUSSION

Increasing use of nanomaterials creates potential danger due excess exposure to nanoparticle which has become a critical issue. However, recent nanotoxicity studies have mainly focused on the health risks to healthy adult population.[23] Silver nanoparticles [AgNPs] are most frequently used nanoparticles to many household products and other consumer. So far, the

knowledge regarding the health and safety aspects of NPs is still in its initial phase and greater efforts are needed to understand interaction of these NPs with the human body[6].

Nutrients, drugs and noxious substance that entered into the body encounter with principal organ i.e. liver through the hepatic portal vein.[24] Therefore, liver function tests are critical screening tool to ascertain and estimate scathing hepatic dysfunction.[25] Biomarkers of liver like SGOT, SGPT, AP, ALP and bilirubin levels were estimated to reckon the level of destruction led by AgNPs. AST (SGOT) is normally found in a variety of tissues including liver, heart, muscle, kidney, and the brain. It is released into the serum when any one of these tissues is damaged. ALT (SGPT) is, normally found largely in the liver.

The silver nanoparticle treated groups at different dose level showed rise in the level when compared to control which could be attributed to number of metabolic activities and enzyme. Cytoplasm is rich in SGPT and SGOT whereas mitochondria contains high amount SGPT.[26] In the present investigation, the elevated levels of SGOT, SGPT, ALP, AP and bilirubin in nanoparticle treated groups were observed. Liver injury hinders the function of hepatocytes causing leakage in plasma membrane or necrosis which led to increased level of these enzymes in serum due to loss of functional integrity of membrane.[27] Present study showed increased levels of AST which is again the sign of damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury, AST is responsible for conversion of alanine to pyruvate and glutamate and is released in a similar manner. High level of ALP synthesis could be attributed to increased biliary pressure.[28] ALP is integrated with hepatocytes next to the bile containing tube. Increased concentration of the ALP in the circulation may be due to inflammation or obstruction of the biliary tract. ALT, AST, ALP does not specificity for biliary tract disease. ALP is released by osteoblasts, the ileum, and the placenta. Elevated acid phosphatase was found in present study elucidates that damage of the prostate gland by toxicants. Thus acid phosphatases could be explored as serological and histological markers of disease.[29] Kidney has been known to displace off the hazardious material from the blood, NPs in blood can be filtered through it.[30,31] Increase in urea level could be due to its high production within liver which may be attributed to high level of protein, as obtained in present study and reduced glomerular filtration rate. Elevated creatinine reveals the dysfunctioning of glomerular filtration/proximal tubular secretion which results from silver nanoparticles administration. Earlier study has shown high levels of

BUN and CREA in the serum.[32] This high level of creatinine may leds to muscles dystrophy. This is supported by study of Zhang et al (2015).[33]

The level of rat serum bilirubin increased following the administration of AgNPs. Earlier research proven that elevated bilirubin beyond the hepatic function capacity could be due to increased red blood cell hemolysis.[34]

Total protein is composed of albumin and globulin and reflects the balance of protein biosynthesis and catabolism. The highly significant increase in total protein at all both dose levels and size might be due to increased synthesis and decreased catabolism.[35] This further denotes abnormal function of liver.[36] which may be due nanoparticles. Elevated level of protein indicates inflammation or infections.[37]

In the present investigation elucidated that Ag nanoparticles results in alterations in the levels of total cholesterol (TC), phospholipid, triglycerides, HDL, LDL, and VLDL of male albino rats. The imbalances (either increase or decrease) in the lipid profile status of cells could peril to several health repercussion. Increased level of TC, triglycerides, LDL and VLDL could raise the risk of cardiovascular disorder. High total cholesterol and triglycerides in AgNPs treated groups may be gives big picture of reducing activities of fat splitting enzymes such as, lecithin: Cholesterol acetyltransferase (LCAT) and lipoprotein lipase. Esterification of gratis cholesterol is performed by cholesterol acetyltransferase and responsible for controlling level of free cholesterol in cells and tissues. Lipoprotein lipase cleaves triglycerides into free fatty acids and glycerol and act as clearing factor in plasma.[38,39]Another reason for high level of triglycerides may be due to increased synthesis of VLDL cholesterol [40]. Decrease in the phospholipid level may be attributed to the change in anabolism or catabolism of very low-density lipoproteins.[41]

HDL is considered as good lipoprotein because it reduces the chances of atherosclerotic buildup.[42] Therefore, a reduction in the HDL level could be due to acceleration of apoA-I clearance from the plasma.[43] which is hazardous. Down regulation in LDL receptors by cholesterol and saturated fatty acids may leds to high level of LDL.[44]

Histological examination of the liver and kidney was carried out to strengthen the results of the biochemical analysis. Histological changes provides stronger evident of toxic damage in tissues and reduced health[45,46]. The liver is known as the principal organ for metabolism[47].

The present study revealed histological changes in hepatocytes of rats characterized by mild periportal inflammatory cells and cytoplasmic degeneration, congested vessel and interstitial kidney hemorrhage. Observation coincides with earlier findings having histopathological alterations in the liver and kidney[48,49]. Our finding in this present study is also in agreement with the report of Saleh (1993)[50] who reported that liver of treated rats showed degenerative changes in the form of cloudy swelling, hydropic degeneration, chromatolysis, pyknosis, fatty degeneration, necrosis and karyorrhexis.

CONCLUSION

Inimical effects of AgNPs were clearly manifested from serum lipid profile analysis. Kidney functioning was found to be impaired in presence of AgNPs which was confirmed through serum and histopathological examination. Liver was found to be main target organ affected after oral administration of silver nanoparticles. Pronounced toxicity was observed with 20 nm size of nanoparticles in comparison to 40 nm. Serum toxicity of selected nanoparticles was proven fatal for the survival. Languishing effects of AgNPs was clearly reflected in histopathology of liver and kidney.

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