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Original Article

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Silver nanoparticle induced muscle abnormalities : A sub-chronic dermal assessment in guinea pig

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Abstract

Nanosilver has recently been recognized as an antimicrobial agent. Although this nanoparticle can be used in medical applications but its dermal and systemic toxicity via dermal exposure has not been completely determined yet. The aim in this study was to investigate the potential dermal toxicity of Nanosilver in subchronic method. Before the colloidal silver nanoparticle toxicity evaluation, their size was subjected in sizes < 100 nm by Transmission Electron Microscope and revealed that the nanoparticles contained nanosilver by X-Ray Diffraction. The selected animals were 24 male guinea pigs weighing 350-400 g. These animals were exposed to three concentrations of nanosilver (100, 1000 and 10000 μg/ml) by dermal exposure. Toxic responses were assessed based on histopathologic parameters. Significant dose-dependent histopathological changes were observed in the muscle of treated animals. It seems that colloidal silver nanoparticles have the ability to create dose-dependent toxic responses in this organ.

Key words: nanosilver, histopathological changes, sub-chronic toxicity, dermal toxicity

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1. Introduction

Probability of exposure to nanomaterial is increasing by the growing use of this substances in various industries (Stebounova et al., 2011). Some researchers are interested to study the major toxicological responses to nanoparticles that are redox active or pass across cell membranes and enter the tissues and sub-cellular organelles (Teodoro et al., 2011). Whereas the toxicological effects of nanosilver due to oral and inhalation are studied, there is a lack of study on hazards arising from the substance dermal diffusion and assessment of its consequent acute and sub-chronic toxic effects.

Human has used silver compounds in medical applications for centuries. There are several sources in environment (Weast, 1988–1989), which can release silver compounds such as nitrate, chloride, bromide, acetate, oxide, sulfate, and cyanide (Rosenman et al., 1979). Silver could be found in low concentrations in various tissues (Wan et al., 1991; Hollinger, 1996; Sue et al., 2001). Argyria and mild allergic responses are caused by exposure with high doses of silver and its compounds (Stokinger, 1981:).

Silver has been known as an antimicrobial agent for centuries in the various forms such as metallic silver, silver sulfadiazine ointments (Sondi and Salopek-Sondi, 2004; Cho et al., 2005; P et al., 2005; Morones et al., 2005 ; J H Ji JH et al., 2007). However, it is recently reported that colloidal silver nanoparticles has been used as a biocidal material (Nowack et al., 2011). Nanosilver has been recognized as a potent antimicrobial agent and has been used for medical applications in the forms of silver based dressings or silver coated medical devices (band, pad, gloves, and catheter cover, wound dressing and etc.) (Park et al., 1999; MTR, 2006). Silver nanoparticles can damage the bacterial cell walls and cause cell death (Sondi and Salopek-Sondi, 2004). Although nanosilver has a broad therapeutic properties, its safety profile has not yes been completely identified. In our previous study we reported acute and sub-chronic dermal toxicity of nanosilver in skin, liver and spleen (M Korani M et al., 2011). In this study, we present our findings on toxicological effects of silver nanoparticles in muscle.

2. Materials and Methods:

Nanosilver

The silver nanoparticles were purchased from Quantum sphere Inc., (Santa Ana, CA). Then, three different nanosilver solutions (100, 1000 and 10000μg/ml) was provided in the Pharmaceutics Lab., Faculty of Pharmacy, Tehran University of Medical Sciences.

Experimental animals and housing conditions

In present study thirty experimental male Hartley-albino guinea pigs were obtained from Pasteur Institute of Iran at the age of five or six weeks old and weight of 350 -450 g. All guinea pigs were housed in stainless steel cages and allowed to adapt to that condition for 14 days before the experiments. The animals were kept on a 12 h dark/12 h light cycle at controlled temperature (about 22 ± 3 o C) and were allowed free access to standard laboratory diet, vitamin C and tap water. The experimental animals were randomly divided into ten treatment groups, each one containing six animals. Three groups were specified to acute test and the rest were used to sub-chronic dermal toxicity test. All animal studies were treated according to the guidelines of the US National Institute of Health (NIH publication no. 85-23, revised 1985).

Transmission electron microscopy and X-ray diffraction tests

Sizes of silver nanoparticles were determined by X-ray diffraction (XRD) and transmission electron microscopy (TEM). In this study, XRD was used as standard equipment

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(Siemens with Cu source, 40 K V and 30 mA). (Sample picks were determined at 10°- 70°) (Gupta et al., 2007).

Sub-chronic dermal toxicity studies

Thirty guinea pigs were randomly divided into five groups that group embodying six animals. An area in 5cm x 5cm of the animal back surface was shaved for treatment. Continuous back shaving was performed two or three times per week during the test (13 weeks).

After shaving the animals' skin, the shaved area in treatment group was rubbed with 100, 1000 and 10000 μg/ml of nanosilver and in the control group the shaved skin remained untreated. The procedure was repeated once daily for five days per week.

Pathological studies

At the end of the treatment, animals were anesthetized were killed by overdose of formalin. For histopathology studies, we obtained samples of 5-μm thick muscle and stained them with hematoxyline and eosin (H&E). The tissue sections were embedded in paraffin wax and were analyzed by using (×40Olympus-2B microscope).

3. Results

TEM Studies

The size of nanosilver particles was determined

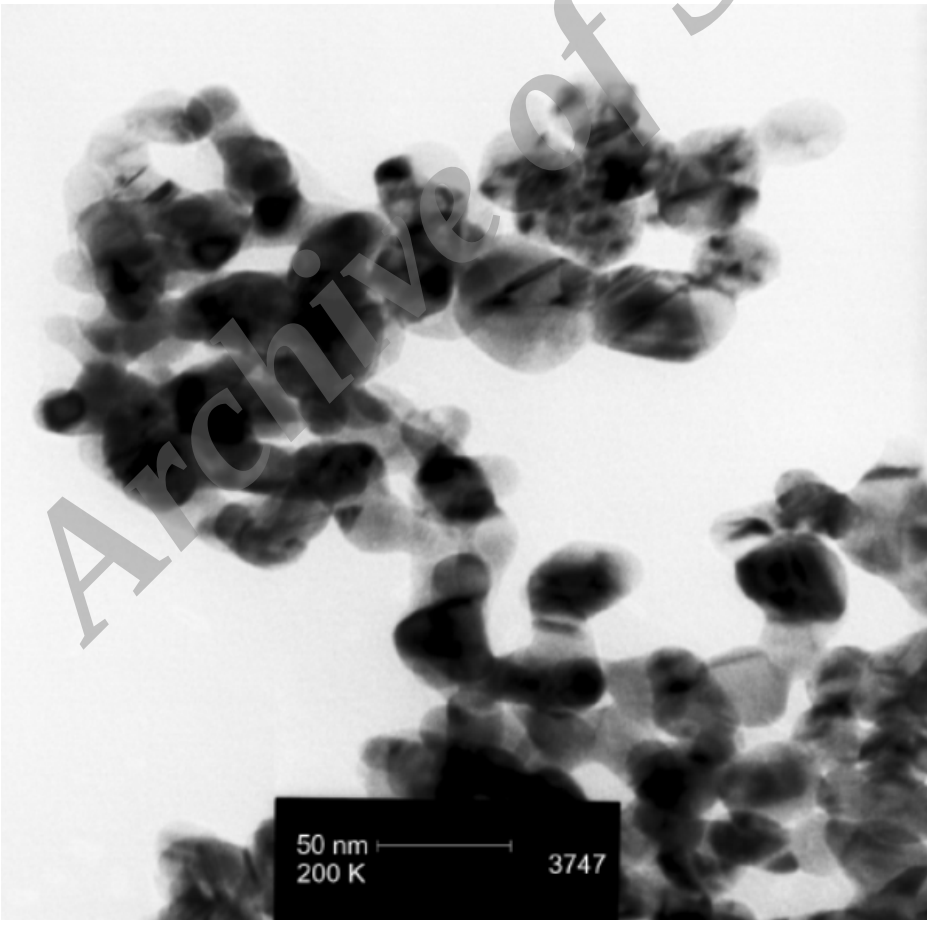

Figure 1: TEM Image of nanosilver depicts particles measures are less than 100nm

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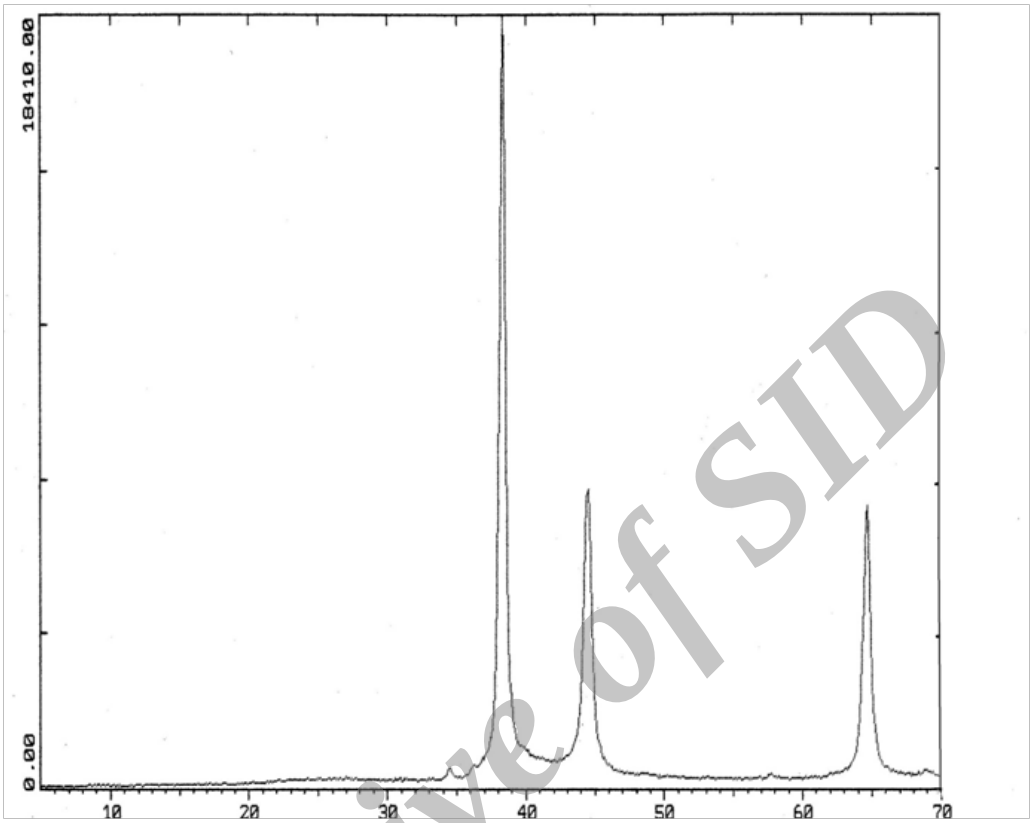
<100 nm.

XRD examination



Figur 2: In XRD model, peak samples at 38, 44 and -64.5 degrees represents existence of nanosilver

XRD patterns of nanosilver particles were observed in the ranges of 39, 45, 65 degrees.

Histopathological findings:

Analysis of abnormalities in muscle using stain of Haematoxylin Eosin (H&E) showed that they were completely normal in control group without inflammation. In this group, peripheral nuclei, endomysium between fibers with reduction of tissue connection were detected and fibers showed striated cytoplasm (Figure 3A). In AgNO3 group, some fibers of muscle contained acidophilic cytoplasm surrounded by macrophages as well as inflammation in their endomysium (Figure 3B). In the group treated with 100 μg/mL solution of nanosilver, fibers of muscle with endomysium and inflammation in edomysium were observed (Figure 3C). In the group treated with 1000 μg/mL solution of nanosilver, the same characterization was recorded. Also in this group, acidophilic cytoplasm of fibers and accumulated myophagocytoses were observed (Figure 3D). In the group treated with 10000 μg/mL solution of nanosilver group, the same pattern was repeated but the histopathological changes were higher than the group treated with 1000 μg/mL group (Figure 3E). All details on histopathological changes of the

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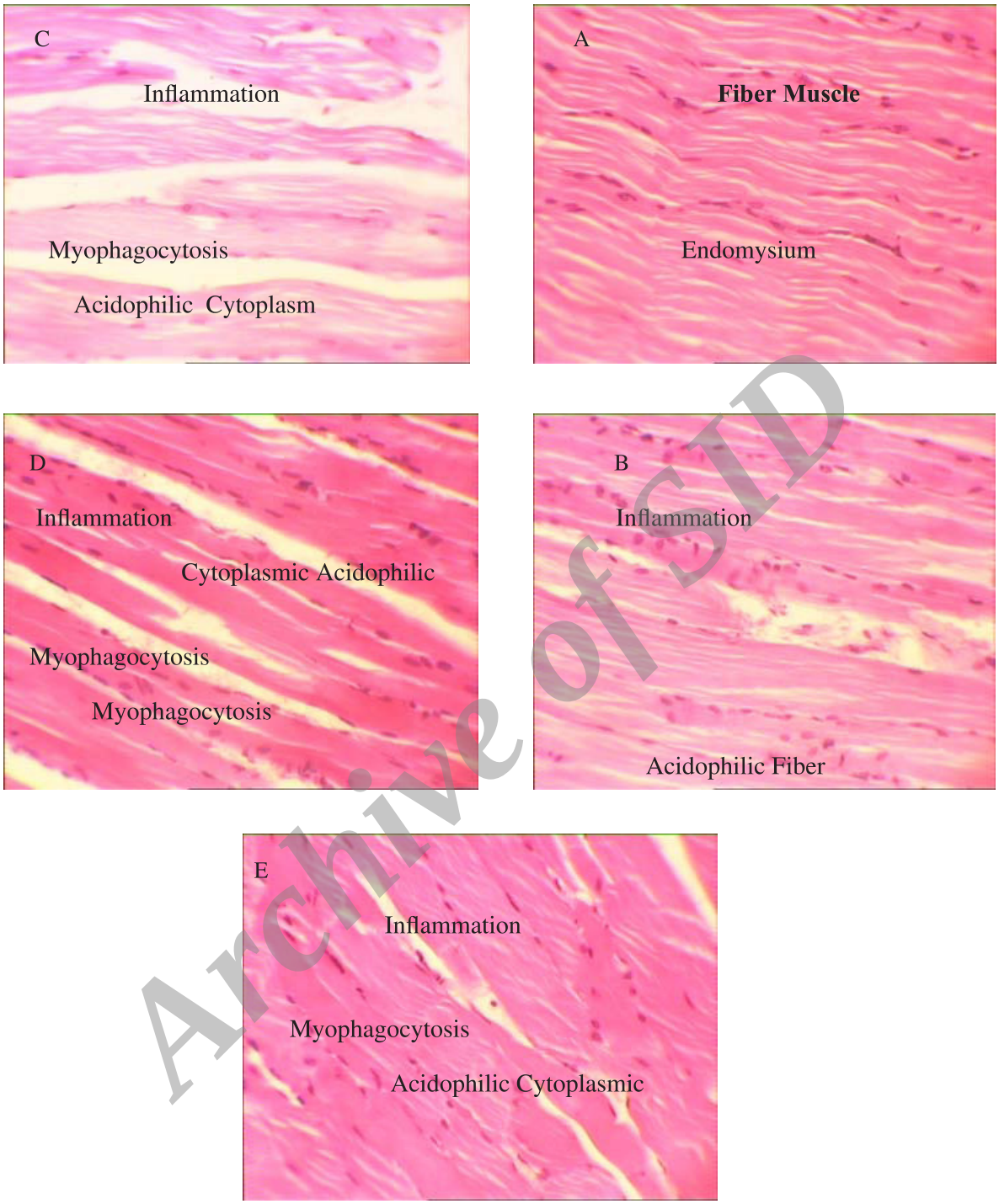

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Figure 3: Histological section of the muscle tissue in a guinea pig treated with different concentrations of nanosilver (A) shows normal cardiomyocyte in control group (B & C) and AgNO3 greoup and low-dose nanosilver group, mild degennation fiber (D) and middle-dose nanosilver group, with mild inflammation, and moderate increased of macrophage (E) high-dose nanasilver with mild inflammation , severe degennation fiber and increased of macrophage (H & E×40).

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Table 1: Summary of muscle abnormal changes

Group	Inflammation	Degennation Fiber	Macropha ge (Myophagocytosis)	Deformation Fiber
control	-	-	-	-
AgNO3	+	+	+	-
nanosilver100ppm	+	+	+	+
nanosilver1000ppm	+	++	++	+
nanosilver10000ppm	+	+++	+++	++

Severe (+++) moderate (++) mild (+) none(-)

spleens are recorded in Table 1.

4. Discussion

In the preliminary study, no mortality was observed among treated animals.

The use of nanosilver as a nanomaterial is grown in various industry such as medical and consumer products because of its bactericidal properties. Thus the potential of exposure with the material is increasing.

Because the potential toxic effects of nanosilver are not sufficiently known (Benn et al., 2010), we evaluated the effects in different concentration of nanosilver by dermal toxicity methods. At present, most of reports on toxic effects of nanosilver are limited to those focusing on substance inhalation (Stebounova et al., 2011) or oral exposure (Kim et al., 2010).

In pervious study, we recorded the toxicity effects of silver nanoparticles on the livers and spleens of guinea pigs. According to the demonstrated results, concentrations >100 could cause inflammation, white pulp atrophy in spleen for all of the test groups. In liver, these concentrations, could cause inflammation, limited pale destruction, hepatocyte degeneration .The experimental model applied in this study was described previously (Blumberg and Carey, 1934).

Our previous study on toxic effects of nanosilver in liver and spleen based on 90- day dermal exposure showed that the consequent histopathological changes are completely dose-dependent (M Korani M et al., 2011). Other studies on sub-acute exposure with nanosilver has identified minimal pulmonary inflammation or cytotoxicity. Our result in this study clearly showed appearance of abnormalities in muscle due to exposure to nanosilver.

In a case study, Blumberg and Carey (1934), reported appearance of argyria in a woman who swallowed a total dose of 6.4g AgNO3 over a one year period. The symptoms were observed after the first six months of exposure (Blumberg and Carey, 1934). Rosenman et al. (1979) found that workers exposed to AgNO3 and Ag2O dusts for 1-10 years show a range of illnesses including respiratory irritation, abdominal pain, decreased night vision (Rosenman et al., 1979). In another study, the same authors observed respiratory irritation, decreased night vision, increased of N-acetyl-B-D glucoseaminidase (NAG) and decreased creatinine clearance in a group of workers exposed to silver compounds (Rosenman et al., 1987). In another study, some workers showed the symptoms of discoloration of conjunctiva and cornea (Moss et al., 1979). Williams et al. (1999) identified argyrosis in 51 years old man exposed to silver compounds (Williams, 1999). A case study on a 59-year-old man ingested colloidal silver two to three times per year for two years (Chang et al.; 2006) detected appearance of hypertension, diabetes, hyperlipidemia as well as a blue-grey signs on face (Chang et al., 2006). Tang et al.

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(2008) reported neurological symptoms and myoclonic seizures in a 75-year-old man who had experienced self-medication with colloidal silver for a long period (Tang and Xi, 2008).
Stepien et al. demonstrated that many medical devices could release silver ions (Ag+) which may be absorbed into blood and be accumulate in some organs such as liver and kidney and induce toxicity in tissues. Also exposure to a certain doses of Ag+ has been reported to cause death (Stepien et al., 2009). In present study we demonstrated that silver nanoparticles with properties similar to Ag+ could be translocated into the body and cause histopathological abnormalities in the tissues such as kidney but these changes are not similar to those arisen by AgNO3 through the same route of administration.
Kim et al. (2010), examined oral toxicity of nanosilver in rat for 90 days using different concentrations and reported histopathological abnormalities in various organs such as kidneys, spleen, heart, liver and etc. In the kidneys, a slight increase of minimal tubular basophilia. In the liver, focal, multifocal, lobular necrosis, bile –duct hyperplasia were observed (Kim et al., 2010) too.
Braydich-Stoll et al. (2005) showed potential cytotoxicity of different concentrations of nanosilver to mammalian stem cells. They reported apoptosis and necrosis in cells exposed to high doses of nanosilver (≥10 μg/ml). At nanoparticle concentrations of 1-5 μg/ml mitochondrial function and cell viability were reduced (Braydich-Stolle et al., 2005).
We used three concentrations of nanosilver solution (10000, 1000 and 100 μg/ml) without recording any mortality in animals. While this is the first study on dermal and systemic toxicity of nanosilver in subchronic treatment, the nanoparticle concentrations were high and unrealistic therefore it seems necessary to estimate the NOAEL (no observable adverse effect level) of nanosilver by dermal application.

5. Conclusion

Nanosilver targets the skin, liver, spleen, and muscle in the male guinea pig in dermal application. It seems that proposed doses of present study was not safe for dermal application and may cause muscle damages. Although no mortality was observed in all nanosilver groups significant dose-dependent abnormalities were recorded.

Conflict of interests : None declared.

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