

Original Research

## Histopathological effects of nanosilver (Ag-NPs) in liver after dermal exposure during wound healing

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### Abstract

**Objective(s):** With the advent of nanotechnology, significant progress has been made in the area of nanoscale materials such as nanosilver (Ag-Nps). These nanoparticles have a wide range of applications and been used for antimicrobial purposes for more than a century. However, little

attention has been paid to the toxicity of nanosilver wound dressing. This study was designed to investigate the possible histopathological toxicity of Ag-NPs in liver of mice during wound healing.

**Materials and Methods:** A group of 50 female BALB/c mice of about 8 weeks were randomly divided into two groups: Ag-NPs and control groups (n=25). After creating similar wound on the backs of all animals, the wound bed was treated in Ag-NPs group, with a volume of 50 microliters of the nanosilver solution (10ppm), and in control group, with the same amount of distilled water. The experiment lasted for 14 days. Histopathological samplings of liver were conducted on days 2, 7 and 14 of the experiment.

**Results:** Histopathological studies demonstrated time-dependent changes in mice liver treated with Ag-NPs compared to control group. Some changes include dilation in central venous, hyperemia, cell swelling, increase of Kupffer and inflammatory cells.

**Conclusion:** This study suggests that use of nanosilver for wound healing may cause a mild toxicity, as indicated by time-dependent toxic responses in liver tissue. However, this issue will have to be considered more extensively in further studies.

**Keywords:** Dermal toxicity, Liver, Nanosilver (Ag-NPs), Wound healing

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### Introduction

Silver and silver compounds have been used as antimicrobial agents (in the form of metallic silver and silver sulfadiazine ointments) (1).

Nano-silver or silver nanoparticles (Ag-NPs) have a large number of physicochemical characteristics and biological effects including optical and electronic properties, catalytic and antibacterial activities (2). Thus, Ag-NPs have become one of the most widely used nanomaterials in consumer products due to its strong antiseptic and antibacterial properties (3). Ag-NPs are being used in wound dressings and bandages (4). However, there are many concerns about their toxic properties (5).

Both *in vitro* and *in vivo* studies have shown that Ag-NPs may have negative effects in human health through the skin (dermal absorption), along with inhalation and may increase risk of chronic diseases using medicinal products containing nanosilver (6). For example, it has been reported that some nanoparticles can be toxic by generating free radicals and reactive oxygen species (ROS) and therefore cause intracellular damage (7). The results of studies suggest that Ag-NPs can be causing a decrease of reduced glutathione (GSH), increased levels of ROS, lipid peroxidation, and genes responsible for ROS production (8). However, nanoparticles toxicity depend on their route of entry into the living system (7). It has been reported that smaller Ag-NPs are more toxic than larger ones (9). Silver compounds can be absorbed through the gastrointestinal tract, skin, respiratory tract and other mucous membranes (10). They can accumulate in the liver and kidneys and can cause organ damage, (11) such as liver failure (12). In a previous study reported in 2005 that Ag-NPs may damage the liver through the induction of oxidative stress (13).

In other study on toxicity of Ag-NPs in human liver tumor cells (HepG2), the authors found that the accumulation of Ag-

NPs in the cytoplasm and nuclei of treated cells induced intracellular oxidative stress, which is independent of the toxicity of Ag (+) ions (14).

However, the same cytotoxic effects have been reported for Ag ions. It has been reported that Ag ions were released from Ag-NPs surfaces, and they could show cytotoxicity, apoptosis and induction of stress response genes (15).

In a study of Ag-NPs toxicity in human hepatoma cell line (HL-7702), researchers reported that Ag-NPs can cause membrane damage, leakage of lactate dehydrogenase (LDH), and reduced superoxidase dismutase and glutathione peroxide activity. Besides, cell viability was reduced in a dose and time-dependent manner (16).

Because of widespread application of Ag-NPs in medicine and their effects on human health, we have designed to investigate the possible histopathological effects of Ag-NPs in liver of mice after dermal exposure during wound healing.

### Materials and Methods

#### *Preparation of Ag-NPs*

Nanosilver solution (4000 ppm) was purchased from Nano Pars Co., Iran, with a purity of 95% and with feature sizes less than 30 nm.

Nanosilver has a strong bactericidal effect in the range concentrations of 10–50 ppm (17). Thus, in this study, the final concentration of solution was 10 ppm.

#### *Mice holding*

A group of 50 female BALB/c mice of about 8 weeks (weighting  $24.2 \pm 3.0$  g) were purchased from Medical Faculty of Shahrekord University, and then transferred to the laboratory.

The animals were in a single group and maintained on commercial pellet diet, given deionized water *ad libitum* and kept in plastic cages in a  $20 \pm 2$  °C, 50–70% relative humidity room with a 12-h light/dark cycle.

After 2 weeks acclimation, these mice were randomly divided into two groups: Ag-NPs and control groups (n=25).

#### ***Excisional wound model & experiment***

Anesthesia for experimentation was achieved with an intramuscular injection of 10 ml ketamine, 0.5 ml acepromazine, 2 ml diazepam and about 0.5 ml xylazine solution at a dose of 50 mg/Kg. The dorsal area of each mouse was carefully shaved ( $2.0 \times 2.0 \text{ cm}^2$ ) after anesthesia, then the skin was disinfected with iodine. A circular full-thickness excisional wound  $10 \pm 2 \text{ mm}$  was created.

Once a day at the same time, the wound bed was treated in Ag-NPs group, with a volume of 50  $\mu\text{l}$  of 10 ppm Ag-NPs solution (diluted with distilled water), and in control group, with the same amount of distilled water (the treatment was done by sampler).

Mice were housed separately. The experiment lasted for 14 days. This study was conducted according to the NIH guideline.

#### ***Pathological study of liver***

On days 2, 7 and 14 of the experiment, six animals from each group were killed by ether inhalation in a closed space. The livers were removed. Then, small pieces of liver tissue were fixed in 10% buffered formalin and dehydrated in a graded series of alcohol.

The samples were sectioned at a thickness of 5  $\mu\text{m}$  and stained with hematoxylin and eosin (H&E) for histopathologic studies.

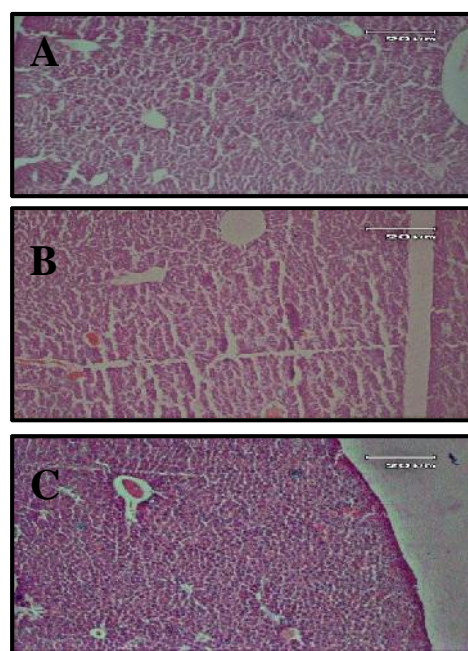
#### **Results**

In all days of sampling (days 2, 7 and 14), control group showed normal liver tissue with no specific injury (Figure 1).

In contrast, the pathological results of Ag-NPs group showed the pathological changes in the liver, including dilation in central venous, hyperemia, cell swelling, increase of Kupffer and inflammatory cells and fatty change in a time-dependent manner.

Therefore, on day 2, in Ag-NPs group, minor dilation in central venous and hyperemia, in addition, an increase of Kupffer and inflammatory cells was seen. The inflammatory cells were mostly mononuclear lymphocytes, eosinophils and plasma cells. On day 7, in Ag-NPs group, dilation in central venous, hyperemia was seen. In addition, the radial arrangement of liver cells was lost and cloudy swelling in the hepatic cytoplasm was observed.

After 7 days, Ag-NPs group showed a significant increase in the number of inflammatory cells and also Kupffer cells. On day 14, in Ag-NPs group, besides dilation in central venous, hyperemia and a significant increase of Kupffer and inflammatory cells, a slight fatty change in some hepatocytes was seen (Figure 2). A comparison of liver histopathologic changes between two groups of mice in 2, 7 and 14 days of study are shown in table 1

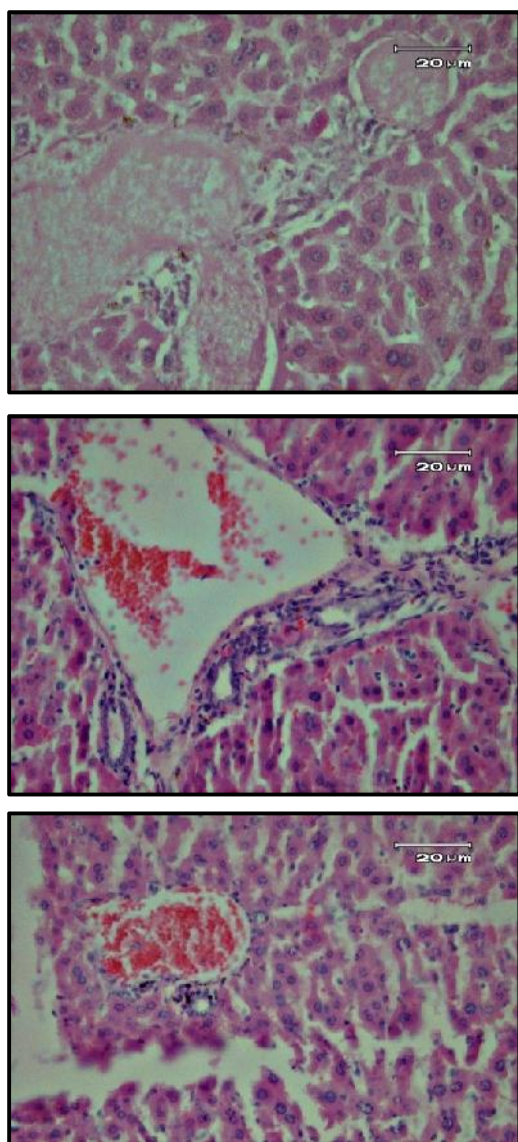


**Figure 1.** H&E stained liver sections of mice of control group in 2,7 and 14 days of study ( $\times 10$ ). A) control group-day 2; B) control group-day 7; C) control group-day 14. Liver tissue is normal in the control groups.

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**Table 1.** Comparison of liver histopathologic changes between two groups of mice in 2,7 and 14 days of study. Severe (+++), moderate (++), mild (+), none (-).

Group	Day	Inflammatory cells	Kupffer cells	Fatty change	Dilatation of the central vein
Control	2 day	-	-	-	-
	7 day	-	-	-	-
	14 day	-	-	-	-
Nanosilver	2 day	+	+	-	+
	7 day	++	++	-	++
	14 day	+++	+++	+	+++



**Figure 2.** H&E stained liver sections of mice of treatment (nanosilver) group in 2,7 and 14 days of study ( $\times 40$ ). A) Treatment group-day 2 with minor dilatation of the central vein, minor hyperemia, and inflammatory cells.; B) Treatment group-day 7 with dilatation of the central vein, hyperemia, and cell swelling; C) Treatment group-day 14 with dilatation of the central vein, hyperemia, inflammatory cells, increase kupffer cells, and slight fatty change.

## Discussion

The present study clearly showed that Ag-NPs used for wound healing in adult mice can produce the histopathological abnormalities in the liver in a time-dependent manner.

Ag-NPs can be absorbed through the skin. Studies indicate that Ag-NPs can enter the bloodstream when they used for burn wounds. These results were confirmed by use of Acticoat™ as a wound dressing, for patients with burns in Western Australia. On the skin uptake, Ag-NPs with very small diameters may have profound effects on brain, kidneys and liver (10).

The liver normally contains high levels of thiol-rich proteins such as glutathione (11). Thus, Ag-NPs accumulate mainly in the liver (18).

Likewise, the results of a study showed that Ag-NPs accumulated mainly in the liver and spleen (19).

It has been reported that Ag-NPs (7-20 nm) with final concentrations of 10-200  $\mu\text{g/ml}$  (10-200 ppm) can cause oxidative stress, apoptosis, and decreased cell viability in fibroblasts and liver cells of isolated from Swiss albino mice (20). Also, a study on the toxicity of Ag-NPs wound dressing after 21 days in rats have demonstrated minor pathological changes in liver tissue and also a significant increase in serum levels of alanine aminotransferase (ALT) in Ag-NPs group, that it confirms the hepatotoxic effect of Ag-NPs by dermal exposure (21). In the present study also we observed hepatotoxicity. Other studies have also shown that Ag-NPs can cause a dose-and time-dependent changes.

Loghman et al. (2012), reported the dose-dependent lesions in liver cells of broiler chickens after 42 days of oral Ag-NPs administration.

They observed hyperemia and cell swelling at low concentration (4 ppm), dilated central vein, hyperemia and severe fatty change at more concentrations (8 and 12 ppm) and an increase in connective tissue (fibroplasia) and focal necrosis of hepatocytes at the highest concentration (12 ppm) (22). Koohi et al. (2010) showed the pathological changes in the liver of rabbits by dermal exposure of Ag-NPs (23). In one study, the oral toxicity of Ag-NPs in rats was investigated for 28 days.

Researchers found dilated central vein and bile duct hyperplasia in the liver of rats (24). In another study (2012) (25) the oral toxicity of Ag-NPs (70 nm) was studied for 30 days in rats. At the highest concentration (2 mg/kg), liver damage, necrosis and apoptosis in liver cells took place. Yousef et al. (2012) investigated the toxicity of Ag-NPs (< 50 nm) through an intraperitoneally injection in different concentrations for 30 days in rats.

They reported that the walls of most sinusoids showed numerous Kupffer cells (26). In the present study, over-production of Kupffer cells occurred in mice liver of Ag-NPs group. It has been shown that Kupffer cells are important for removing nanoparticles (27). When nanoparticles are removed from the liver by macrophages (due to phagocytosis), the generation of free radicals greatly increases.

The increase in oxidant production may contribute to damage the cell membrane and ultimately impair liver function (25). Thus, the number of Kupffer cells can show the amount of damage in liver tissue. Korani et al. (2011) studied dermal toxicity of Ag-NPs (< 100 nm) in guinea pig.

They reported the histopathological changes in the liver and also over-production of Kupffer cells in a dose and time-dependent manner (28).

In the present study, also a significant increase in the number of Kupffer and inflammatory cells after 7 days was seen, that can show the pathological liver injury in a time-dependent manner.

Based on previous microscopic studies it was observed that Ag-NPs caused mild to severe dose-dependent lesions in liver cells such as accumulation of inflammatory cells, necrosis in liver cells and an increase in connective tissue (fibroplasias) (29). The results of present study, the histopathological abnormalities in the liver with no sign of necrosis, can indicate a mild histological damage in the liver.

### Conclusion

This study suggests that use of Ag-NPs for wound healing may cause a mild toxicity, as indicated by time-dependent toxic responses in liver tissues. However, in this study, it was better that the amount of silver was measured in liver and bloodstream. Therefore, this issue will have to be considered more extensively in further studies.

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