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RESEARCH ARTICLE

EFFECTS OF SILVER NANOPARTICLES ON LIVER FUNCTIONS OF MALE ALBINO RAT

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ABSTRACT

Present investigated was performed to study the toxic effects of oral administration of silver nanoparticles on liver and serum parameters of male rat. Six healthy Wistar rats weighing between 150-200 g were randomly selected and divided into three groups. Group I serve as control group and received distilled water while treatment groups were administered 1 mg kg^{-1} b.wt. silver nanoparticles (AgNPs) for 30 days. After 24 hrs of experiments completion, rats were sacrificed using diethyl ether. Blood was collected by cardiac puncture to extract out serum for biochemical estimation. Liver was excised and used for histopathological examination. Exposure of AgNPs results in significant alteration in levels in ALT, AST, ALP, bilirubin and acid phosphatase. The histopathological examination of liver revealed congestion, inflammation and cellular damage due to exposure to the AgNPs. Thus it can be concluded that silver nanoparticles at dose level 1 mg kg^{-1} b.wt could be harmful if exposed for month which proven by serum biochemical and histopathological alterations in liver.

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INTRODUCTION

Nanotechnology has wide range of potential applications as it deals at the atomic and molecular level in formation of new materials and devices (Joshi and Kaushik, 2013). Due to its innovativeness and fascinating physicochemical properties improves its efficiencies and increases its use in biomedical, agriculture, industrial, manufacturing, military sectors (Robertson *et al*., 2010; Kisin *et al*., 2007; Stark, 2011; Tholoul *et al*., 2008). Among different types of nanoparticles that are used silver nanoparticles (AgNPs) is most widespread due to its novelty and control over the size which made its use in number of consumer products which impart antimicrobial properties like in bedding, paints, washers, water purification filter, shampoo, humidifiers, coatings, appliances, toys, deodorants , glues, fabrics, inks, pastes, polymers, tooth paste, etc., to make them thinner conducting pastes and coatings (Kaushik and Joshi, 2015; Joshi and Kaushik, 2013). But excessive use of AgNPs causes its release into the environment intentionally or unintentionally which is matter of concern. Ag nanoparticles being nano in size finds its way in body more easily than bulky material of same composition. Interaction of Ag nanoparticles inside body with biological molecules and any toxicity caused by it is still in learning phase. Study has shown that oral, inhalation, or subcutaneously administration of Ag-NPs results in hepatotoxicity (Kim *et al*., 2009). AgNPs in circulation appeared to be deposited in liver (Takenaka *et al*., 2001). Thus it is high time to think and explore the harmful effects silver nanoparticles and upto what extent its exposure may pose threat to the internal machinery of living system. Present investigation focus on investigating the toxic effects on serum biochemical parameter of liver and its histopathology after 30 days of oral administration of 1 mg kg^{-1} b.wt silver nanoparticles.

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MATERIALS AND METHODS

Nanoparticles and its preparation

The silver nanoparticles (20 and 40 nm) were purchased from Nanobeach, New Delhi, India and were at least 99.98% pure. Silver nanoparticles were uniformly suspended using water bath ultra sonicator.

Experimental animals

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

The details of animal groupings were as shown below:

Group 1: Served as the control and received distilled water
Group 2: Received 1 mg kg–1 b.wt. of AgNPs (20 nm) for 30 days
Group 3: Received 1 mg kg–1 b.wt. of AgNPs (40 nm) for 30 days

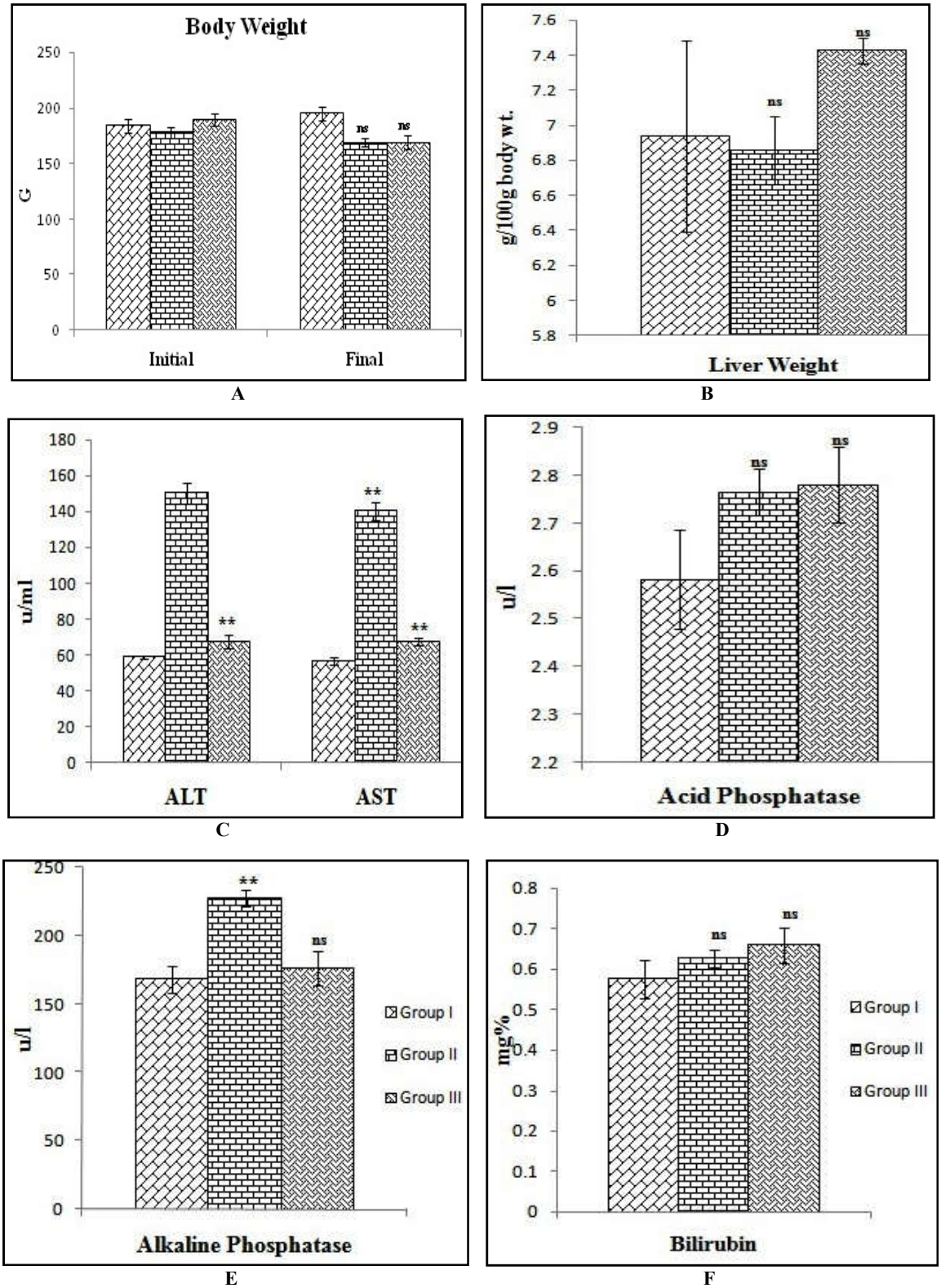

Figure 1 (A-F): Effects of AgNPs on body and liver weight, ALT and AST, alkaline phosphatase, acid phosphatase, bilirubin of Wistar rats. Data were expressed as the mean ± SEM (n= 6). Group II and III compared with group I and expressed as ns- non-significant, P<0.05 significant and P<0.01 highly significant compared with control group

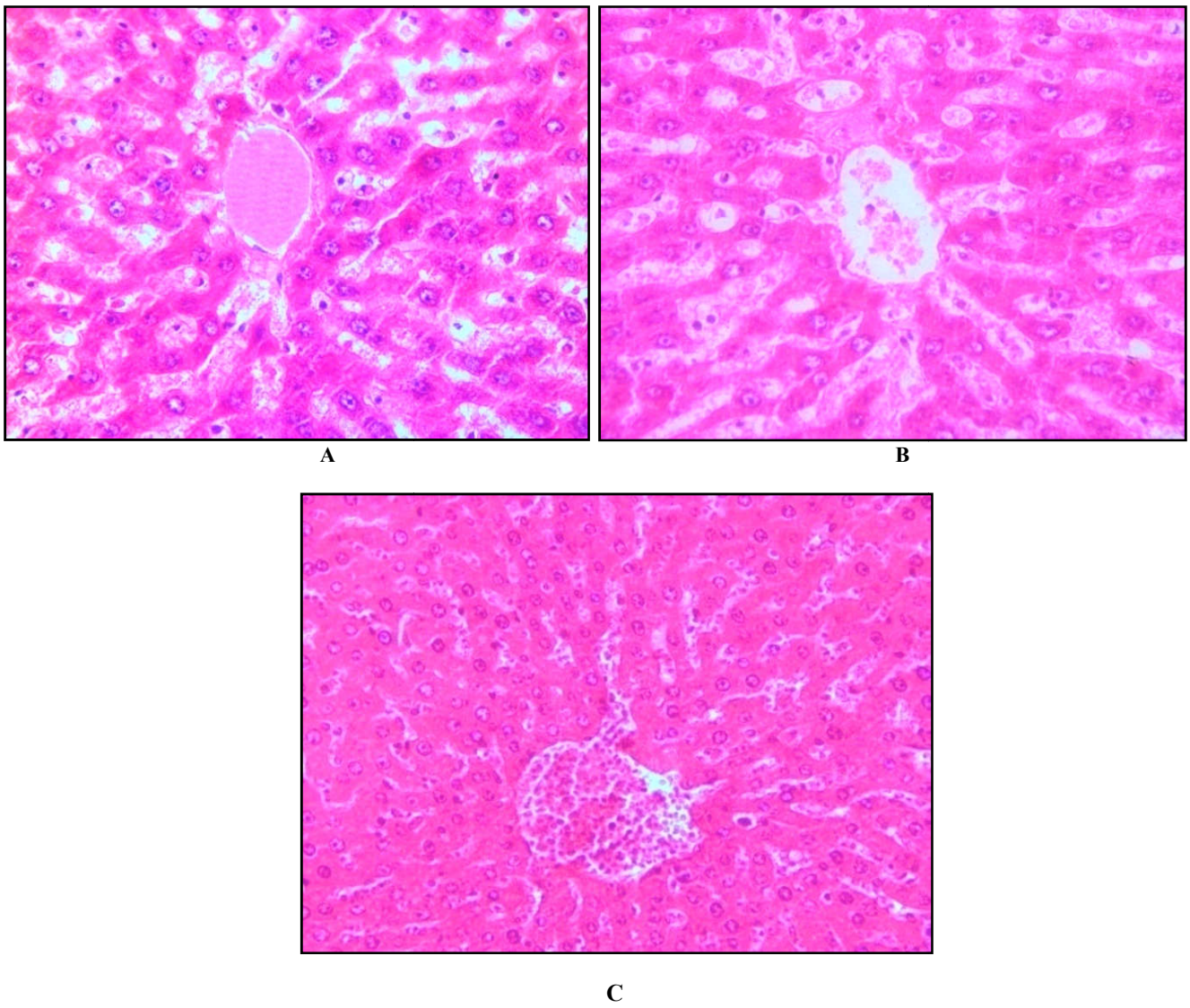

Figure 2. Liver microphotograph at 40x: A. group I (control) B. group II C. group III

A. control liver showing normal histoarchitecture. Central vein (CV) filled and lined with endothelium. 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. Inflammatory cells and apoptotic hepatocytes are present in both the groups. Disorganization of hepatocytes and hypertrophy is noticeable in group II. Congestion of sinusoid is clearly visible in group III.

All doses were orally administered by means of pearl point needle. Treatments were daily and lasted for 30 days. On 31st day treated animals along with control were weighed and sacrificed using light ether anesthesia. Blood was collected by cardiac puncture and serum was then separated by centrifugation at 4000 rpm.

Serum biochemical analysis

Serum so harvested was immediately utilized for biochemical analysis like alanine aminotransferase (ALT) (Reitman and Frankle, 1957), aspartate aminotransferase (AST) (Reitman and Frankle, 1957), alkaline phosphatase (ALP) (King and Jagathesan, 1959), acid phosphatase (King and Jagathesan, 1959) and total bilirubin (TBILI) (Malloy and Evelyn, 1937) are estimate for liver functional test.

Histological examination

The preparation of tissue sections for histological examination under light microscope followed the standard embedding and H-E staining protocol.

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

Present study investigated the toxic effects of silver nanoparticles on functioning of liver enzyme analysis and its histopathological studies of male albino rats.

Body Weight and Organ Weight

Body weight of control and treated groups male albino rats after pre and post treatment was assessed. Non-significant ($P<0.05$) decrease in body weight (graph 1A) was observed in group II and group III as compared to control. However, liver weight was decreased significant ($P<0.05$) in group III whereas group II showed non-significant ($P<0.05$) decrease compared to control (graph 1B).

Liver enzymes

Highly significant ($P<0.01$) increase in ALT and AST level was found in both the treatment groups compared to control (Graph 1C). Similar pattern was observed in alkaline phosphatase showing highly significant ($P<0.01$) increase in group II whereas non-significant ($P>0.05$) increase in group III compared to control (graph 1D). Acid phosphatase and Bilirubin level was found to be increased in both treatment group but non-significant ($P>0.05$) as compared to control (Graph 1E and 1F).

Histology of liver

Control rat showed normal histology whereas oral administration of AgNPs has shown damages to the liver tissue. Necrosis, congestion, inflammation of hepatocytes and neutriphil infiltration in liver tissue. Infact, all treated mice exposed to silver nanoparticle hadminimal to moderate lymphocyte aggregation in hepatic area. Damaged caused by silver nanoparticles of size 20 nm are more determental then 40 nm AgNPs (Figure 2A-C).

DISCUSSION

In coming year's nantechnology will spread its roots in each and every sector. Consumer products containing nanoparticles is increasing day by day. Thus the risk associated with their also increases. Nanoparticles has been reported to induce oxidative stress (Hudecova *et al*., 2012; Adeyemi and Faniyan, 2014). Toxic effects of silver nanoparticles are observed different tissues (Xia *et al*., 2006). Body weight is indicator of good health. In present study administration of silver nanoparticles of different size i.e. 20 and 40 nm has shown to reduce the weight in treatment animals. Decline in weight may be preliminary sign of nanoparticles toxicity. Earlier studies have shown toxicity of drugs or chemicals associated with changes in body weight (Orisakwe *et al*., 2004; Adeyemi and Sulaiman, 2014). Liver is vital organ of biological system which play important role in defending body from xenobiotic compunds. Enzymes of liver are marker, as their concentration increase in blood and thus to predict any damage or cellular toxicity. The administration of Ag nanoparticles increased the activities of AST and ALT in rat serum relative to the control. Free radicals released by stressing hepatocytes into the blood serum could leads to elevation in ALT in the present study (Cheraghi *et al*., 2013; Woodrow Wilson International Center, 2010, Farkas *et al*., 2011; Griffitt *et al*., 2009). Elevated level of serum ALT has been linked with hepatic injury (Adeyemi and Akanji, 2012; Sulaiman *et al*., 2014). AST level was also found to be increased in present investigation which is consistent with earlier reports showing potential of metal nanoparticles to change activity of transaminases (Salma *et al*., 2011). ALP is integrated with hepatocytes next to the bile containing tube. Inflammation or obstruction of the biliary tract is related with increased concentration of the ALP in the circulation. Elevated acid phosphatase indicates prostate gland damage by toxicants. Thus acid phosphatases act as marker for serum and histological alterations (Agbafor, 2015). Administration of Ag nanoparticles leads to change in level of rat serum bilirubin which could be related to increased destruction of red blood cell (Adeyemi *et al*., 2012). Histology provides complementary evidence to the serological parameter analysis. Present investigation observed inflammation, congestion neutrophil infiltration, cellular degeneration, all of which are the sign of hepatotoxicity due to nanoparticle administration. Silver ions binds thiol group in liver results transfer of glutathione to bile bladder and decreases its concentration which necessary to remove peroxides, thus increase toxicity (Hendi, 2010; Campen, 2003).

Conclusion

AgNPs toxicity was clearly manifested from serum analysis. Oral administration of silver nanoparticles showed that liver was severely damaged. Toxic effects were stronger with 20 nm size of nanoparticles in comparison to 40 nm. Toxicity of selected nanoparticles was clearly reflected in histopathology of liver and supporting the serum analysis results.

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