

Cytotoxicity of Silver Nanoparticles in Mice Liver Cells: An Ultrastructure Study

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Abstract

Introduction: Nanoparticles of silver have many important applications and are among the most commonly used nanomaterials. They are increasingly used in a variety of both medical and consumer products which includes: spectrally selective coating for solar energy absorption and intercalation material for electrical batteries, as optical receptors, polarizing filters, catalysts in chemical reaction and bio-labeling. Nanosilver (Ag-NP) has both antibacterial and antiviral activity. Yet, the knowledge about the systemic toxicity of nanosilver is relatively limited.

The aim of work: To evaluate the potential toxicity of small size 10nm silver nanoparticles using two different doses (0.1 ml and 0.4 ml) focusing on the ultrastructural changes occurring in mice hepatocytes.

The methods: This study was performed using three groups of mice. The animals of the first group were given a daily intravenous injection of 0.1 ml of silver nanoparticles for 28 consecutive days. The second group was treated with 0.4 ml of silver nanoparticles for 28 consecutive days. The third group served as a control group in which the animals did not receive any vehicle. The study was focused on the ultrastructure of the liver.

The results: Ultrastructure observations of liver cells of mice Treated with any of the two doses (0.1 and 0.4 ml) of 10 nm Ag-NP indicated severe accumulation of dark deposits of Ag-NP in the cytoplasm and the cell organelles.

Conclusion: Our study revealed that nanosilver used in doses of 0.1 and 0.4 ml led to deposits in the cells and induced damage of cell components especially the nucleus, mitochondria and chromatin.

Key words: nanosilver , cytotoxicity, liver, mitochondria

Introduction

Over the past few years, synthesis and characterization of nanoparticles have gained increasing momentum due to their large surface area to volume ratio because of which nanoparticles exhibit novel and new properties than their macroscopic counterparts.⁽¹⁾

Nanoparticles (NPs) are defined as materials that measure between 1 and 100 nm. Given that the diameter of many cellular macromolecules such as DNA (2 nm), ATP synthase (10 nm), and synaptic vesicles (40 nm) are also in this size range, it is perhaps unsurprising that NPs can exhibit bioactivities that are lacking in corresponding bulk materials. NPs can be synthesized using a variety of base materials in different sizes and shapes, with each parameter conferring specific physical and bioactive properties.⁽²⁾

Silver nanoparticles (AgNPs) are one of the most commonly used nanomaterials. AgNPs are

known to have antioxidant and antimicrobial properties.⁽³⁾

Silver nanoparticles (AgNPs) are presently the most commercialized of all the nonmaterial. According to the Woodrow-Wilson database, about 24% of all consumer products - claiming to contain engineered nonmaterial - have nanosilver. AgNPs have a wide range of applications in electronics, paints, clothing, food, cosmetics, and medical devices that are contributed to their catalytic, optic, magnetic and antibacterial properties.⁽⁴⁾

A small minority of studies reported on the toxicity of supernatants or filtrates of AgNPs solutions so that the toxicity of applied AgNPs can be evaluated separately from the toxicity of

impurities acquired during synthesis, or Ag⁺ released during storage. ⁽²⁾

Techniques have demonstrated that AgNPs can be synthesized using chemical and physical methods; but due to the fact of usage of a huge amount of toxic chemicals and high temperature conditions, it became a mandate to find an alternative method. ⁽³⁾

Nevertheless, biological and toxicological studies on AgNPs are still rather few, and the toxic properties of various forms of AgNPs have not been clearly defined yet. *In vivo* experiments on mice and rats have suggested that inhaled AgNPs are able to reach several organs, including the lungs and the liver, and to cross the blood-brain barrier. ^(4, 5, 6)

In vitro studies demonstrated that Ag-NPs are cytotoxic by their effects on cell metabolism and membrane integrity; they also inhibit embryonic stem cell differentiation. ⁽⁷⁾

In the present study, intravenous administration of nano-silver particles (Ag-NPs) in two doses (0.1 and 0.4 ml) was used for 28 consecutive days to evaluate their potential toxicity. Special emphasis was on the effects of these nanoparticles on the nucleus, mitochondria and chromatin materials.

Material and Methods

Animals

Twenty five adult male albino mice *Mus musculus* were used in this study with body weight (BW) ranging from 25-30 g. They were housed in the animal house located at King Saud University, Faculty of Science, Females Section, Riyadh City, KSA. The animals were kept under good hygienic conditions. Drinking water and conventional feed were provided *ad libitum*. The animals were divided into three groups; the first group was the control group comprised five mice that did not receive any vehicle. The second group of animals was exposed to 0.1 ml of 10 nm nanosilver *via* intravenous injection and the third group was exposed to 0.4 ml of 10 nm nanosilver *via* intravenous injection. The treatment continued for 28 consecutive days.

Chemicals

BioPure silver nanoparticles 10 nm diameter in 2 mM phosphate buffer were obtained from Nano Composix, San Diego, CA, USA.

Methods

According to Hayat (1986), liver tissues were cut into small pieces, immediately fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) at 4°C for 24 hours and were post-fixed in 1% osmium tetroxide 0.1M phosphate buffer at 4°C for one hour. After fixation, dehydration in graded alcohol, then embedding in araldite cy 212 were done. Semi-thin sections were cut and stained for examination. Ultra-thin sections were cut, picked up on copper grids and then were double-stained with uranyl acetate and lead citrate. The sections were examined and photographed using Zeis 100s transmission electron microscope. ⁽⁸⁾ These procedures were carried out in the Central Laboratory, the Female Section, KSU.

Results

Ultrastructure observations of the control liver sections

The normal structure of the liver section was noted. The nuclei are spherical and almost central in position, with normal chromatin, nuclear envelope and intact nucleolus (Fig. 1). The cytoplasm of the hepatocytes contains numerous mitochondria dispersed all over the cytoplasm. The mitochondria are spherical or ovoid in shape with well-developed cristae. The rough endoplasmic reticulum consists of closely packed parallel and flattened cisternae studded with ribosomes (Fig. 2).

Marked ultrastructural changes were observed in nanosilver - treated animals with the dose 0.1 ml. These changes represented by irregular shape nucleus, abnormal nuclear envelope with deposited silver nanoparticle, fragmented chromatin and disintegrated nucleolus. In addition, the cytoplasm revealed numerous deposits of silver nanoparticles (Fig. 3). The cisternae of some parts of rough endoplasmic reticulum were dilated with deposited silver nanoparticles (Fig. 4).

Size (nm)	Mass Concentration (mg/ml)	Atomic (Ag) Molarity (mmol/l)	Particle Concentration (particles/ml)	Ag Mass Percent (%)	Max. Optical Density (cm ⁻¹)	Peak Wave-length (nm)

10	1.0	9.27	1.8×10^{14}	0.1	125	395
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The mitochondria were aggregated and numerous nanosilver particles appeared deposited nearby the outer mitochondrial membrane (Fig. 5). The mitochondrial cristae appeared fragmented with nanosilver particles deposited on the outer membrane (Fig. 6).

Marked ultrastructural changes were observed in 0.4 ml nanosilver-treated animals. The nuclei appeared irregular in shape and varied in size with abnormal nuclear envelope, concentrated border lined heterochromatin and disintegrated nucleolus. Electron-dense mitochondria and dilated endoplasmic reticulum. Numerous nanosilver particles appeared deposited in the cytoplasm nearby the organelles; some of them appeared in the outer membrane or inside the organelles (Fig.7).

Silver nanoparticles appeared as singlet deposits on the nuclear membrane, mitochondrial membranes and the rough endoplasmic membrane (Fig. 8).

Particle aggregates were most often localized inside the mitochondria either on the cristae or on the inner surface of their membranes. In some mitochondria, these aggregates were so large that they occupied almost the entire organelle; but sometimes single NPs were also found inside the mitochondria. Both the mitochondria that closely interacted with particles and those that were free from direct contact with them demonstrated signs of destruction (Fig. 9).

Silver nanoparticles - found as singlet and droplets in the cytoplasm and in the rough endoplasmic reticulum membranes - were demonstrated in Fig. 10.

Discussion

Our study revealed that silver nanoparticles deposits in the liver cell organelles - either as singlet or as aggregated dense particles - were located deep in the cytoplasm nearby the nuclear and organelles membrane especially in those treated with 0.4 ml silver nanoparticle. These could be observed even in the inner membrane of mitochondria, in the mitochondrial matrix and in the nuclear envelope and chromatin. Marked ultrastructural changes was observed with AgNP depositions such as disintegrated nucleolus and fragmented

chromatin, which led to DNA damage, the most affected organelle was mitochondria with fragmented cristae and homogeneous matrix. These results were also reported by McShan *et al*⁽⁹⁾ who recorded that nanosilver particles penetrate the cell and become internalized. One of the main mechanisms of toxicity is that it causes oxidative stress through the generation of reactive oxygen species which cause damage to cellular components including DNA, depletion of antioxidant molecules (e.g., glutathione), binding and disabling of proteins, and damage to the cell membrane. O₂ and other molecules attributed these changes to the properties of the surface of nanosilver that can be easily oxidized in the environmental and biological systems leading to the release of Ag⁺ which is a known toxic ion. The surface oxidation rate is closely related to the nanosilver surface coating, coexisting molecules, especially thiol-containing compounds, lighting conditions, and the interaction of nanosilver with the nucleic acids, lipid molecules, and proteins in a biological system. Kim *et al*⁽¹⁰⁾ performed a proteomic analysis of liver, lung, and kidney tissues in rats exposed to approximately 50 nm SNPs by intravascular injection and indicated that SNPs of AgNP can be distributed to different body tissues after uptake, and SNPs were detected in various tissues and organisms after inhalation, oral ingestion, and contact with the skin. Katsnelson *et al*⁽¹¹⁾ suggested that the damage of nuclear DNA by the action of SNPs is not a direct interaction but is mediated by the mitochondrial reactive oxygen species generation under the effect of SNP (oxidative stress). Reactive oxygen species penetrating into the nucleus from the cytoplasm cause nuclear DNA damage. Hisn *et al*⁽¹²⁾ stated that nanosilver acts through ROS to induce apoptosis *via* the mitochondrial pathway. Our study shows that the most affected organ was the mitochondria which revealed fragmented cristae and homogeneous matrix. This was confirmed by Katsnelson *et al*¹¹ who concluded that NS particles accumulate in greater quantities within mitochondria and cause more marked destruction of their membranes and cristae, and

that they tend to show greater tropism towards the mitochondria.

Chae, *et al*⁽¹³⁾ reported that AgNPs cause DNA damage as well as oxidative and carcinogenic stress in Japanese medaka; while Ag⁺ only induced inflammation, metallic detoxification responses, and a low overall stress response.

On the other hand, our results indicated that the higher silver nanoparticle-dose the higher accumulation rate of deposits of silver nanoparticles in the hepatocyte. This is in agreement with data obtained by Hunt *et al.*,⁽²⁾ Hadrup *et al.*⁽¹⁴⁾ and Zande *et al.*⁽¹⁵⁾ who revealed that Ag⁺ exposure results in higher levels of tissue deposition and further growth inhibition relative to AgNPs exposure.

Park *et al*⁽¹⁶⁾ Park *et al*⁽¹⁷⁾ and Kim *et al*⁽¹⁸⁾ stated that size - dependent bioactivity of AgNPs has been observed in orally exposed mice as smaller AgNPs been associated with increased toxicity and higher levels of silver uptake. Also, Wang *et al*⁽¹⁹⁾ found that silver accumulation in the tissues increases with increased exposure concentration and with smaller AgNPs size. According, to Gliga *et al*⁽²⁰⁾ small size silver nanoparticles are cytotoxic for human lung cells and that the toxicity observed is associated with the rate of intracellular Ag release.

Conclusions

In the present study where rats were intravenously administrated with 0.1 ml or 0.4 ml of 10 nm Ag-NP it was found that the accumulation rate was increased with 0.4 ml AgNPs than with 0.1 ml. The cytotoxic effects are represented by deposition of silver nanoparticles in the hepatocytes especially in the cytoplasm, nuclear envelope, chromatin, the mitochondrial inner, outer membranes and the matrix; there were associated with significant cellular damages.

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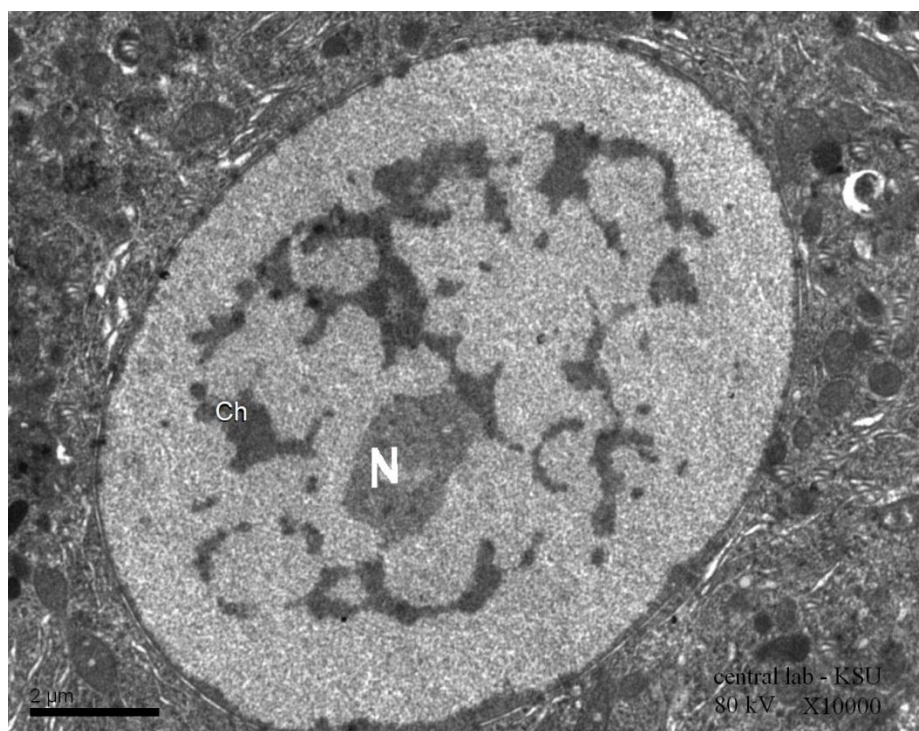


Fig. 1 An electron micrograph of control mouse showing normal nucleus, nucleolus (N) and chromatin (Ch) . (Original mag. X10000)

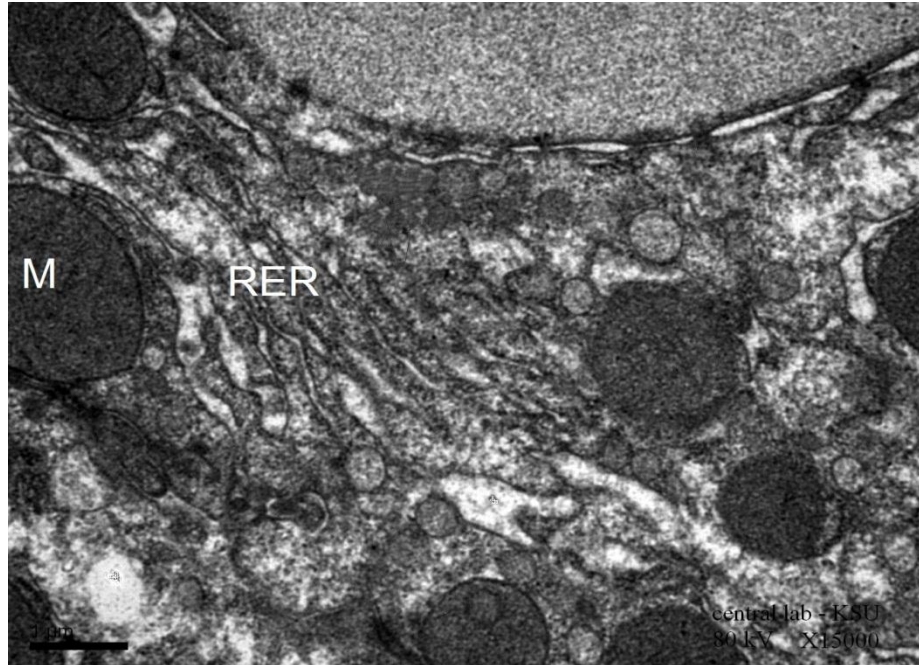


Fig. 2 An electron micrograph of control mouse showing normal mitochondria (M) and rough endoplasmic reticulum (RER) (Original mag. X 15000)

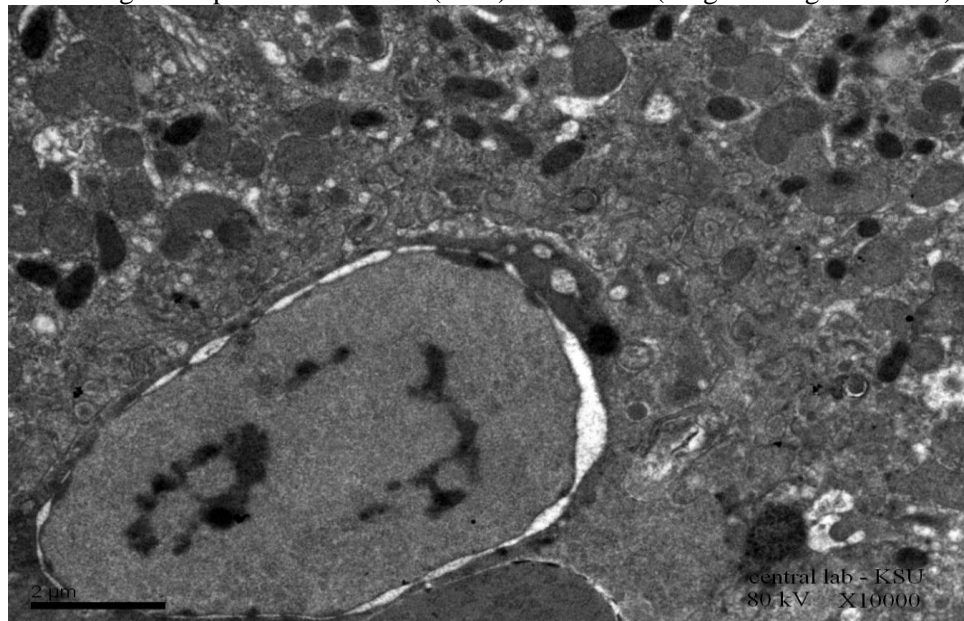


Fig. 3 An electron micrograph of 0.1ml SNP- treated mouse showing abnormal nucleus, irregular nuclear envelope and disintegrated chromatin (Original mag. X10000)

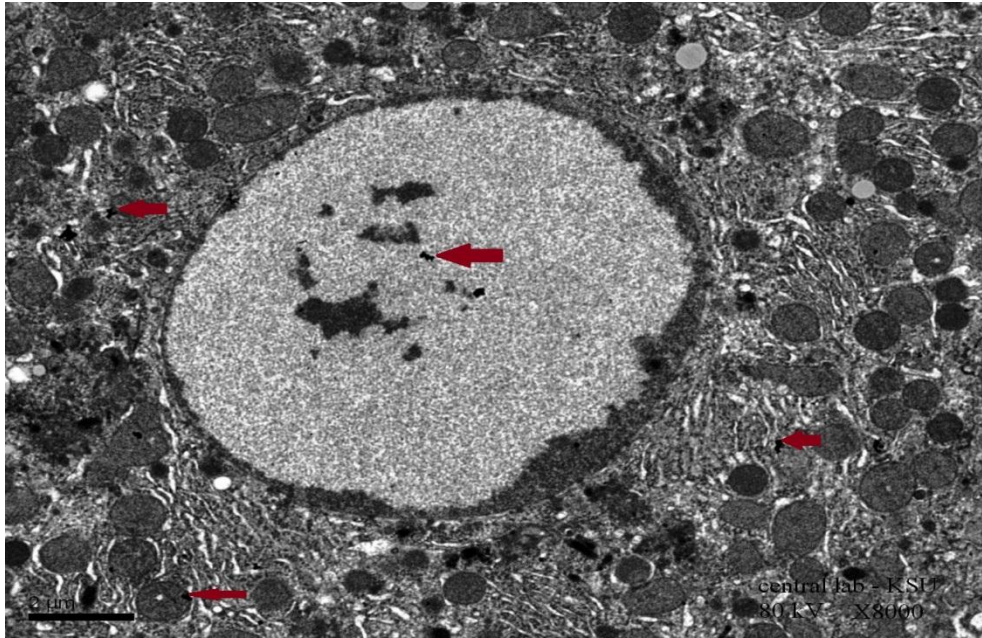


Fig. 4 An electron micrograph of 0.1ml SNP- treated mouse-showing aggregations of SNP deposits(←) in the cytoplasm and in the nucleus (Original mag. X 8000)

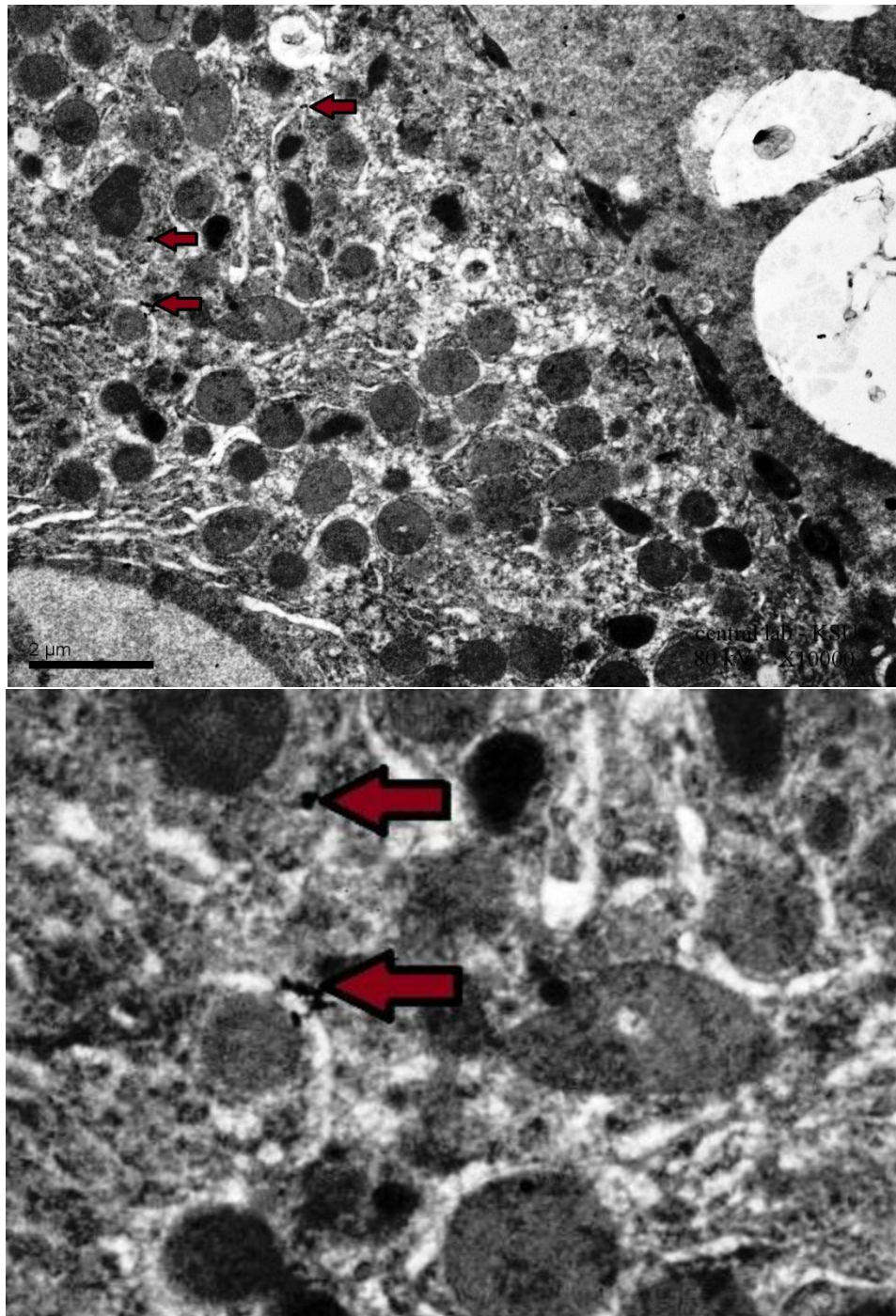


Fig. 5 An electron micrograph of 0.1ml SNP - treated mouse showing aggregations of SNP deposits(←) in the cytoplasm nearby the mitochondrial membrane (Original mag. X 10000)

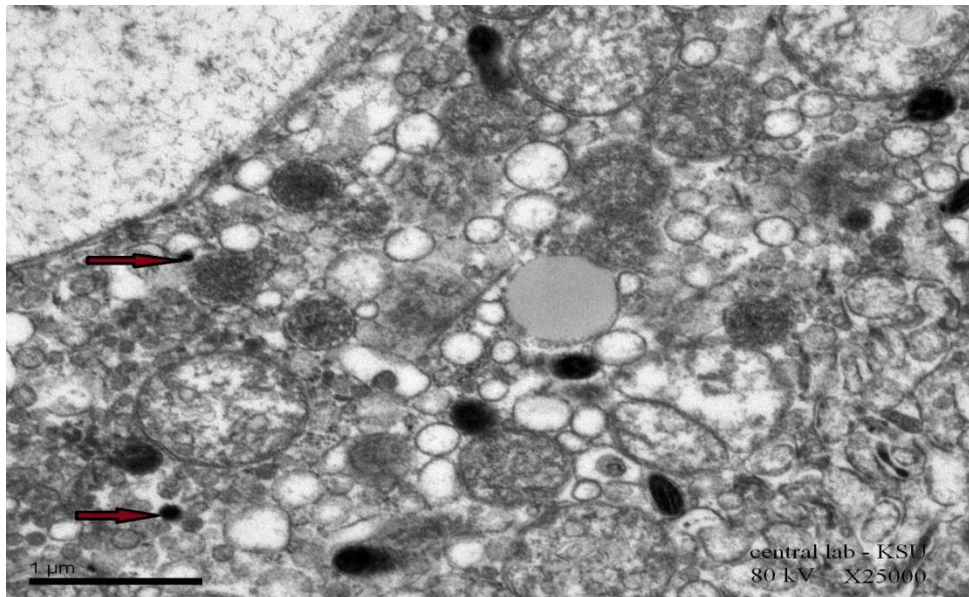


Fig. 6 An electron micrograph of 0.1ml SNP - treated mouse showing aggregations of SNP deposits(→) in the cytoplasm nearby the mitochondrial membrane. Notice the fragmented cristae. (Original mag. X 25000)

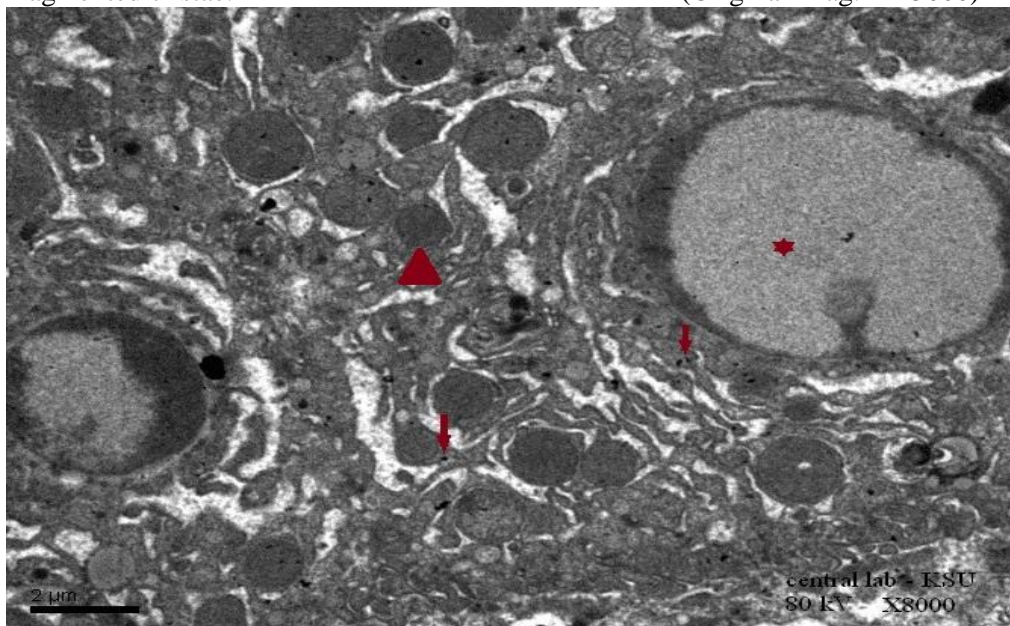


Fig.7 An electron micrograph of 0.4ml SNP - treated mouse showing aggregations of SNP deposits in the cytoplasm nearby and in the organelles(↓). Notice the fragmented nuclear chromatin (*), electron - dense mitochondria (arrow - head) and dilated rough endoplasmic reticulum. (Original mag. X 8000)

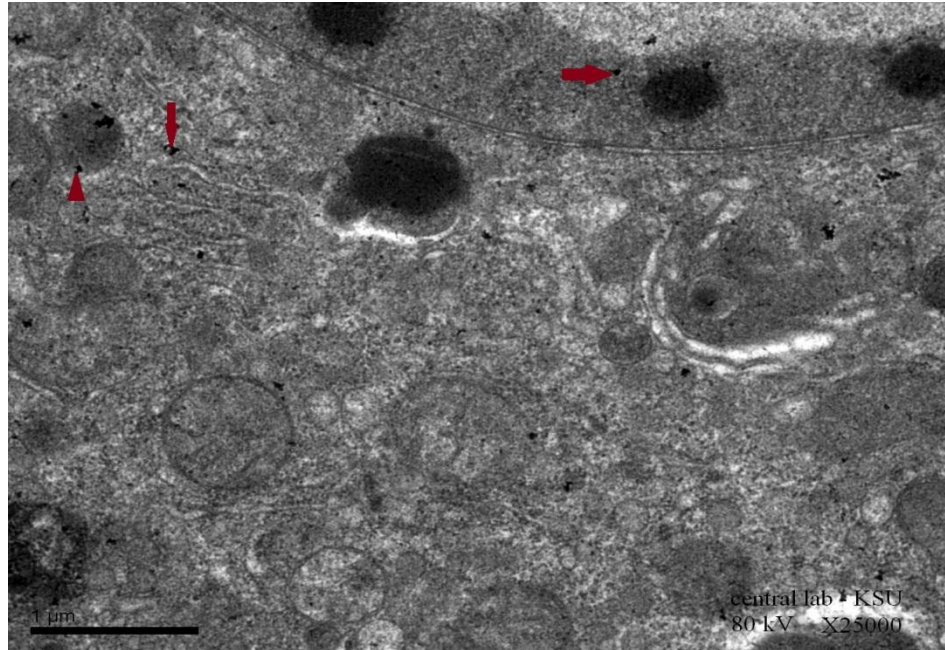


Fig. 8 An electron micrograph of 0.4ml SNP treated mouse showing SNP singlet in the mitochondrial membrane (arrow head), in the rough endoplasmic reticulum membrane (↓) and in the nuclear chromatin (→) (Original mag. X 25000)

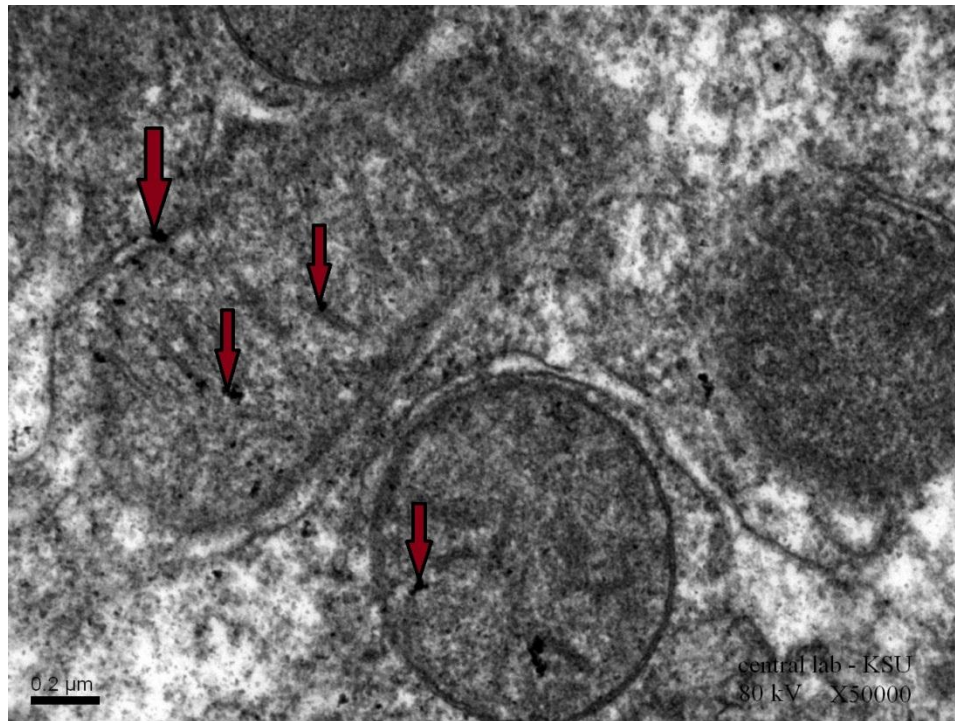


Fig. 9 An electron micrograph of 0.4ml SNP treated mouse showing aggregations of SNP singlet in the outer and inner membranes of the mitochondria (↓). (Original mag. X 50000)

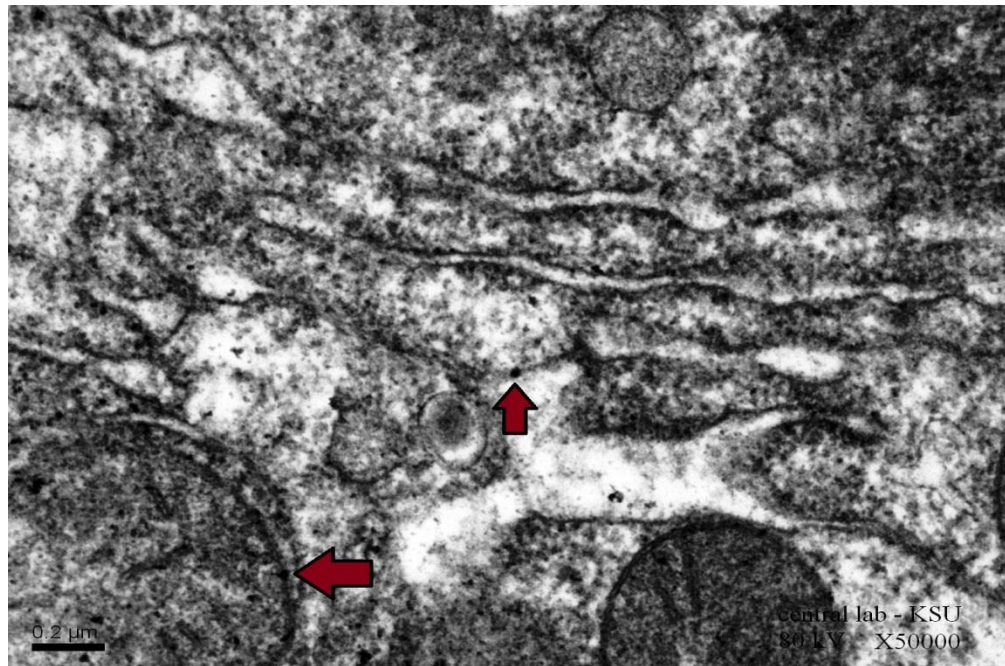


Fig. 10 An electron micrograph of 0.4ml SNP treated mouse showing aggregations of SNP singlets in the rough endoplasmic reticulum membrane(↑).
(Original mag. X50000)

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