

Silver oxide nanoparticles induced toxicity: A histopathological study

Silver oxide nanoparticles toxicity

Mubin Mustafa Kiyani¹, Syed Ali Imran Bokhari¹, Asmara Syed², Hamza Rehman¹, Ekramy Elmorsy^{2,3}, Syed Sajid Hussain Shah²¹Department of Biological Sciences, International Islamic University, Islamabad, Pakistan²Department of Pathology, Faculty of Medicine, Northern Border University, Arar, Saudi Arabia³Department of Clinical Toxicology, Faculty of Medicine, Mansoura University, Egypt

Abstract

Aim: The current study aimed to identify the toxic effects of oral ingestion of different doses of silver oxide nanoparticle, for 21 days, on the liver, kidney and muscle tissues of mice by histopathological examination.

Materials and Methods: The nanoparticles of silver oxide (30 -70 nm in diameter) were prepared by physical methods and administered orally to 3 experimental groups of BALB/c mice in the strength of 250, 375, and 500 mg/kg, respectively for 21 days. Liver, kidney, and muscle tissue specimens from control and nanoparticles exposed groups have been collected for histopathological evaluation.

Results: The liver and kidney tissue of the mice fed with 500 mg/kg of silver nanoparticles revealed portal inflammation in the liver tissue and mild interstitial inflammation in the renal tissue. There is no histopathological evidence of any toxic effects in the other groups. The muscle tissue from all experimental groups is unremarkable.

Discussion: The dose, size of nanoparticles, duration, the route of exposure and limiting factors for nanoparticles induced toxic effects. Hence further studies with different variables regarding the nanoparticles exposure will be helpful for a better understanding of toxicity caused by nanoparticles.

Keywords

Silver oxide; Nanoparticles; Histopathology, Toxicological effects

DOI: 10.4328/ACAM.20148 Received: 2020-03-03 Accepted: 2020-04-11 Published Online: 2020-04-19 Printed: 2020-06-30 Ann Clin Anal Med 2020;11(Suppl 3): S197-201

Corresponding Author: Ekramy Elmorsy, Department of Pathology, Faculty of Medicine, Northern Border University, Arar, Saudi Arabia.

E-mail: ekramyelmorsy@mans.edu.eg P: 00966501275835

Corresponding Author ORCID ID: <https://orcid.org/0000-0002-7444-2499>

Introduction

Nanotechnology is a rapidly growing field of science that is revolutionizing today's medical and industrial applications. Silver nanoparticles represent around 25% of all nanoproducts with wide diagnostic and therapeutic applications as wound healing and arthritic pain [1]. The extensive research in the field of nanoscience in the current decades revealed that nanoparticles of many chemicals yielded antibacterial, antiviral, antifungal and antiparasitic properties [2,3]. The antimicrobial action of the silver nanoparticles depends upon the size as well as the surface area of these nanoparticles [4]. It has been documented in the scientific literature that the polylactic acid porous fibers with silver ions are quite useful for the dressing of wounds due to their effective antimicrobial activity [5-7]. Silver nanoparticles have been found to have very potent antimicrobial actions against the certain dental bacterial strains [8]. Silver nanoparticles prepared from the *Erythrina indica* exhibited potent antibacterial activity. In addition, these nanoparticles were used in some industrial applications as textile fabrics, cosmetic products, antiseptic as well as a preservative to treat dermal problems.

There has been extensive advancement in the development and application of nanoparticles in various fields including medicine. One of the major challenges regarding the use of nanoparticles is the assessment of biological safety associated with the use of these particles [9]. Different in-vitro cytotoxicity's assays have reported the Cytotoxic and genotoxic effects of silver nanoparticles using different models including liver cells HepG2 cells [10], retinal endothelial cells [11], breast cancer cells MCF-7 [12] cervical cancer cells HeLa and lymphoma cells U937 [13]. Another study showed that the silver nanoparticles prepared by actinomycetes HGG 16n strain are an effective antibacterial agent. These cell line studies data had reported different underlying cytotoxic mechanisms that are induced by the tested nanoparticles including inflammatory responses, impairment of the cell membrane, oxidative stress, cell cycle arrest, chromosome aberration, DNA damage and genotoxicity, and apoptosis [10-14]. The silver nanoparticles exposure can induce the changes of cell shape, reduce cell viability, enhances the release of lactate dehydrogenase enzyme so resulting in cell apoptosis or necrosis [14,15]

In vivo, after the absorption, the metallic nanoparticle which includes silver, gold, zinc, titanium and iron etc, accumulates mainly in the liver, spleen and lymph nodes because of reticuloendothelial uptake but may be distributed in the whole body [16]. Hence, the aim of the present study is to assess the histopathological evidence of untoward toxicities on the livers, kidneys, and muscles of silver oxides nanoparticles treated mice.

Material and Methods

Production of silver oxide nanoparticles

Silver oxide nanoparticles were synthesized by preparing two solutions in deionized water which were as follows: The first solution contained 100 mM of AgNO₃ while the second solution contained 100 mM of Sodium borohydride. Sodium borohydride solution was added into silver nitrate solution drop by drop using air as a medium under vigorous stirring by using a magnetic stirrer. The reaction was stopped when the pH 11 was

achieved. The mixture obtained was filtered and washed several times with distilled water. The mixture obtained was calcined at 300°C for 2 hours. Using pestle and mortar the powder obtained was ground until it became homogeneous apparently [17]. These synthesized nanoparticles were subjected to various characterization techniques such as Scanning electron microscope (SEM) and Energy-dispersive X-ray spectroscopy (EDS) and X-ray diffraction analysis (XRD).

Group formation

This study was conducted after receiving the ethical approval of the research project from the International Islamic university ethical committee.

The BALB/c mice are segregated into four groups (A, B, C and D). The animals which were fed with a diet without silver oxide nanoparticles comprised in Group A which was a control group. The Group B mice were fed with the diet containing 250 mg/kg body weight, silver oxide nanoparticles and Group C were mice fed with diet containing 375 mg/kg body weight silver oxide nanoparticles while Group D mice were fed with diet containing 500 mg/kg body weight silver oxide nanoparticles.

Histopathological examination of specimens

After three weeks of oral administration of silver oxide nanoparticles in the experimental groups, the liver, kidney, and muscle tissue specimens from all groups of animals have been examined microscopically for the assessment of any morphological alteration due to the toxicity. The liver tissue was evaluated for any alteration in the liver architecture and for any evidence of inflammation, necrosis, apoptosis, and fibrosis. In the renal tissue, the glomeruli, tubules, interstitium, and blood vessels have been evaluated histopathologically for any morphological alterations and similarly, the muscle tissue was assessed for any degenerative changes. The acquired specimens from liver, kidney and muscle tissue are processed in an automated tissue processor after fixation in 10% buffered formalin. After completing the tissue processing, the tissues are embedded in paraffin wax and tissue blocks were prepared. Three to four micron thick sections have been cut from these tissue blocks and stained with hematoxylin and eosin. The slides from the liver, kidney and muscle tissue have been examined by two histopathologists [18].

Results

Characterization of synthesized silver oxide NPs

Some peaks at the 2θ of x-axis at different Bragg angles were observed for the Silver oxide NPs XRD analysis [Figure 1A]. The synthesized nanoparticle was indicated but the highest peak was around the 2θ of 38.1538°. Using the Debye-Scherrer equation for average size calculation, it was observed that the nanoparticles have an average size around 40 nm.

Scanning electron microscopy of silver oxide NPs showed that the synthesized nanoparticles articles are in nano range but agglomerated as can be observed in Figure 1B. These nanoparticles were slightly spherical in shape and were highly packed with each other. Diameter measurement showed that the diameter of these particles ranged between 30 nm to 70 nm.

EDX spectroscopy of silver oxide NPs showed that the silver and oxygen were present in acceptable amounts. Traces of chloride and carbon were also observed (Figure 1C).

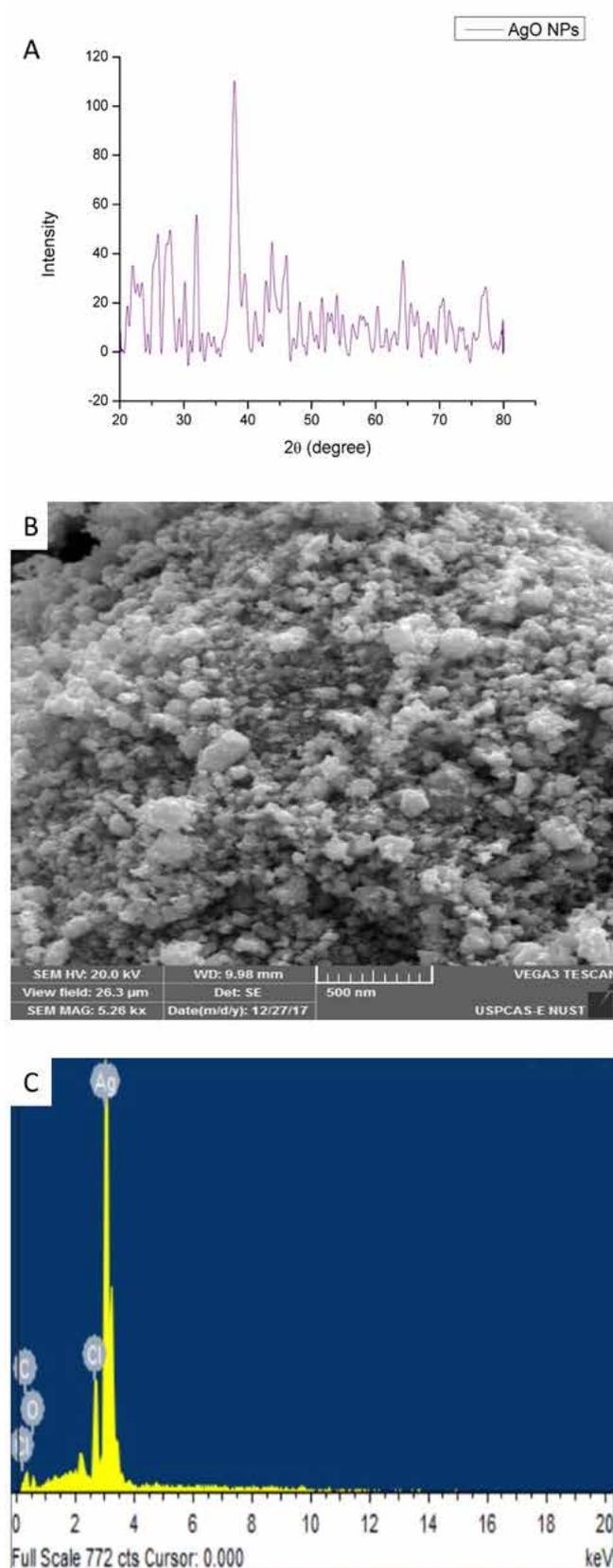


Figure 1. A: X-ray diffraction pattern of synthesized silver oxide nanoparticles. B: Scanning Electron Microscope (SEM) Image of synthesized Silver oxide nanoparticle. C: Energy Dispersive X-ray Spectroscopy pattern of synthesized Silver Oxide nanoparticles

Histopathological evaluation

The hematoxylin and eosin-stained slides prepared from the liver tissue specimens of these four groups of mice have been evaluated for the presence of any histopathological abnormalities in the liver such as hepatocyte balloon degeneration, necrosis, apoptosis, infiltrate of inflammatory cells in portal tract and lobule and fibrosis. The sections liver tissues of mice (Group D) fed with 500 mg/kg of silver oxide nanoparticles revealed mild portal inflammation while the sections of liver tissues from other groups (control (A) and experimental group B and C) revealed no significant histopathological abnormality (Figures 2A, 2B). The sections from the renal tissue of mice of Group D showed mild inflammatory cellular infiltrate in the interstitium while other renal tissues obtained from other groups (A, B, C) were histopathologically unremarkable (Figures 2C, 2D). The muscle tissue of mice obtained from all four groups revealed no evidence of any morphological abnormality.

Discussion

Nano-science is a rapidly developing field of science with promising medical and industrial applications. The important route of entry of nanoparticles in the human being is oral ingestion, inhalation, and skin. After absorption from these routes, the nanoparticles may circulate in the blood and may accumulate in different tissues and organs. The higher level of accumulation of metallic nanoparticles occurs in the liver which makes the liver the most susceptible to the toxic effects of nanoparticles [19]. The liver has also got vital importance in the process of detoxification of exogenous substances. Similarly, the kidney is also a very vital organ which is related to the elimination of waste products from the body and it plays an important role in the clearance of the nanoparticles from the body [20]. During this process, the kidneys may be affected by the nanoparticles. The current study investigated the effect of daily exposure of mice to silver nanoparticles (30-70 nm in size) for 3 weeks in the concentration range of 250-500 mg/kg followed by histopathological evaluation of toxic effects on the liver, kidney and muscle tissues. The smaller dosage (250-375 mg/kg) of silver oxide nanoparticles revealed no histopathological abnormality while the higher dosage of nanoparticles (500 mg/kg) revealed mild inflammatory cellular infiltrate in the portal tracts of liver tissue and mild interstitial inflammation in the renal tissues. This observed inflammatory reaction is in accordance with Cha et al. (2008) [21], who reported lymphocytes infiltration in livers of male balb/c mice 3 days after exposure treated with 13 nm silver nanoparticles (single dose of 2.5 g directly to their stomach). This inflammation was explained by the nanoparticles induced expression of the inflammatory genes in the treated mice. Also, the current data regarding silver nanoparticles hepatic and nephrotoxicity are in accordance with the results of the study conducted on Sprague-Dawley rats by Wen H et al. (2017) which showed that a single intravenous administration of Silver nanoparticles (containing Ag nanoparticles and released Ag⁺, 5 mg/kg) silver nanoparticles caused extensive damage in the liver, kidney, thymus, and spleen without marked effect on lungs and hearts. Their data showed degeneration, necrosis and hemorrhages in the liver and hyaline degenerations in the epithelial

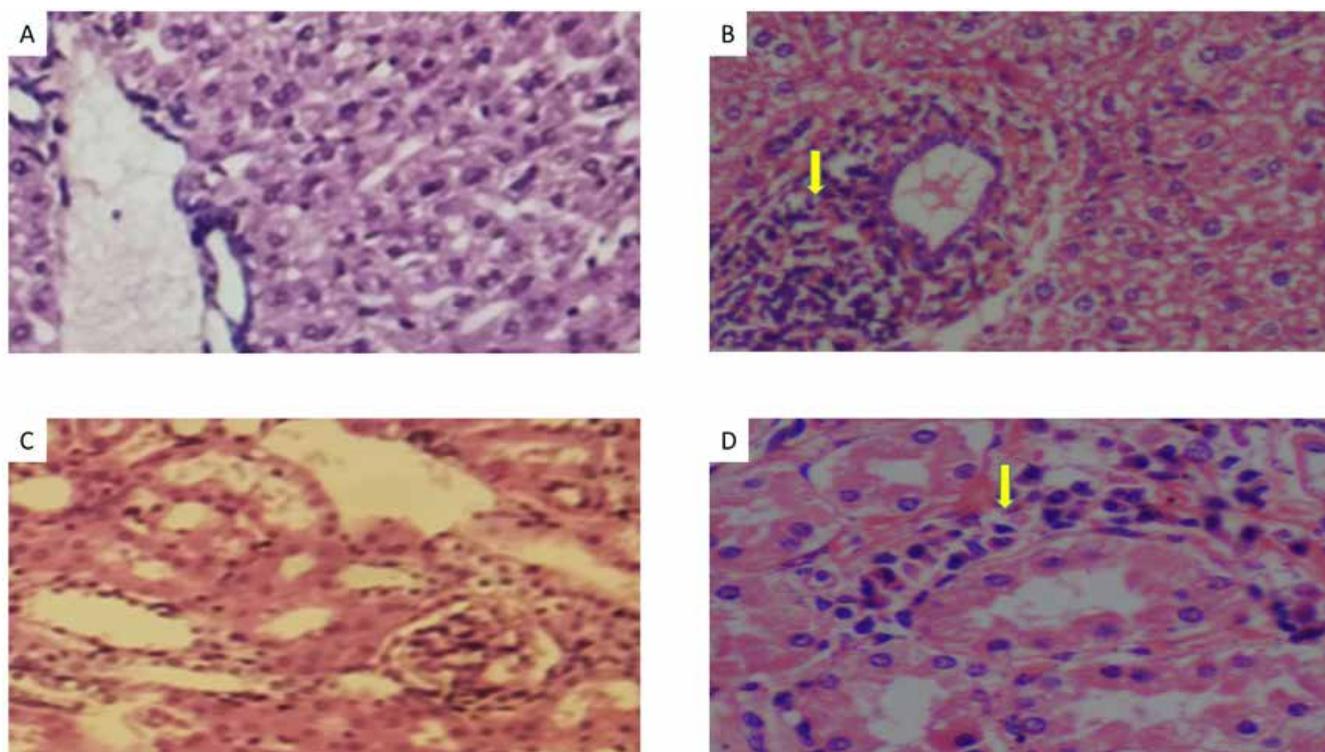


Figure 2. A: Liver tissue with no significant histopathological changes in mice ingested 250 and 375 mg/kg daily for 21 days (H&E x 100). B: Liver tissue exhibiting portal inflammation after oral exposure to 500 mg/kg of silver oxide nanoparticle for 21 days (yellow arrow) (H&E x 200). C: Kidney tissue with no significant histopathological changes in mice ingested 250 and 375 mg/kg daily for 21 days (H&E x 100). D: Kidney tissue exhibiting interstitial inflammation after exposure to 500 mg/kg of silver oxide nanoparticle for 21 days (yellow arrows) (H&E x 400).

cells of renal tubules [22]. The morphological alterations in the study by Wen H et al. are different from our study which may be attributed to the different mode of administration and duration exposure of silver nanoparticles. In our study, the mode of administration is oral and the duration of exposure is three weeks. Against Wen data, acute lung toxicity and cardiac ischemic perfusion injuries were reported in Male Sprague–Dawley rats that were exposed to intratracheal instillation of 200 μg of 20 or 110 nm polyvinylpyrrolidone (PVP) or citrate capped AgNP [23]. These discrepancies in data highlight the role of route of administration and dosage of nanoparticles as limiting factors for their toxic effects.

In another study which was carried out by Roda E et al, they revealed that the intratracheal administration of silver nanoparticles (50 $\mu\text{g}/\text{rat}$) caused morphological changes in the glomeruli of rat [24]. They observed that the glomeruli of the rat after exposure to silver nanoparticles showed Bowman capsule enlargement with shrinkage of mesangial cells and glomerular capillaries along with edema and interstitial micro-hemorrhages. These alterations in the renal glomeruli were observed at day 7 and persisted for 28 days post-exposure.

A study published by Gherkholagh MH et al revealed that significant histological abnormality was detected in the renal tissue of rats which were given 30, 125, 300, and 700 mg/kg of silver nanoparticle (200–300 nm) orally for 28 days. The group of 700 mg/kg concentration showed more necrosis and congestion while the 30 mg/kg concentrations show significantly lower numbers of the glomerular mesangial cells, while no inflammatory cells were reported with all studied concentrations [25]. These findings are contrary to our observations as in our

study, as the inflammatory cells were the main finding in our treated animals.

The current data are important as they show that daily prolonged oral exposure to silver nanoparticles (30–70 nm in size) can be hepatotoxic and nephrotoxic. Secondly, the distribution of nanoparticles is limiting factors for the site of their toxic effect, so Liver and kidney are affected with the silver nanoparticles while the muscles are spared as they are not main sites for toxicants distribution. Thirdly, the data support the conclusion that the dose, route on administration and size of the particles are main limiting factors for their toxic effect. Hence other studies using the different concentration and routes are of value and more meticulous data are required regarding the biohazard of the nanoparticles on different tissues and organs with respect to the size, dose and route of administration of the silver nanoparticles in terms of patient safety.

Conclusion

The dose, route, size of nanoparticles and durations of exposure are limiting factors for the toxic effect of the nanoparticles. Liver and kidney are major targets for nanoparticles toxicity in cases of oral exposure as they are main sites of their distribution. Any data regarding the toxicity of nanoparticles with different dose, route, size of nanoparticles and durations of exposure will contribute to better understanding of the safe use of these nanoparticles.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: None

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

- Leon L, Chung EJ, Rinaldi C. A brief history of nanotechnology and introduction to nanoparticles for biomedical applications. In: Chung EJ, Leon L, Rinaldi C, editors. *Nanoparticles for Biomedical Applications*. 1st ed. Cambridge: Elsevier; 2020.p. 1-4).
- Duran N, Duran M, de Jesus MB, Seabra AB, Favaro WJ, Nakazato G. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomedicine*. 2016;12(3):789-99. DOI: 10.1016/j.nano.2015.11.016.
- Lara HH, Ixtepan-Turrent L, Jose Yacamán M, Lopez-Ribot J. Inhibition of *Candida auris* Biofilm Formation on Medical and Environmental Surfaces by Silver Nanoparticles. *ACS Appl Mater Interfaces*. 2020; DOI: 10.1021/acsami.9b20708
- Duran N, Nakazato G, Seabra AB. Antimicrobial activity of biogenic silver nanoparticles, and silver chloride nanoparticles: an overview and comments. *Appl Microbiol Biotechnol*. 2016;100(15):6555-70. DOI: 10.1007/s00253-016-7657-7.
- Rathi Sre PR, Reka M, Poovazhagi R, Arul Kumar M, Murugesan K. Antibacterial and cytotoxic effect of biologically synthesized silver nanoparticles using aqueous root extract of *Erythrina indica* lam. *Spectrochim Acta A Mol Biomol Spectrosc*. 2015;135:1137-44. DOI: 10.1016/j.saa.2014.08.019.
- Buszewski B, Railean-Plugaru V, Pomastowski P, Rafinska K, Szultka-Mlynska M, Kowalkowski T. Antimicrobial Effectiveness of Bioactive Silver Nanoparticles Synthesized by Actinomycetes HGG16n Strain. *Curr Pharm Biotechnol*. 2017;18(2):168-76. DOI: 10.2174/1389201018666170104112434
- Sun Z, Fan C, Tang X, Zhao J, Song Y, Shao Z, et al. Characterization and antibacterial properties of porous fibers containing silver ions. *Appl Surf Sci*. 2016; 387: 828-38.
- Manikandan V, Velmurugan P, Park JH, Chang WS, Park YJ, Jayanthi P, et al. Green synthesis of silver oxide nanoparticles and its antibacterial activity against dental pathogens. *3 Biotech*. 2017; 7(1):72. DOI 10.1007/s13205-017-0670-4.
- Bueno J. Nanotoxicity: the impact of increasing drug bioavailability. In: Shegokar R. editor. *Nanopharmaceuticals*. 1st ed. Cambridge: Elsevier; 2020.p. 121-133.
- Mishra AR, Zheng J, Tang X, Goering PL. Silver nanoparticle-induced autophagic-lysosomal disruption and NLRP3-inflammasome activation in HepG2 cells is size-dependent. *Toxicol Sci*. 2016;150(2):473-87.
- Liu W, Wu Y, Wang C, Li HC, Wang T, Liao CY, et al. Impact of silver nanoparticles on human cells: effect of particle size. *Nanotoxicology*. 2010;4(3):319-30.
- Sriram MI, Kalishwaralal K, Barathmanikant S, Gurunathani S. Size-based cytotoxicity of silver nanoparticles in bovine retinal endothelial cells. *J Exp Nanosci*. 2012;1(1):56-77.
- Kaba SI, Egorova EM. In vitro studies of the toxic effects of silver nanoparticles on HeLa and U937 cells. *Nanotechnol Sci Appl*. 2015;8:19.
- Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. *Arab J Chem*. 2017; DOI:10.1016/j.arabjc.2017.05.011
- Akter M, Sikder MT, Rahman MM, Ullah AA, Hossain KF, Banik S, et al. A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives. *J Adv Res*. 2018 1;9:1-6.
- Zhoumeng Lin Z, Monteiro-Riviere NA, Riviere1 JE. Pharmacokinetics of metallic nanoparticles. *WIREs Nanomed Nanobiotechnol*. 2014. DOI: 10.1002/wnan.1304
- Fatima M, Zaidi NU, Amraiz D, Afzal F. In Vitro Antiviral Activity of Cinnamomum cassia and Its Nanoparticles Against H7N3 Influenza A Virus. *J Microbiol Biotechnol*. 2016;26(1):151-9. DOI: 10.4014/jmb.1508.08024.
- Gamble M. *Haematoxyline and eosin. Theory and practice of histological techniques*, 6th ed. New York, USA: Churchill Livingstone; 2008.p.121-36.
- Yao Y, Zang Y, Qu J, Tang M, Zhang T. The Toxicity of Metallic Nanoparticles on Liver: The Subcellular Damages, Mechanisms, and Outcomes. *Int J Nanomedicine*. 2019; 14:8787-8804. DOI:10.2147/IJN.S212907
- Du B, Yu M, Zheng J. Transport and interactions of nanoparticles in the kidneys. *Nat Rev Mater*. 2018;3(10):358-74. DOI: 10.1038/s41578-018-0038-3
- Cha K, Hong HW, Choi YG, Lee MJ, Park JH, Chae HK, et al. Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. *Biotechnol Lett*. 2008;30(11):1893-9.
- Wen H, Dan M, Yang Y, Lyu J, Shao A, Cheng X, et al. Acute toxicity and genotoxicity of silver nanoparticle in rats. *PLoS One*. 2017;12(9):e0185554. DOI:10.1371/journal.pone.0185554

23. Holland NA, Thompson LC, Vidanapathirana AK, Urankar RN, Lust RM, Fennell TR, et al. Impact of pulmonary exposure to gold core silver nanoparticles of different size and capping agents on cardiovascular injury. *Part Fibre Toxicol*. 2015;13(1):48.

24. Roda E, Barni S, Milzani A, Dalle-Donne I, Colombo G, Coccini T. Single Silver Nanoparticle Instillation Induced Early and Persisting Moderate Cortical Damage in Rat Kidneys. *Int J Mol Sci*. 2017;18(10):2115. DOI:10.3390/ijms18102115.

25. Gherkholagh MH, Asari MJ, Alizadeh Z, Sohrabi M. In Vivo Induced Nephrotoxicity of Silver Nanoparticles in Rat After Oral Administration. *J Res Med Dent Sci*. 2018; 6 (1): 43-51. DOI: 10.24896/jrmds.2018619.

How to cite this article:

Mubin Mustafa Kiyani, Syed Ali Imran Bokhari, Asmara Syed, Hamza Rehman, Ekramy Elmorsy, Syed Sajid Hussain Shah. Silver oxide nanoparticles induced toxicity: A histopathological study. *Ann Clin Anal Med* 2020;11(Suppl 3): S197-201