

RESEARCH ARTICLE

Dermal toxicity of Colloidal Nanosilver in Albino Rabbit: A New Approach to Physicochemical Properties

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ARTICLE INFO

Article History:

Received 30 April 2017

Accepted 21 June 2017

Published 10 July 2017

Keywords:

Dermal toxicity

Irritation, corrosion

Nanosilver

AgNPs

Commercial products

Albino rabbit

ABSTRACT

Objective(s): Silver nanoparticles have been widely used as new potent antimicrobial agents in cosmetic and hygienic products, as well as in new medical devices. Serious concerns have been expressed on the potential health risks of dermal applications of nanosilver containing consumer products (AgNPs), therefore regulatory health risk assessment has become necessary for the safe usage of AgNPs in biomedical products with special emphasis to their dermal toxicity potentials. We aimed in the present study to compare the dermal toxicity of three different AgNP containing disinfectants in an albino rabbit model and tried to determine the role of size and other physicochemical properties on their possible dermal toxicity.

Methods: After the characterization of all three samples by transmission electron microscopy (TEM), X-Ray Diffraction (XRD) and Dynamic Light Scattering (DLS), corrosive and irritant potentials of AgNPs in three different sizes of three colloidal AgNPs were scored by the OECD 404 guideline with necessary modifications and were applied under the specified concentrations via nanosilver skin patches on the shaved skin of young female albino rabbits. All skin reactions were recorded in 3 min as well as in 1, 4, 24, 48 and 72 hours from the application and compared with the control group and followed up for 14 days.

Results: Although short-term observations didn't show any significant changes in the weight of animals and macroscopic variables, long-term histopathological abnormalities were seen in the skin of all test groups, which was not associated with the size and other physicochemical properties of AgNP samples. The toxicity manifestations were dry skin, scaling in doses lower than 100 ppm and erythema in higher doses up to 4000 ppm which was reversed.

Conclusions: This finding creates a new issue in the possible dermal effects of all colloidal AgNPs, containing nano health products, which should be considered in future studies by focusing on other physicochemical properties of AgNPs and possible underlying mechanisms of toxicity by conducting cellular models.

How to cite this article

Raesian A, Arbabi Bidgoli S, Rezayat Sorkhabadi S M, Dermal toxicity of Colloidal Nanosilver in Albino Rabbit: A New Approach to Physicochemical Properties. *Nanomed Res J*, 2017; 2(3):142-149. DOI: 10.22034/nmrj.2017.03.001

INTRODUCTION

Increased utilization of nanoparticles in recent years due to significant advances in nanotechnology have an impact on industrial technology with increased risk of human exposure to nanoparticles

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through occupational, environmental or medical routes [1,2]. One of the most commonly used engineered nanomaterials (ENMs) is Nanosilver (AgNP), which has unique physicochemical properties and antibacterial potentials [3]. It plays



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an impressive role in many new industries especially textiles production [4], because textiles can make suitable substrates to grow micro-organisms especially at special humidity and temperature in contact to human body [5].

The key to AgNP related successful antibacterial activity is the multifaceted mechanism by which AgNP acts on microbes and because of multiple mechanisms of antibacterial activities and lower resistance to AgNP than other antibacterial agents [5]. For these reasons, AgNPs are utilized in antibacterial coatings of different medical devices to decrease the level of nosocomial infections [6,7]. Owing to their potent antibacterial activity, different AgNP containing products are attracting public interests for a variety of health applications, and recently, the local market of AgNP containing textiles has been improved for more than hundred new healthcare products [8].

AgNPs are incorporated into many consumer and medical products due to their antimicrobial properties; however, the potential environmental risks of AgNPs are yet to be fully understood. In fact, the AgNP production, environmental release, and fate, predicted environmental concentrations in surface water, sediments, as well as possible human health effects are not clarified exactly [9]. The antibacterial properties of AgNPs are mainly attributed to their high surface area to volume ratio but this potential may cause their higher reactivity to macromolecules especially DNA reactions, their possible genotoxic effects, mutagen induced health risks properties and carcinogenic properties as well [10]. Although different size dependent genotoxic effects from AgNP are reported by different methods in a dose-dependent manner [10], other physicochemical properties including zeta potential, shapes (rods, triangles, spherical particle) and aggregation capacities have been considered for their toxic potentials [11].

We showed that silver nanoparticles could be found in skin, liver, spleen of guinea pig [12] as well as heart, bone, kidney and skin damages by dermal exposures [13]. Due to the significance of dermal exposure to silver nanoparticles in different nano health products and due to the knowledge gaps in this regard, we aimed in this study to compare the dermal effects of a wide range of doses of three different commercially available colloidal AgNPs in similar sizes on the basis of their physicochemical

properties in rabbit model according to the OECD 404[14] guideline.

MATERIALS AND METHODS

Materials

AgNPs commercial products

Three types of prevalent commercial products of colloidal silver nanoparticles were used for this study. The first commercial product of AgNPs was a yellowish-brown product, purchased from a local manufacturer in Tehran and coded as A-AgNP. According to the information provided by the manufacturer, it was a water-based colloid which contained 4000 mcg/ml spherical uncoated AgNPs. The second commercial AgNPs was donated by another local manufacturer in Isfahan and coded as B-AgNP. According to the information provided by the manufacturer, it was water-based plant coated colloid contained 187.5 mcg/ml of spherical silver nanoparticles. The third colloidal silver nanoparticle was purchased from a local manufacturer. This product was a water based colloidal AgNP containing 1000 mcg/ml spherical silver nanoparticles with 99.9% purity, according to the information provided by the supplier. The size and zeta potential of these samples were provided by TEM and DLS methods respectively (Fig 1). Table 1 compares the physicochemical properties of three test AgNPs samples.

Characterization of nanoparticles

The concentration of samples was determined by Atomic Absorption Spectroscopy (AAS). AgNPs were homogeneously dispersed in sterile distilled water by sonication for 30 minutes and filtered. Transmission electron microscopy (TEM) was used to estimate the size, shape, and composition of the AgNPs. The distribution of the AgNPs, hydrodynamic size and zeta potential was evaluated using Dynamic light Scattering (model ZEN3600; Malvern instrument Ltd., Tokyo, Japan). The X-ray diffraction pattern was performed in order to characterize nanoparticle structure. Experimental methods were used to optimize the concentrations of each serially diluted samples and to assure the quality of provided data. All experiments were repeated at least three times to confirm the accuracy and reproducibility of this method. Standard gold nanoparticles with pre-determined size were used to validate the instrument. Both above parameters

Table 1: Physicochemical properties of three test samples

Parameter	C-AgNP	B-AgNP	A-AgNP
Chemical composition	Ag: 4000ppm	Ag: 425 ppm Pb: 0.06 ppm	Ag:50/100/1000/2000/ 4000 ppm
Particle size	Diam(nm): 7.768 Width(nm):2.17 3 50nm	Width(nm): 5.64 Diam.(nm):7.332 100-300nm	Diam(nm): 9.142 Width(nm): 2.23 80-100nm
Physical form	Colloidal silver	Colloidal silver	Nano colloid silver - silver L2000
Wall Zeta Potential (mV)	-3.24	-14.4	9.33
Dispersant Name	Water	Water	Water
pH	3.30	4.27	5.3 = 50ppm 4.38= 1000ppm 2000ppm 5=100ppm 4= 3.67= 4000ppm
Viscosity(cp)	1.0031	1.003	0.8872
Conductivity (mS/cm)	5.17	2.88	3.97

(size and zeta potential) were measured at least three times for each sample. The data were calculated as the average size or zeta potential of AgNPs.

Selection of animal species

According to the test guideline (OECD 2002), 3 healthy young adult rabbits were used to test each dilution from each sample. Animals were obtained from Pasteur Institute of Iran at ages of 1-3 months and identified by color coding. To preclude mix-up, the animal number and group number also appeared on the outside of each cage.

Animal Housing and Maintenance

Individually, housed Female rabbits were in separate quarters in solid bottom cages. The animal room environment was adjusted accordingly (relative humidity 30-70%, temperature 22°C to 25°C) and monitored daily, the photocycle being 12 hours light and 12 hours dark. Upon arrival, all animals were examined generally and all were found healthy and admitted. Diet and water were offered ad libitum throughout the acclimatization and study periods. Air filtration and recirculation, the cage cleaning schedule, health checks and facility maintenance were carried out in accordance with the IAUPS Standard Operating Procedures,

recorded in the animal room records.

Preparation of the animals

By closely clipping the dorsal area of the trunk of the animals, fur was removed approximately 24 hours before the test. Care was taken to refrain from abrading the skin, and the inclusion of animals with normal skin was assured. For the Limit Test, all animals were fasted overnight. Food but not water was withheld from 5:00 p.m. on the day preceding dosing. Animals were offered a 10% w/v aqueous solution of glucose during this period for minimizing the stress caused by fasting.

Application of the test substance

The test substance was applied to a small area (approximately 6 cm²) of skin and covered using a gauze patch, being held in place with non-irritating tape. In case the direct application was not possible (liquid insecticide), the test substance was first to be applied to the gauze patch, which was then applied to the skin.

Observations

Observation of the animals were done individually at least once during the first 30 min after dosing, periodically during the first 24 hr

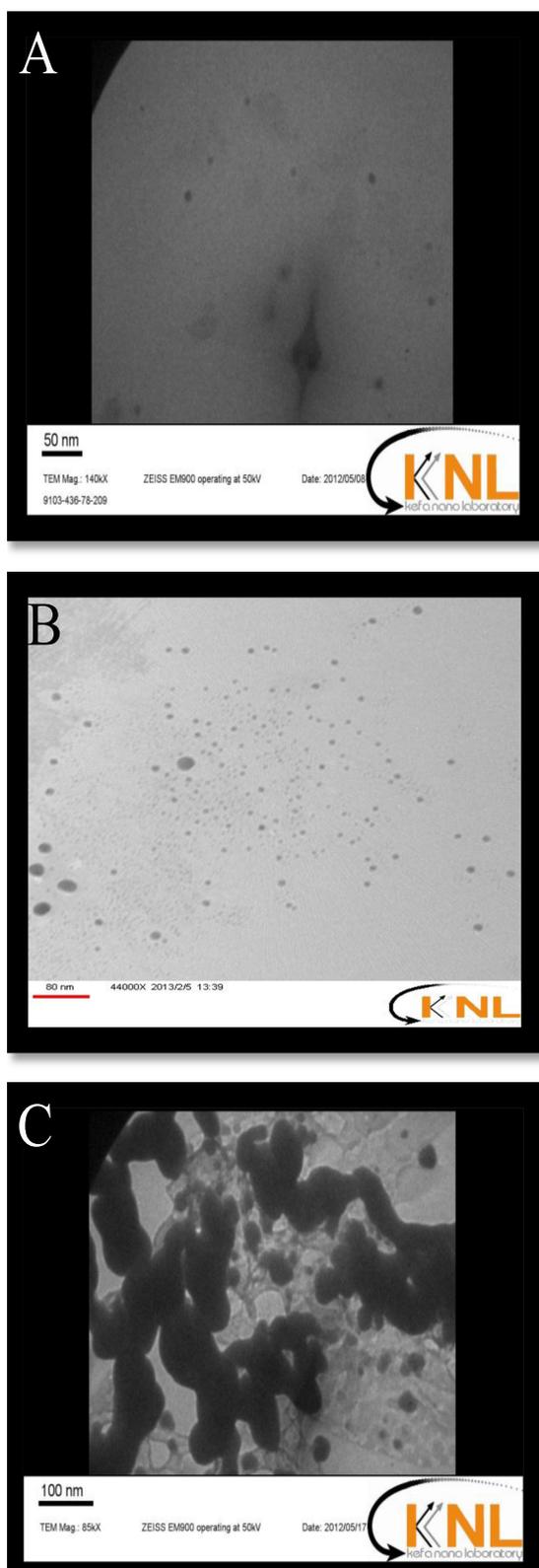


Fig. 1. TEM studies showed nanosilver particles in sizes of 50 nm (A), 80 nm (B) and 100 nm (C).

(during the first 4 hours with special attention), and daily thereafter, for a total of 14 days, except when they needed to be removed from the study and humanely killed for animal welfare reasons or were found dead. Systematically, all observations were recorded with individual records being maintained for each animal. Examination of all animals was done considering the signs of erythema and edema, and the responses scored at 60 minutes and then at 24, 48 and 72 hours after patch removal. For the initial test in one animal, the test site was also examined immediately after the patch was removed. Observations were continued until day 14 to determine the reversibility of the effects, if there was damage to the skin, which couldn't be identified as irritation or corrosion at 72 hours. In addition to the observation of irritation, all local toxic effects, such as defatting of the skin, and any systemic adverse effects (e.g., effects on clinical signs of toxicity and body weight), were fully described and recorded.

Statistical Analysis

Values were expressed as mean \pm SD. To compare groups, homogeneity of variances was evaluated first. When variances were not significantly different, data were analyzed by one-way analysis of variance (ANOVA) and the Student's t-test. When variances were considered significantly different, Man-Whitney U test for comparison of two variables and Kruskal-Wallis H test for comparison of more than two variables were used. A significant difference was accepted at $P < 0.05$. All statistical methods were performed by SPSS 21.0.0.

RESULTS AND DISCUSSION

TEM Studies

By using TEM analysis nano silver particles, less than 100 nm in size, were detected (Fig. 1).

XRD examination

Peaks of nanosilver by XRD were observed within the ranges of 30, 44, 64.5 degrees (2θ) (Fig. 2).

Mortality rate

Because no mortality was recorded during dermal application of different concentrations of nanosilver at doses of up to 10000 $\mu\text{g/ml}$ ppm in the preliminary study on rats, we considered all samples as practically nontoxic agents in acute

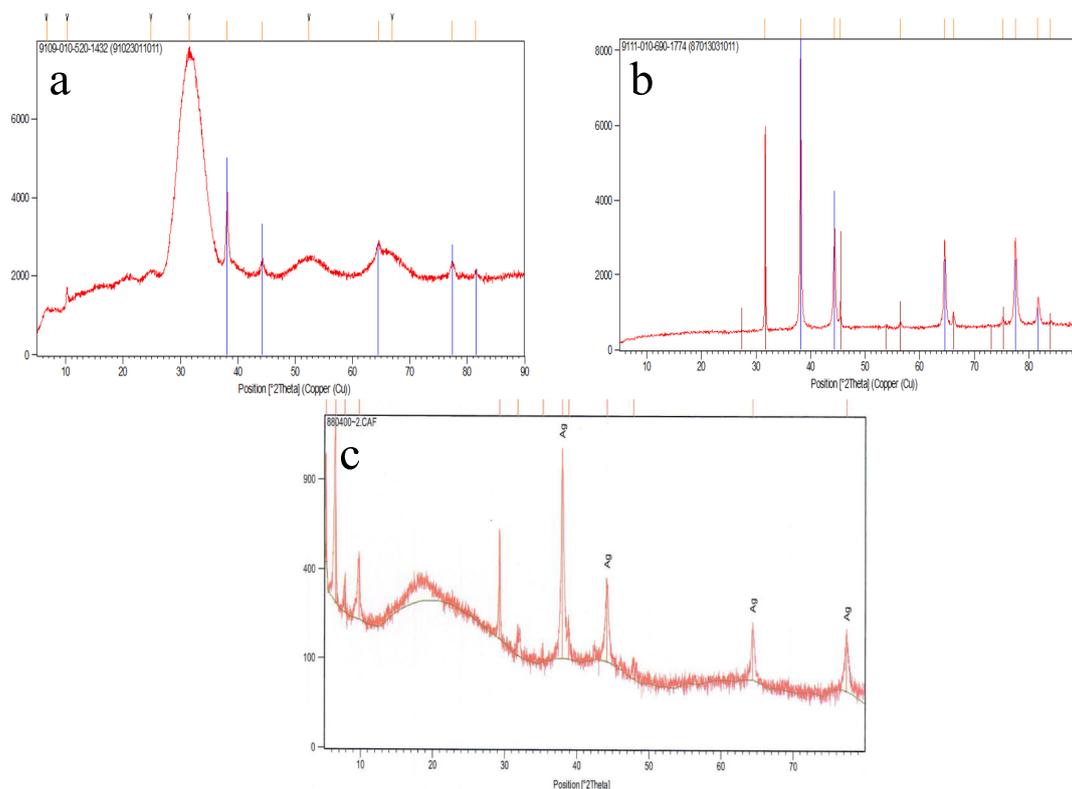


Fig. 2. XRD examinations on 3 AgNPs test samples:

a) Nanosilver particles were observed in the ranges of 38, 44, 64.5, 77.5 degrees (2θ) (A-AgNP). b) Nanosilver particles were observed in the ranges of 38, 44, 64.5, 77.5, 81.5 degrees (2θ) (B-AgNP). c) Nanosilver particles were observed in the ranges of 38, 44, 64.5, 77.5 degrees (2θ) (AgNP-C).

dermal exposures and focused on the OECD guideline 404 to identify the irritation and corrosion potentials of test samples.

Skin irritation and corrosion

A-AgNP: The skin application of test sample was started by the lowest dilution (50ppm). The lowest dilution (50ppm) showed clinical manifestations of moderate irritation by the total score of 18 after long-term dermal exposure (>24 hrs), which was intensified by persistent (>72 hrs) exposure. This sample at this dilution was considered a moderate irritant, according to the OECD scoring method. Due to ethical rules, higher dilution wasn't considered for the same test. According to the observations at 72 hours in all other dilutions of the test substance, the toxic reaction in animals eventually led to skin dryness and scaling and when considering the 1st and the 4th hour, symptoms were more evident (Fig. 3). All irritant effects

recovered to a normal state after patch removal on day 14 of study.

B-AgNP: The skin application of test sample was started by the lowest dilution (15ppm). The lowest dilution (15ppm) showed clinical manifestations of moderate irritation by the total score of 18 after long-term dermal exposure (>24 hrs) which was intensified by persistent (>72 hrs) exposure. This sample at this dilution was considered a moderate irritant, according to the OECD scoring method. Due to ethical rules, higher dilution wasn't considered for the same test. According to the observations, at 72 hours compared with the dilution of the test substance, the toxic reaction shown in animal led to skin dryness and scaling eventually. At the 1st hour and 4th hour, symptoms were more evident. All irritant effects recovered to a normal state after patch removal on day 14 of study.

C-AgNP: The skin application of test sample was started by the lowest dilution (100 ppm). The lowest

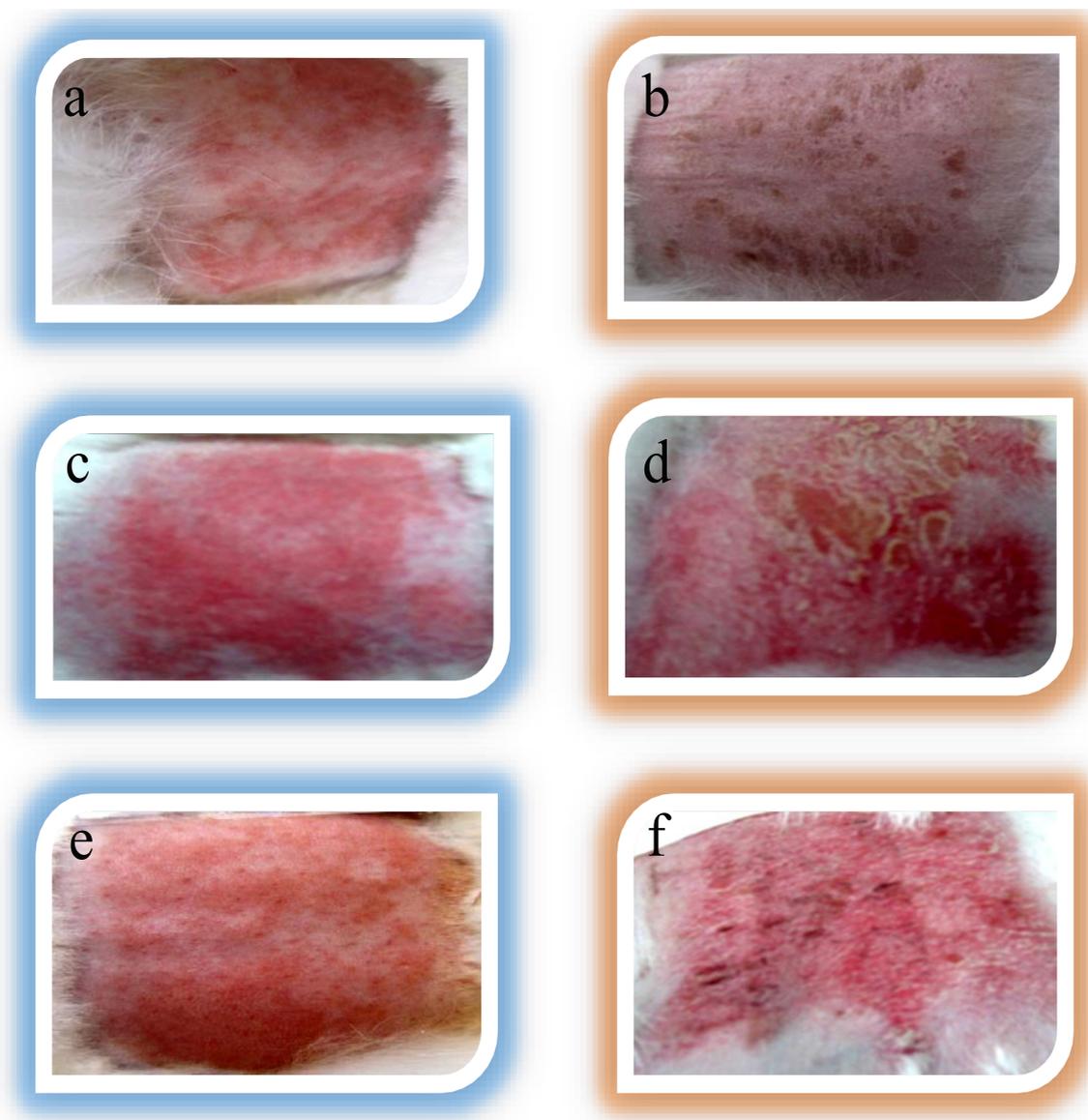


Fig. 3. Skin reactions to AgNP samples in short term (1-4hours) and long term (>24 days) dermal exposure.
a) A-AgNP after short term dermal exposure. b) A-AgNP after long term dermal exposure.
c) B-AgNP after short term dermal exposure. d) B-AgNP after short term dermal exposure.
e) C-AgNP after short term dermal exposure. f) C-AgNP after long term dermal exposure

dilution (1000 ppm) showed clinical manifestations of severe irritation by the total score of 30 after long-term dermal exposure (>24 hrs), which was intensified by persistent (>72 hrs) exposure. This sample at this dilution was considered a severe irritant according to the OECD scoring method. Due to ethical rules, higher dilution wasn't considered for the same test. According to the observations, at 72 hours compared with the dilution of the test substance, the toxic reaction

is shown in animal eventually led to skin dryness, scaling, and erythema which was evident from 1st hour of exposure. Irritant effects were partially recovered to a normal state after patch removal on day 14 of study.

Nanosilver has become one of the most commonly used nanomaterials in consumer products for its antiseptic and antimicrobial properties. However, public concern over the potential adverse effects of nanosilver [17] had encouraged us to conduct

the present study. In this research, we analyzed the toxicity potentials of various concentrations of nanosilver by dermal application for the first time, because to our knowledge, most of the dermal toxicological studies on nanosilver are limited to rat [18], Guinea Pig models [13,14], inhalational [19], and oral routes [19] of administration. In inhalational and oral studies, minimal pulmonary inflammation and cytotoxicity following sub-acute exposures were shown in animals exposed to nanosilver. However, longer term exposures showed higher body burdens of nanosilver. We have to ensure that there are possible chronic effects on organs through dermal exposure and we have to evaluate the possible translocation of nanosilver to some organs in rabbit model as we have performed on other animal models previously [12, 16].

The present study lucidly showed that dermal contact to colloidal nanosilver might cause dermal abnormalities of the skin at exposed area, magnified by increased concentration in longer term (72 hr) exposure. The research in present has emphasized also that dermal toxicity of colloidal nanosilver may not be affected by physicochemical properties, comparing to mutagenic effects [19], genotoxicity, and cytotoxicity of AgNP [20]. According to the recent studies, silver ions (Ag^+) could be distributed to the other organs such as liver and kidney through blood circulation, in case medical devices loaded with colloidal nanosilver were used. It may induce hepatotoxicity or renal toxicity and may lead to death in some situation, extremely exposed to a certain dose of Ag^+ . We proved in present study that silver nanoparticles with close properties to silver ions could act on epithelial cells of albino rabbit and cause local irritation and corrosive effects in a dose and time-dependent manner. We used 100, 50, and 15 $\mu g/ml$ solutions as the minimum and maximum doses without recording any sign of mortality in animals. Although the high dose of nanosilver was unrealistic to our knowledge, this was the first study on the dermal toxicity of nanosilver in a rabbit model. It seems necessary to conduct the same study on lower doses and find the NOAEL (No observable adverse effect level) of nanosilver by dermal application.

CONCLUSIONS

Compared to the control group, although no sign of mortality via dermal application was

detectable in all treatment groups, significant dose-dependent dermal toxicity was observed in three treatment groups comparing to controls. Results obtained from the present study indicated that dermal exposure to more than 15 ppm of silver nanoparticles might result in moderate irritation and damage to the skin. The conclusion of this study could be that the toxicity profile of nanosilver was different from silver through the same route of administration. Considering the present findings, it is vital to find the correlation between the period of exposure and histopathological changes with lower doses for different duration of time. Detection of the role of shape and particle size on the toxicity profile of nanosilver by different routes of administration is highly recommended for the future studies.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGMENT

The work was financially supported by Islamic Azad University Pharmaceutical Sciences Branch, Further; we acknowledge the staff of Iran Nanohealth committee for providing the samples of the present study.

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