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RESEARCH PAPER

Investigation on the Possible Cutaneous Toxicity of Silver Nanoparticles in Swiss Albino Rats

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ABSTRACT

Due of its potent antibacterial effect, nanosilver is among the most commonly used nanomaterials. As a result of this, there is a growing concern regarding the potential adverse effects of these nanoparticles due to the rising likelihood that humans may be exposed to nanosilver. In this research, we studied the possible cutaneous toxicity of silver nanoparticles in rats. The statistical population of this study consisted of 30 adult male Swiss albino rats (100-150g). They were acquired from animal farm of the Egyptian Organization for Biological Products and Vaccines (VACSERA Holding Company), Cairo, Egypt. For this experiment, they were split into the treatment and control groups (n = 15) at random. Anesthetics were administered to all animals, and the backs were shaved around the spinal column in the nanosilver group and the control group, respectively. The sterile bandages of rats were then moistened with distilled water (in control) or a volume of 55µl of 15µg/ml of nanosilver solution (in treatment) before being adhered to the skin with cloth glue. The bandages were removed after 4 and 8 days, and standard kits were used to determine the serum levels of two groups of rats' aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cr), and blood urea nitrogen (BUN). BUN, AST, and ALT levels in the treatment group over the experiment periods were significantly higher than those in the control group ($p<0.05$). According to the results of this study, 15µg/ml nanosilver (45nm) absorbed through the skin can cause renal toxicity and hepatotoxicity in rats.

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INTRODUCTION

Utilizing nanoparticles in new goods is a field of nanotechnology that is expanding quickly [1,2]. A growing number of novel nanodevices that may be utilized in a variety of pharmaceutical, biomedical, biological, and physical applications are being made with the help of nanosized inorganic particles, whether they are composite or simple in nature [3–5]. These particles also exhibit distinctive chemical and physical properties.

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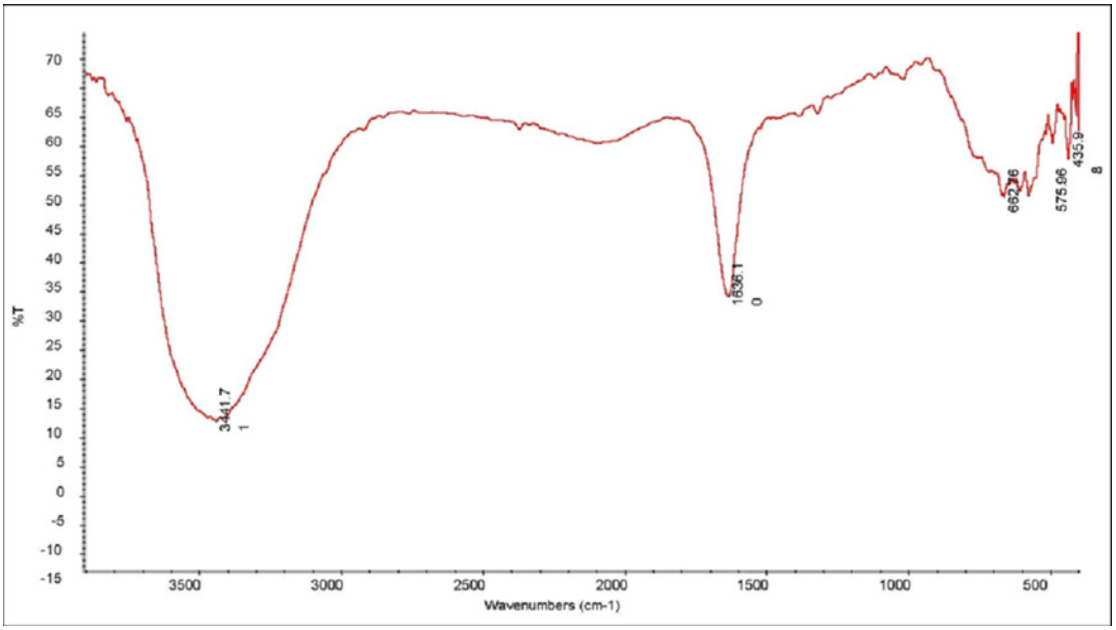
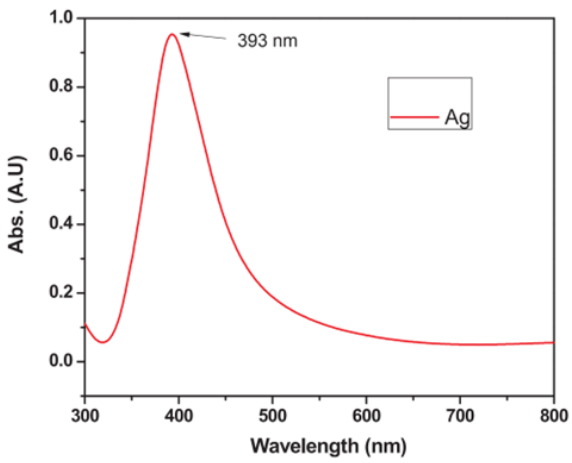
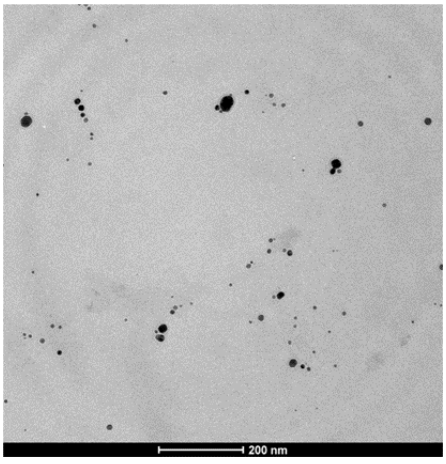
By adjusting a compound's size to change its effect, nanotechnology provides opportunity to reexamine the biological characteristics of already established antibacterial substances [6,7]. According to earlier research, bactericidal materials made of antimicrobial compositions in the form of nanoparticles could be employed successfully [8–11]. It is commonly acknowledged that silver-based compounds and silver ions are extremely poisonous to microorganisms, with up to 16 different species of bacteria, including E. coli, being particularly susceptible to their biocidal effects [12,13]. Thus, coatings for medical devices, ion exchange fibers, and dental resin composites all use silver ions as an antibacterial component [14–16]. Due to its anti-inflammatory and antibacterial characteristics, silver has been employed in human health care for ages [17]. In order to prevent



(a)





(b) (c)

Fig. 1. Analysis results of nanosilver (a) FTIR spectroscopy, (b) absorption spectra, and (c) HRTEM image..

bacterial infections in burns and wounds, silver and its compounds were administered [18]. Silver's commercial use has recently seen a renaissance thanks to the development of nanotechnologies that extensively utilize silver nanoparticles (NPs) [19]. Materials are considered to be NPs if at least one of their dimensions is below 100nm. Due to its potent antibacterial properties, nanosilver is one of the most popular nanomaterials. For instance, it has been employed in medical items like catheter coatings, surgical instruments, and wound dressings [20]. As a result, potential cutaneous toxicity is a concern. According to research, outer membrane protein and heat shock protein synthesis were both improved by nanosilver [21,22]. Consequently, it is a potent stressogenic factor for bacteria. Nanosilver has also been found to have the ability to accelerate protein construction by binding silver ions to amino acid functional groups [23]. Studies on the toxicity of nanosilver in both in vitro and in vivo settings have revealed that using nanosilver products may have detrimental impacts on human health [24,25]. According to studies, rats exposed to nanosilver orally (60nm) had a dose-dependent buildup of silver in many tissues, including kidneys, lungs, liver, and the blood [26]. In grown mammalian cells, nanosilver has been shown to cause cytotoxicity and primary DNA damage [27]. After being exposed to nanosilver (50nm) for 24 hours, bovine retinal endothelial cells (BRECs) have been shown to induce caspase-3 activation, build DNA ladders, and undergo apoptosis [28]. According to research, after cutaneous absorption of nanosilver (<100nm), it can lead to dose- and time-dependent histopatholgical changes in sleep, liver, and the skin of guinea pigs [29–31]. After systemic exposure to nanosilver (10-15nm), researchers discovered histopathological abnormalities and accumulation in the rat liver [32,33]. Eye discoloration (argyrosis) and pigmentation, which results in skin and mucous membranes turning permanently gray or bluish-gray (argyria), are the most frequent side effects of long-term exposure to silver in humans [34]. Whilst nanosilver has various uses in pharmaceuticals and can be absorbed nearly completely via the skin, there are less research that have been done to evaluate its cutaneous toxicity [35]. Thus, in this research, we provide our study results on the skin toxicity of nanosilver in adult male rats.

MATERIALS AND METHODS

Nanosilver solution was acquired from Pio Chem Cairo, Egypt. Fig. 1 shows the Fourier of silver nanoparticles. Fig. 1 shows the transform infrared spectroscopy (FTIR) spectra, the absorption spectra and the high-resolution transmission electron microscope (HRTEM) image of the nanosilver acquired.

The purity and particle size were 95% and 45nm, respectively. Thirty adult male Swiss albino rats (100-150g) were acquired from animal farm of the Egyptian Organization for Biological Products

Fig. 2. Male rats following full nanosilver coatings.

and Vaccines (VACSERA Holding Company), Cairo, Egypt and randomly divided into two groups (treatment and control). For 14 days prior to the studies, all rats were housed in stainless steel cages to give them time to acclimate to the surroundings. The animals were kept in 24±2°C on a cycle of darkness and light lasting 12 hours, and they had unrestricted access to a laboratory diet consisting of regular foods and running water on demand. As part of the treatment, a section measuring 2cm×2cm on each animal's back was shaved, and the area was located in the vicinity of the spinal column. The sterile bandages of rats were then moistened with distilled water (in control) or a volume of 55µl of 15µg/ml of nanosilver solution (in treatment) before being adhered to the skin with cloth glue. The portions that had been shaved were bandaged cleanly and fastened with cloth glue (Fig. 2).

Remaining test gas was cleaned up with water after exposure periods (4 and 8 days). The variations in the hepatic necrosis biomarkers namely aspartate transaminase (AST) and alanine transaminase (ALT) have been measured by kits from DiaSys (Diagnostic Systems, Holzheim, Germany). The renal function parameters (RFP), namely blood urea nitrogen (BUN) and creatinine (Cr) have been analyzed by standard kits from DiaSys in the blood sera of rats and were compared between groups. The US National Institute of Health criteria were followed for all animal experimentation [36]. One-way analysis of variance (ANOVA) was used to assess the data, and then Tukey's Honest Significant Difference (HSD) post-test was performed. Statistical significance was defined as values of $P<0.05$. The SPSS software version 21 was used to conduct all statistical analyses.

RESULTS AND DISCUSSION

The findings of the research done on nanosilver at a concentration of 15µg/ml demonstrated that the level of BUN, which is a parameter used to measure renal function, was significantly elevated in the treatment group in contrast to the control group throughout the experiment ($P<0.05$). In the treatment and control groups, the BUN level at four days was 23.12±3.51mg/dL and 18.43±2.93mg/dL, respectively. Whereas for the aforementioned groups at eight days, the BUN level was 25.93±3.52mg/dL and 19.06±1.97mg/dL, respectively. Additionally, findings indicated that there was no discernible rise in Cr levels in the treatment group throughout the course of the investigation ($P>0.05$) (Fig. 3).

During the experiment, the treatment group's AST and ALT levels significantly outperformed the control group ($P<0.05$) (Fig. 4). In the treatment

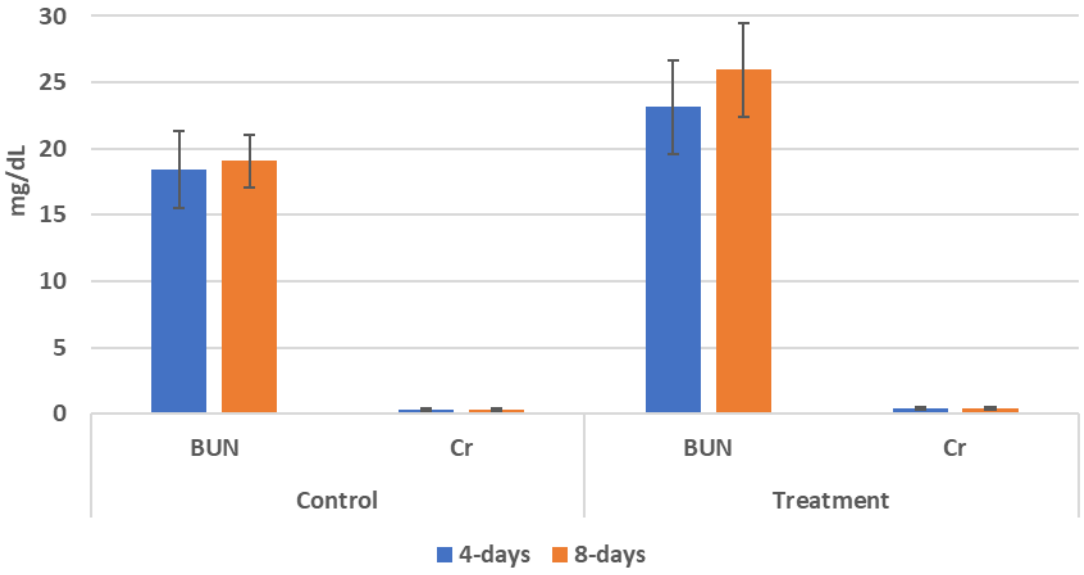


Fig. 3. Variations in the indices of renal function measured four and eight days after receiving nanosilver treatment.

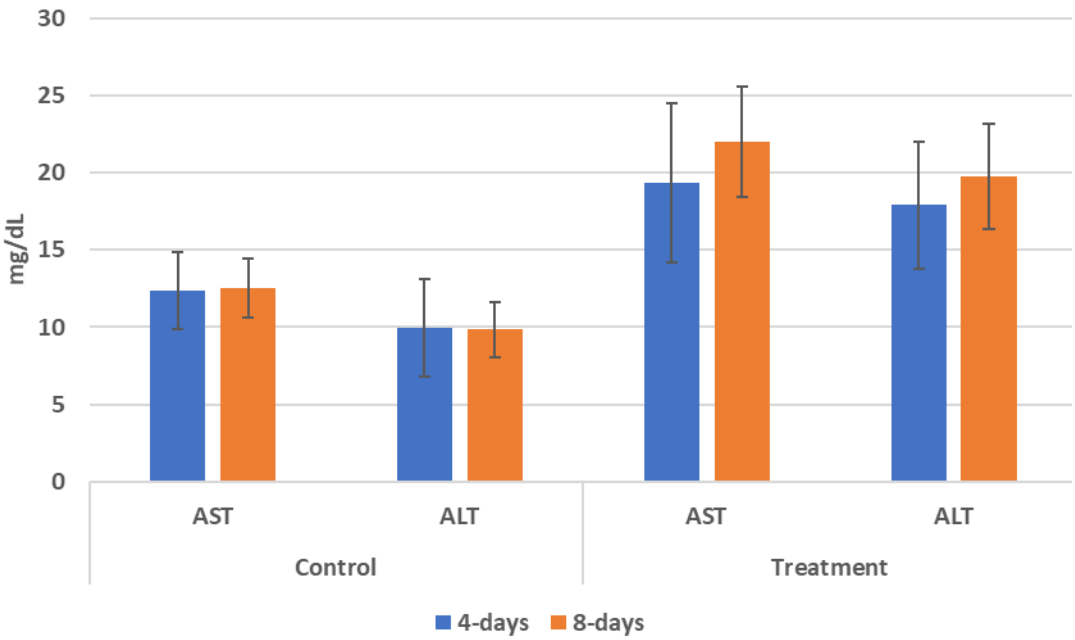


Fig. 4. Alterations in the indicators of liver necrosis four and eight days after nanosilver treatment

group, the AST and ALT concentrations were 19.34±5.12 and 17.91±4.12mg/dL after four days, but in the control group, the concentrations were 12.36±2.15 and 9.96±3.12mg/dL. At eight days, the AST and ALT levels were 21.98±3.58 and 19.76±3.43mg/dL in the treatment group, and 12.56±1.91 and 9.84±1.76mg/dL in the control group.

It is worth mentioning that any rise in the biomarkers for hepatic necrosis and renal function parameters is a sign of both renal and hepatic disease.

In concentrations between 10 and 50g/ml, nanosilver has a potent antibacterial effect [37]. Accordingly, in this investigation, we measured hepatic necrosis biomarkers and renal function indicators in serum to assess the cutaneous toxicity of nanosilver (15g/ml) in rats. The current investigation demonstrated the toxicity of nanosilver to the liver and kidneys. Researches have proven that skin's horny layer is a pathway for nanoparticles to infiltrate the dermis and epidermis layers [38]. Further studies discovered that different nanoparticles can absorb the dermis and then enter the bloodstream [13]. Another research discovered that subcutaneous injection of nanosilver allows it to get into the bloodstream and reach organs like the kidneys and liver [33]. It has also been confirmed that nanosilver poisoning affects the liver and kidneys [30]. Cutaneous absorption of nanosilver (<100nm) can result in dose-dependent histopatholgical problems in bone, heart, and the kidneys of guinea pigs [35]. A considerable increase in the renal function parameter (BUN) was seen in the current investigation as the kidney's toxic response. Furthermore, it was investigated how hazardous burn wound dressings containing nanosilver were to rats' systems after 21 days [13]. However, they discovered a substantial increase in ALT level in the nanosilver group, confirming the hepatotoxic potentials of nanosilver. Data indicated no substantial variation in AST levels and renal function indices (BUN and Cr). In contrast, nanosilver demonstrated renal and hepatotoxicity in the current investigation, which may be related to the various dosages and measurements of nanosilver in dressings. According to reports, the size and concentration of silver utilized in the creation of nanoproducts are among the parameters that affect how well silver penetrates human skin [33]. The vast majority of toxicity studies on nanosilver have only examined oral, inhalational, and intravenous administration methods of usage [32,33]. Swiss mice were used in a study to determine the toxicity of

various nanosilver concentrations entrapped in montmorillonite [20]. They noted several modifications to various biochemical parameters in the blood and urine, including a reduction in urea and creatinine levels in the urine after consuming a large dose of Ag (0)-montmorillonite. Following daily intraperitoneal (IP) injections for 30 days, a study assessed the toxicity of various dosages of nanosilver (5 and 10kg/day). lkaline phosphatase (ALP) and BUN levels in the high-dose and low-dose groups both significantly increased, while Cr levels significantly decreased [30]. According to certain research, medical items with a silver coating can produce silver ions that can enter the bloodstream and collect in several organs, including the liver and kidneys, where they can cause renal toxicity or hepatotoxicity [10]. In addition to causing oxidative stress in mitochondria, silver ions are used to interact with the thiol groups of the inner membrane of the mitochondria [24]. Another research discovered that the shape, size, and diameter of nanoparticles affect how they interact with biological cells [28]. In light of this, the effects of nanosilver on cells, tissues, and organs vary depending on its size, concentration, and ability to produce silver ions.

CONCLUSION

The current findings suggest that hepatotoxicity and renal toxicity may result from cutaneous absorption of 15g/ml nanosilver (45nm). Histology research is suggested, and various nanosilver sizes and concentrations could be used in this study. To corroborate this preliminary data, additional research is required.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Shojaei TR, Salleh MAM, Tabatabaei M, Mobli H, Aghbashlo M, Rashid SA, et al. Applications of Nanotechnology and Carbon Nanoparticles in Agriculture. Synthesis, Technology and Applications of Carbon Nanomaterials: Elsevier; 2019. p. 247-277.
2. Santos AC, Morais F, Simões A, Pereira I, Sequeira JAD, Pereira-Silva M, et al. Nanotechnology for the development of new cosmetic formulations. Expert Opinion on Drug Delivery. 2019;16(4):313-330.
3. Madkour LH. Environmental Impact of Nanotechnology and Novel Applications of Nano Materials and Nano Devices. Advanced Structured Materials: Springer International Publishing; 2019. p. 605-699.
4. Keçili R, Büyüktiryaki S, Hussain CM. Advancement in bioanalytical science through nanotechnology: Past, present and future. TrAC, Trends Anal Chem. 2019;110:259-276.
5. Cardigos J, Ferreira Q, Crisóstomo S, Moura-Coelho N, Cunha JP, Pinto LA, et al. Nanotechnology-Ocular Devices for Glaucoma Treatment: A Literature Review. Current Eye Research. 2018;44(2):111-117.
6. Shah KW, Ong PJ, Chua MH, Toh SHG, Lee JJC, Soo XYD, et al. Application of phase change materials in building components and the use of nanotechnology for its improvement. Energy and Buildings. 2022;262:112018.
7. Ramkumar C, Vishwanatha A, Saini R. Regulatory Aspects of Nanotechnology for Food Industry. Nanotechnology Applications in Dairy Science: Apple Academic Press; 2019. p. 169-184.
8. Munir MU, Ahmed A, Usman M, Salman S. <p>Recent Advances in Nanotechnology-Aided Materials in Combating Microbial Resistance and Functioning as Antibiotics Substitutes. International Journal of Nanomedicine. 2020;Volume 15:7329-7358.
9. Fahmy HM, Salah Eldin RE, Abu Serea ES, Gomaa NM, AboElmagd GM, Salem SA, et al. Advances in nanotechnology and antibacterial properties of biodegradable food packaging materials. RSC Advances. 2020;10(35):20467-20484.
10. Erkoc P, Ulucan-Karnak F. Nanotechnology-Based Antimicrobial and Antiviral Surface Coating Strategies. Prosthesis. 2021;3(1):25-52.
11. Cacciatore FA, Brandelli A, Malheiros PdS. Combining natural antimicrobials and nanotechnology for disinfecting food surfaces and control microbial biofilm formation. Critical Reviews in Food Science and Nutrition. 2020;61(22):3771-3782.
12. Pareek V, Gupta R, Panwar J. Do physico-chemical properties of silver nanoparticles decide their interaction with biological media and bactericidal action? A review. Materials Science and Engineering: C. 2018;90:739-749.
13. Dahm H. Silver Nanoparticles in Wound Infections: Present Status and Future Prospects. Nanotechnology in Skin, Soft Tissue, and Bone Infections: Springer International Publishing; 2020. p. 151-168.
14. Qing Y, Li K, Li D, Qin Y. Antibacterial effects of silver incorporated zeolite coatings on 3D printed porous stainless steels. Materials Science and Engineering: C. 2020;108:110430.
15. Martorana A, Pitarresi G, Palumbo FS, Catania V, Schillaci D, Mauro N, et al. Fabrication of silver nanoparticles by a diethylene triamine-hyaluronic acid derivative and use as antibacterial coating. Carbohydr Polym. 2022;295:119861.
16. Garipov IT, Khaydarov RR, Gapurova OU, Khaydarov RA, Lutfi FM, Efimova IL, et al. Silver Nanoparticles as a New Generation of Antimicrobial Prophylaxis. Journal of Siberian Federal University Biology. 2019:266-276.
17. Tsang K-K, Kwong EW-Y, Woo KY, To TS-S, Chung JW-Y, Wong TK-S. The Anti-Inflammatory and Antibacterial Action of Nanocrystalline Silver and Manuka Honey on the Molecular Alternation of Diabetic Foot Ulcer: A Comprehensive Literature Review. Evid Based Complement Alternat Med. 2015;2015:1-19.
18. Hosny AE-DMS, Rasmy SA, Aboul-Magd DS, Kashef MT, El-Bazza ZE.;The increasing threat of silver-resistance in

clinical isolates from wounds and burns. Infection and Drug Resistance. 2019;Volume 12:1985-2001.
19. Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine. Molecules. 2019;25(1):112.
20. Konop M, Damps T, Misicka A, Rudnicka L. Certain Aspects of Silver and Silver Nanoparticles in Wound Care: A Minireview. Journal of Nanomaterials. 2016;2016:1-10.
21. Xin L, Wang J, Wu Y, Guo S, Tong J. Increased oxidative stress and activated heat shock proteins in human cell lines by silver nanoparticles. Human & Experimental Toxicology. 2014;34(3):315-323.
22. Girilal M, Krishnakumar V, Poornima P, Mohammed Fayaz A, Kalaichelvan PT. A comparative study on biologically and chemically synthesized silver nanoparticles induced Heat Shock Proteins on fresh water fish Oreochromis niloticus. Chemosphere. 2015;139:461-468.
23. Burcza A, Gräf V, Walz E, Greiner R. Impact of surface coating and food-mimicking media on nanosilver-protein interaction. Journal of Nanoparticle Research. 2015;17(11).
24. Rezvani E, Rafferty A, McGuinness C, Kennedy J. Adverse effects of nanosilver on human health and the environment. Acta Biomater. 2019;94:145-159.
25. Gupta I, Duran N, Rai M. Nano-Silver Toxicity: Emerging Concerns and Consequences in Human Health. Nano-Antimicrobials: Springer Berlin Heidelberg; 2011. p. 525-548.
26. Dziendzikowska K, Gromadzka-Ostrowska J, Lankoff A, Oczkowski M, Krawczyńska A, Chwastowska J, et al. Time-dependent biodistribution and excretion of silver nanoparticles in male Wistar rats. J Appl Toxicol. 2012;32(11):920-928.
27. You C, Han C, Wang X, Zheng Y, Li Q, Hu X, et al. The progress of silver nanoparticles in the antibacterial mechanism, clinical application and cytotoxicity. Mol Biol Rep. 2012;39(9):9193-9201.
28. Sriram MI, Kalishwaralal K, Barathmanikanth S, Gurunathani S. Size-based cytotoxicity of silver nanoparticles in bovine retinal endothelial cells. Nanoscience Methods. 2012;1(1):56-77.
29. Paknejadi M, Bayat M, Salimi M, Razavi V. Concentration- and Time-Dependent Cytotoxicity of Silver Nanoparticles on Normal Human Skin Fibroblast Cell Line. Iranian Red Crescent Medical Journal. 2018;In Press(In Press).
30. Samani PY, Samani PY, Arabi M, Shadkhast M, Samani PY, Pivaei E. Repeated-Dose Toxicity in Mouse Liver and Kidney after Skin Exposure to Silver Nanoparticles. JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH. 2018.
31. Ferdous Z, Nemmar A. Health Impact of Silver Nanoparticles: A Review of the Biodistribution and Toxicity Following Various Routes of Exposure. Int J Mol Sci. 2020;21(7):2375.
32. Shahare B, Yashpal M. Toxic effects of repeated oral exposure of silver nanoparticles on small intestine mucosa of mice. Toxicology Mechanisms and Methods. 2013;23(3):161-167.
33. Munger MA, Radwanski P, Hadlock GC, Stoddard G, Shaaban A, Falconer J, et al. In vivo human time-exposure study of orally dosed commercial silver nanoparticles. Nanomed Nanotechnol Biol Med. 2014;10(1):1-9.
34. Skalska J, Strużyńska L. Toxic effects of silver nanoparticles in mammals – does a risk of neurotoxicity exist? Folia Neuropathol. 2015;4:281-300.
35. George R, Merten S, Wang TT, Kennedy P, Maitz P. In vivoi analysis of dermal and systemic absorption of silver nanoparticles through healthy human skin. Australas J Dermatol. 2013;55(3):185-190.
36. Frequently Asked Questions About the Public Health Service Policy on Care and Use of Laboratory Animals: Prepared by the staff of the Division of Animal Welfare, Office for Protection from Research Risks, National Institutes of Health, Bethesda, Maryland. ILAR Journal. 1993;35(3-4):47-49.
37. Agnihotri S, Mukherji S, Mukherji S. Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. RSC Adv. 2014;4(8):3974-3983.
38. Knorr F, Patzelt A, Meinke MC, Vogt A, Blume-Peytavi U, Rühl E, et al. Interactions of Nanoparticles with Skin. Biological Responses to Nanoscale Particles: Springer International Publishing; 2019. p. 329-339.