Nanomed Res J 5(3):288-297, Summer 2020

RESEARCH ARTICLE

Sub-chronic intraperitonealy toxicity assessments of modified silver nanoparticles capped coated Myrtus communis-derived the hydrolyzable tannins in a mice model

Shirin Tavakoli[1], Mohammad Ali Ebrahimzadeh[2], Emran Habibi[3], Pourya Biparva[4], Hamidreza mohammadi[1], Afshin Zahedi Mazandarani[5], Soheil Vafaeinejad[2,6], Ali Ziar[1], Shahram Eslami[2*]

[1] Pharmaceutical Sciences Research Center, Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

[2] Pharmaceutical Sciences Research Center, Hemoglobinopathy Institute, Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

[3] Pharmaceutical Sciences Research Center, Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

[4] Department of Basic Sciences, Sari University of Agricultural Sciences and Natural Resources, Sari, Iran

[5] Department of Medical Entomology and Vector Control, School of Public Health and Health Sciences Research Center, Mazandaran of University of Medical Sciences, Sari, Iran

[6] Department of Biology, Sana Institute of Higher Education, Sari, Iran

ARTICLE INFO

Article History:
Received 18 May 2020
Accepted 20 Jul 2020
Published 01 Aug 2020

Keywords:
Green synthesis
Silver Nanoparticle
Myrtus communis
Hydrolyzable tannins
sub-chronic
toxicity

ABSTRACT

Objective(s): The use of silver nanoparticles in the field of biomedicine is increasing day by day, but less attention has been paid to its toxicity. In this paper, the ability of the silver nanoparticles produced by a green synthesis procedure to protect the liver and its effects on liver function in male mice was investigated in a sub-chronic toxicity study.

Methods: The silver nanoparticles functionalized the hydrolyzable tannin fraction of *Myrtus communis* (MC-AgNPs) were used for testing in vivo sub-chronic toxicity in mice model. The MC-AgNPs and Ag^{+} were intraperitoneally injected with different doses 5 times a week over 90 days. The biochemical, hematological factors were determined using an autoanalyzer following the routine procedures. In addition, histopathological test of liver tissue in laboratory mice were examined through haematoxylin & eosin staining.

Results: The obtained results showed that liver enzymes (AST, ALT, and ALP) were decreased. The mean value ± standard deviation of white blood cells, lymphocytes, red blood cells and Hb were increased, while red blood cells and hemoglobin decreased. Histopathological investigations indicated no obvious effect on hepatic cyto-architecture in the group receiving silver nanoparticles (50 mg/kg), and mild inflammation in the port space. In the groups receiving silver nanoparticles (100 and 200 mg/kg), mild inflammation, and moderate inflammation were observed in the port space and pre portal, respectively.

Conclusions: The findings indicated that AgNPs could be safe even for long-term use in a therapeutic period if hybridized with active biomolecules.

How to cite this article
Tavakoli Sh., Ebrahimzadeh MA., Habibi E., Biparva P., mohammadi HR., Zahedi Mazandarani A., Vafaeinejad S., Ziar A., Eslami Sh. Sub-chronic intraperitonealy toxicity assessments of modified silver nanoparticles capped coated Myrtus communis-derived the hydrolyzable tannins in a mice model. Nanomed Res J, 2020; 5(3): 288-297. DOI: 10.22034/nmrj.2020.03.009

* Corresponding Author Email: *eslamish540@gmail.com*

INTRODUCTION

Nowadays, nanotechnology has emerged as one of the most promising and effective strategies for possible functions in a range of fields including biology, the environment, and in particular medicine [1-3]. The use of nanomaterials is a concern in terms of safety considerations, which is much attention [4]. The anti-pathogenic properties of silver nanoparticles have made them one of the most extensively used products in the field of nanotechnology [5, 6]. The shape, size, and chemistry of silver nanoparticles in general are such that after entering the bloodstream, they can cross cell membranes and interact with intracellular structures [7]. For example, AgNPs triggered alterations in the number of white and red blood cells and increased platelet agglomeration in mice [8-10].

Many studies have focused on the harmfulness of AgNPs *in vivo* through administration of AgNPs by means of various courses such inward breath, intravenous infusion, or oral gavage; however, few studies suggested that the use of AgNPs is intraperitoneally. Intraperitoneal course of organization no longer exclusively allows for shirking of changeability in assimilation from the ingestion locales, anyway furthermore speaks to a possible presentation course of AgNPs for their logical and symptomatic applications. In this manner, the (Thusly, the) production of large-scale silver nanoparticles and their application in various fields emphasizes the importance and need for this research [11]; therefore, the study of the toxic effects of AgNPs seems to be a fundamental and necessary issue. Inflammation in the body is a natural defense mechanism against an external substance. When an external substance is detected by the body's immune system, the inflammatory waterfall becomes active, which may result in inflamed, abnormal/damaged cells [12]. Inflammation suggests the interplay of nanoparticles with the immune system; in other words, it is regarded as an indicator of immune toxicity [13]. It has been established that size, form, and functionalization of the metallic nanoparticles can have an effect on their destiny and mobile toxicity, perchance due to altered immunological interactions [14, 15].

Therefore, there is a growing need for antioxidants, especially the natural antioxidants (e.g., polyphenolic compounds and phytochemicals) [16]. Polyphenolic compounds antioxidant activity is due to several different mechanisms, such as quenching free radical, chelating iron ion, donating hydrogen atoms and activating antioxidant enzymes. The predominant protection mechanism of polyphenolic compounds is to cancel reactive oxygen species (ROS) generation and accordingly keep away from oxidative harm formation [17]. Capping ligands are used to enhance the surface compatibility of AgNPs and lengthen their half-life in circulation to shield particles from any damage or clearance until they reach their destination [18]. There are exclusive sorts of coating layers such as organic conjugates (e.g., amino acids and antibodies), polymers (e.g., PLGA) and herbal polymers (e.g., polyphenolic compounds), which are chosen based on their unique functions [19]. Plant extracts are one of the most commonly used capping agents for AgNPs. Plant extracts additionally improve AgNPs stability and biocompatibility each interior and outdoor the cells, which make a contribution to environmentally friendly and efficient delivery to their target sites [20]. The association of tannic acid with AgNPs confirmed the apparent improvements in damaged liver tissue. The shielding impact of tannic acid in opposition to the toxicity of AgNPs may be because of its antioxidant features and scavenging competencies towards reactive free radicals [21]. Among the various polyphenolic compounds, hydrolyzable tannins have shown stronger antioxidant activity than flavonoids and phenolic acids [22], and they can be considered as a very suitable potential antioxidant for preparation and stabilization of silver nanoparticles. Therefore, tannin-rich plant extracts might be capable of preventing the toxicity of iron-mediated Fenton reaction and reactive oxygen species (ROS) generation. *Myrtus communis methanolic* leaf extract (MCLE), with 80% hydrolyzable tannins content, was used to produce the silver nanoparticles used in the study [23, 24]. *Myrtus communis* has been used as a medicinal herb for different purposes such as peptic ulcer, inflammation, diarrhea, hemorrhoid, skin, and pulmonary sicknesses [25]. The use of the plant has been considered not only for the synthesis of silver nanoparticles but also for the prevention of oxidative stress-related disorders caused by nanoparticles.

Given the attainable oxidative consequences of AgNPs, this paper aimed to check out the feasible poisonous impact of hybrid AgNPs (nano Ag + tannin molecules) following 90 days frequent intraperitoneal injection on, hematological,

biochemical factors and histopathological alterations in mice's liver.

MATERIALS AND METHODS

Substances used

The substances used in this find out about had been from an analytical classification with a high purity proportion and did no longer require re-purification for use. Silver nitrate and sodium hydroxide were acquired from Merck Company (Darmstadt, Germany). Distilled deionized water used to be organized by means of Ultrapure™ water purification equipment of model Millipore SAS 67120 (Millipore Inc., Molsheim, France). Other chemical substances used in the experiment have been in terms of analytical grade or at the best possible grade on the market.

Bio-synthesis of the silver nanoparticles

Silver nanoparticles were synthesized in the same way as in our previous work [26]. Briefly, to the $AgNO_3$ solution (40mM), a clear solution of the extract ($4x10^{-3}$ gL^{-1}, PH=10) with a volume equal to the volume of silver salt solution was added drop by drop under lightless conditions (200rpm, 65 °C, 20 min). Then, the purified pellets were dried via a vacuum freeze dryer for 24h and used for further studies.

The hydrodynamic diameter of spherical MC-AgNPs measured by the dynamic light scattering technique was 142 nm (Core-Shell structure; core= nano Ag = 30 nm (TEM) and sell= tannin biomolecules = 112 nm), which corresponds to the effective size of nanoparticles in drug delivery (50-200 nm), (Fig. 1), [27-29].

In vivo experimental procedure of treating with the nanoparticles

All manipulations of the animals were carried out according to the Mazandaran Medical University's Animal Experimental Ethical Committee guidelines of Laboratory Animal Research Center (Iran, approval no. 911, 2012).

The mice were organized into six trial groups (6 mice per group), with intraperitoneal injection based on the following: group 1—control (normal saline), group 2—Ag^+ (2 mg per kg per day), group 3—MC-AgNPs (50 mg per kg per day), group 4—MC-AgNPs (100 mg per kg per day), group 5—MC-AgNPs (200 mg per kg per day), group 6—MCLE (200 mg per kg per day) groups. Infusions were performed five a week for 3 subsequent months.

All animals were euthanized via diethyl ether after 3 months (end of the trial time frame), and the blood was obtained from heart using a 27 gauge needle. The serum of samples was collected for measuring the serum (AST, ALT, and ALP) levels. Livers of each group were expelled and stored in supported formalin (10%) for histopathological assessment [30].

Evaluation of hematological parameters

The blood samples taken in tubes were used for estimation of hematological parameters using an automated hematology analyzer (ABX Pentra XL 80, France) following the routine procedures.

Histopathological examination of the mice liver via haematoxylin & eosin staining

Histological examination was performed at "Avicenna" hospital (Iran, Sari). The fixed liver tissues were embedded in paraffin wax and 5 mm thickness segments have been cut. The slides were organized with the aid of staining approach with the assist of hematoxylin and eosin for histological testing [31, 32]. The marked tissue slices were surveyed underneath a light microscope (Lx 400, Labomed, California, USA).

Statistical analysis

Data have been analyzed through Prism software program (GraphPad, model 8.0.2 (263), USA). All of the information with typically disbursed are introduced as the mean ± standard deviation (m ± SD). One-way evaluation of variance and Tukey exams has been used. Statistically significant variations have been well-known as $P < 0.05$.

RESULTS AND DISCUSSION

Sub-chronic toxicity assessment (three months)

In this part, the possible outcomes of MC-AgNPs on the hepatotoxicity of exposed mice are investigated by histopathological assessment based on liver enzyme levels and tissue inflammation compared to control mice.

Assessment of changes in liver functional enzymes at serum level

The liver is an effector in opposition to inflammation prompted by means of silver nanoparticles that inspires irregularities in liver function by imposing modifications on the level of functional liver enzymes [33].

Alterations in liver function were evaluated by

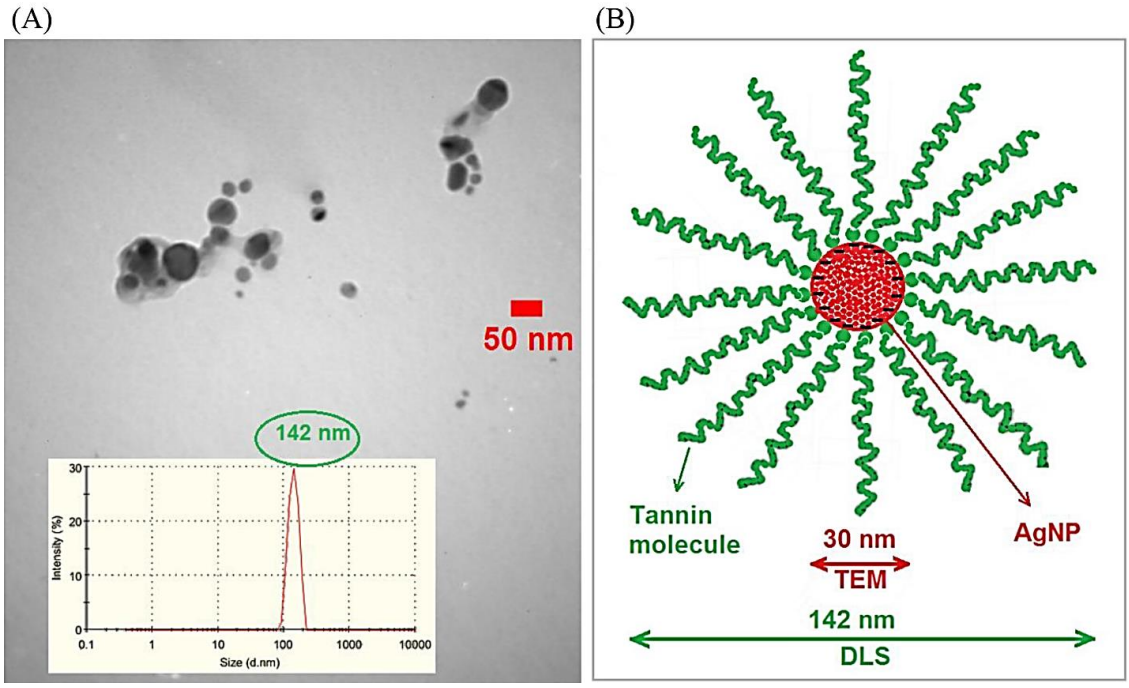

Fig. 1. The TEM image of MC-AgNPs and the transparent layer of tannin molecules coating around the AgNPs (A), model of a hybrid *Myrtus communis*-silver nanoparticle (B) and inset showing the hydrodynamic diameter of MC-AgNPs measured by the dynamic light scattering technique.

way of the degrees of these enzymes, which were enhanced in Ag^+ group comparative with the control group (AST; P < 0.0001, ALP; P = 0.0574 and ALT; P = 0.5291), (Fig. 2A–C). Compared with the Ag^+ group, Ag^+ group, treatment with the MC-AgNPs dose-dependently reduced the degrees of AST; $P < 0.0001$, ALT; $P < 0.001$ and ALP; $P < 0.0001$. Simultaneously, AST, ALT and ALP values likewise decreased in MC-AgNPs-treated mice, compared with the control group dose-dependently. Thus, the mice treated with MC-AgNPs at doses of 100 and 200 mg kg^{-1} revealed a significant lessening in ALT and ALP levels than the control group (ALT; $P < 0.05$ and ALP; $P < 0.001$). In short, treatment of mice with the MC-AgNPs weakened the liver dysfunction (ALT/AST/ALP values), especially ALP, and AST enzyme levels were close to the control group in all three doses. Similar observations have been reported by Dhilil et al. (2020) after treatment of mice by green synthesized nanoparticles using *Indigofera oblongifolia* leaf extract [34]. In addition, Rai et al. (2017) reported lower levels of hepatic enzymes in mice treated by AgNPs (50, 100, and 150 µg/kg) than the control group revealing an achievable protecting role of AgNPs in combination with critical oils [33]. Other observations have been reported by Ansari et al. (2014) after treating mice with silver nanoparticles synthesized using a non-green synthesis method (Silver nanoparticles (Sigma Aldrich, USA) with the manufacturer stated particle size <100 nm). In this way, the enzyme AST decreased with increasing dose of silver nanoparticles, but the enzymes ALT and ALP increased than the control group [35]. In the present study, AgNPs were able to lessen alterations in AST, ALT, and ALP. This might be because of the attendance of tannins in *Myrtus communis* extract as the effective phytochemical components.

Assessment of hematological factors

Fig. 3 represents the mean values of the hematological factors (white blood cells; WBCs, red blood cells; RBC, hemoglobin; Hb, Lymphs, lymphocytes of male mice treated with Ag^+, MCLE, and MC-AgNPs for 90 days. The results showed that treatment with MC-AgNPs resulted in an increase in WBCs compared to the control group as dose-dependent, which was significant at doses of 100 and 200 mg/kg (P <0.0001) and significantly lower than the Ag^+ group (P <0.01). The results showed that treatment with MC-AgNPs caused a

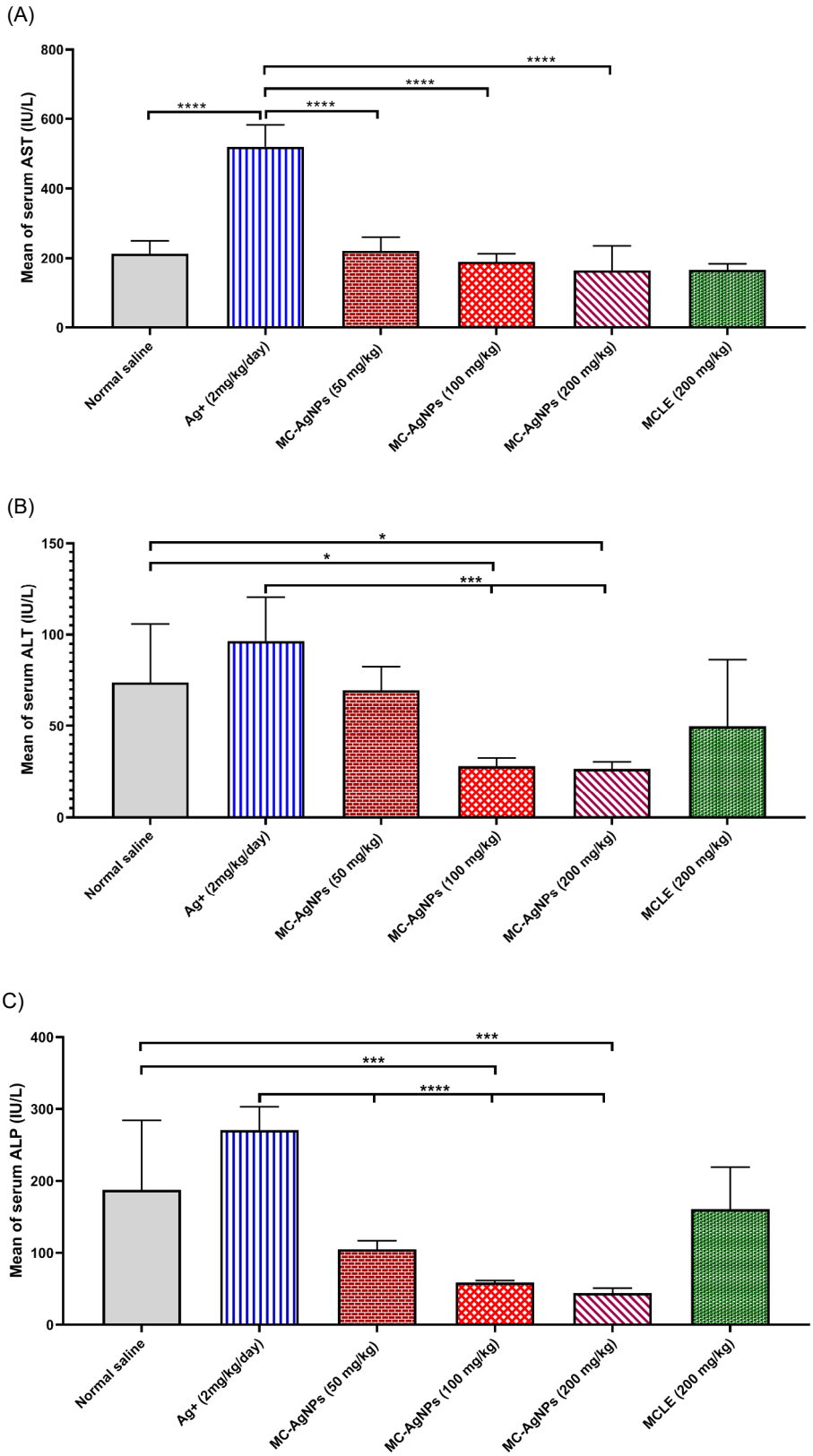

Fig. 2. Serum concentrations of AST (A), ALT (B) and ALP (C) in the *Myrtus communis*-encapsulated Ag nanoparticles- / Ag^{+}-treated and control mice (n = 6). All values are expressed as mean ± standard deviation. $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), $P < 0.0001$ (****).

slight increase in lymphocytes when compared to the control group, which was itself lower than the Ag^+ group. In comparison with the control group, in the group treated with silver nanoparticles, a decrease in platelet count was observed as dose-dependent. However, the number of lymphocytes in the control group was higher than the Ag^+ group. More importantly, treatment with MC-AgNPs increased RBCs and Hb compared to the control group and the Ag^+ group, which is different with the results reported by the Israa et al. (2019) after treating mice with synthesized silver nanoparticles using a non-green synthesis method that were spherical in the range of 17-51 nm [5].

Overall, a comparison of our results with those reported by Israa et al (2019), suggests that the enclosure of silver nanoparticles in the middle of the coating of tannin molecules (Fig. 1B) can prevent the release of silver ions and may reduce the toxic activity of silver nanoparticles. On the other hand, the hybrid tannin-silver nanoparticles were stable. Importantly, the zeta potential of the nanoparticles used to be decided to be −42.5 mV, so they are incredibly anionic [36], and may additionally be regarded as totally non-toxic [37].

Histopathology: photomicrographs analysis

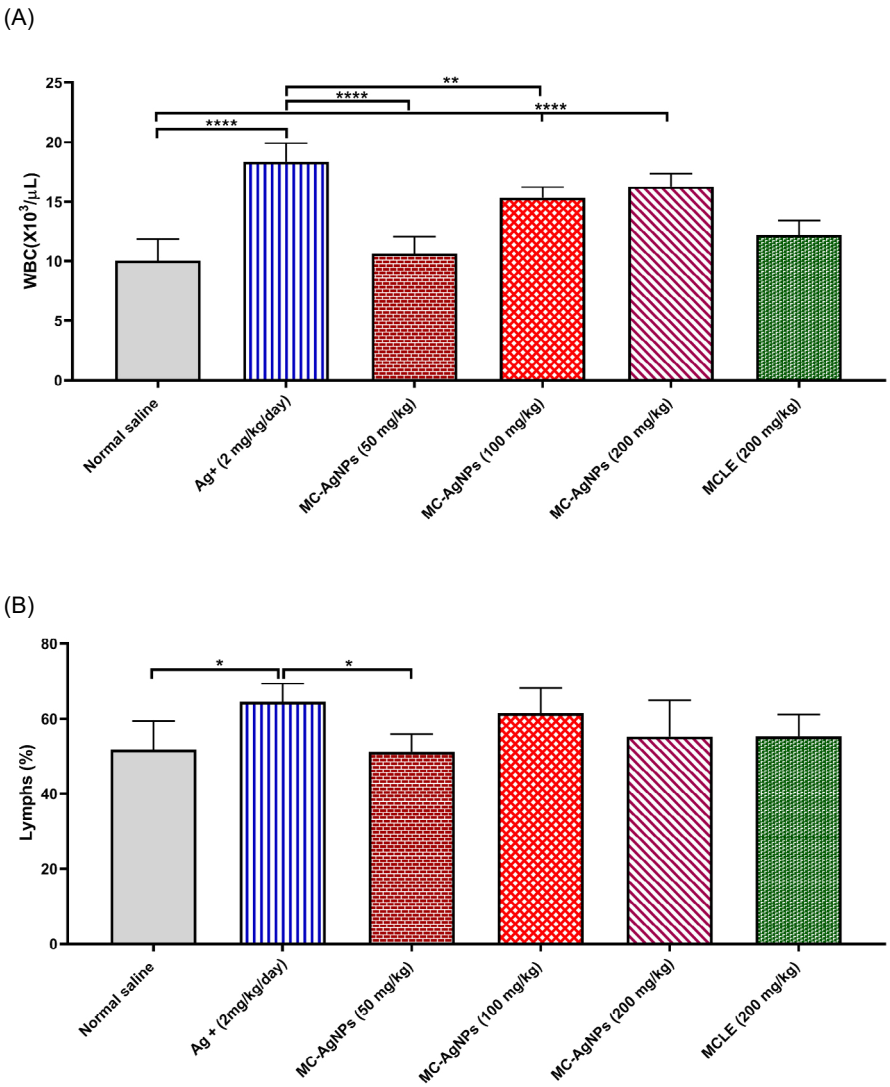

Fig. 3. Hematological factors of male mice treated with Ag^+, MC-AgNPs and MCLE (90 days); mean value ± standard deviation of white blood cells (A), lymphocytes (B), platelets (C), red blood cells (D), hemoglobin (E).

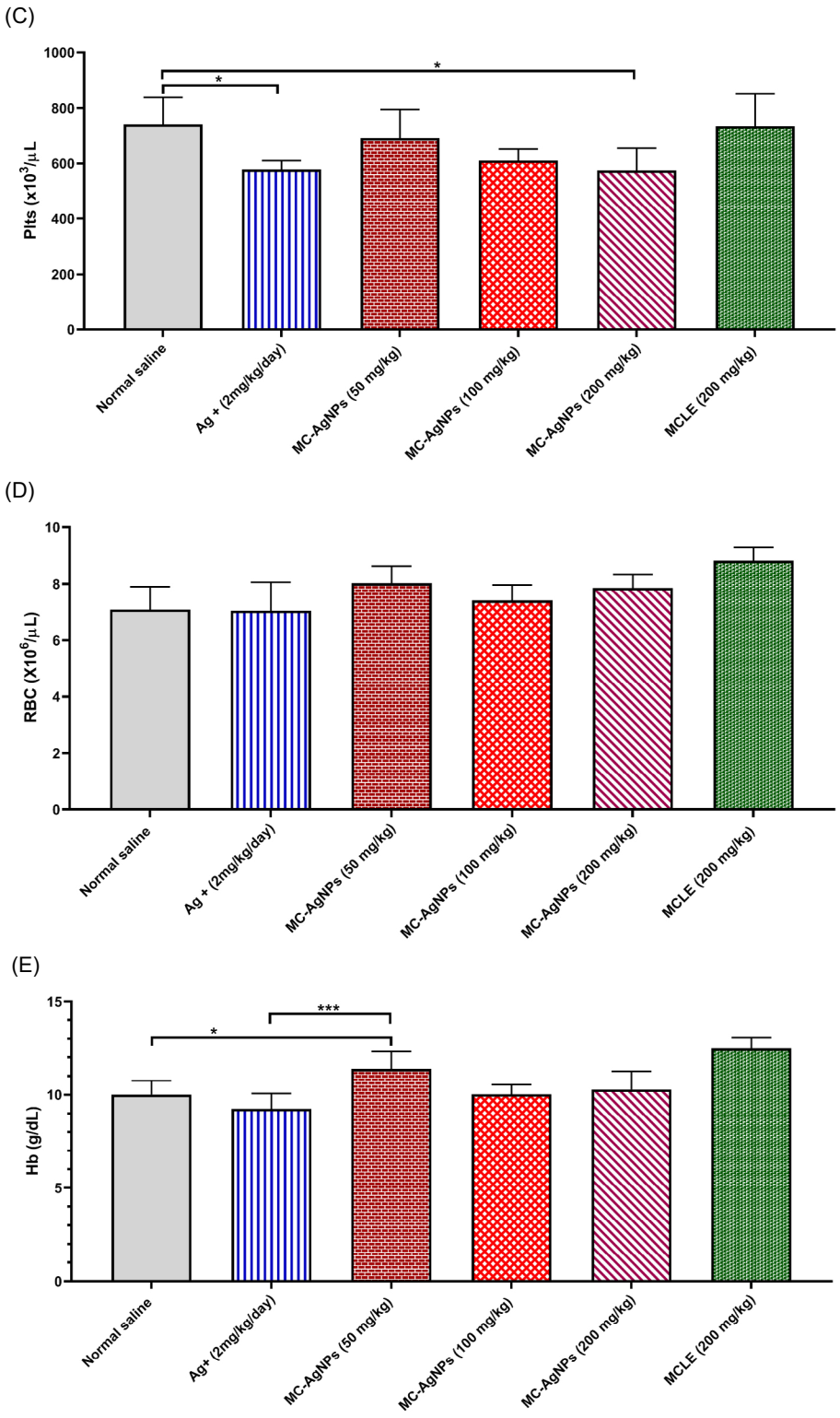

Fig. 3. Hematological factors of male mice treated with Ag^+, MC-AgNPs and MCLE (90 days); mean value ± standard deviation of white blood cells (A), lymphocytes (B), platelets (C), red blood cells (D), hemoglobin (E).

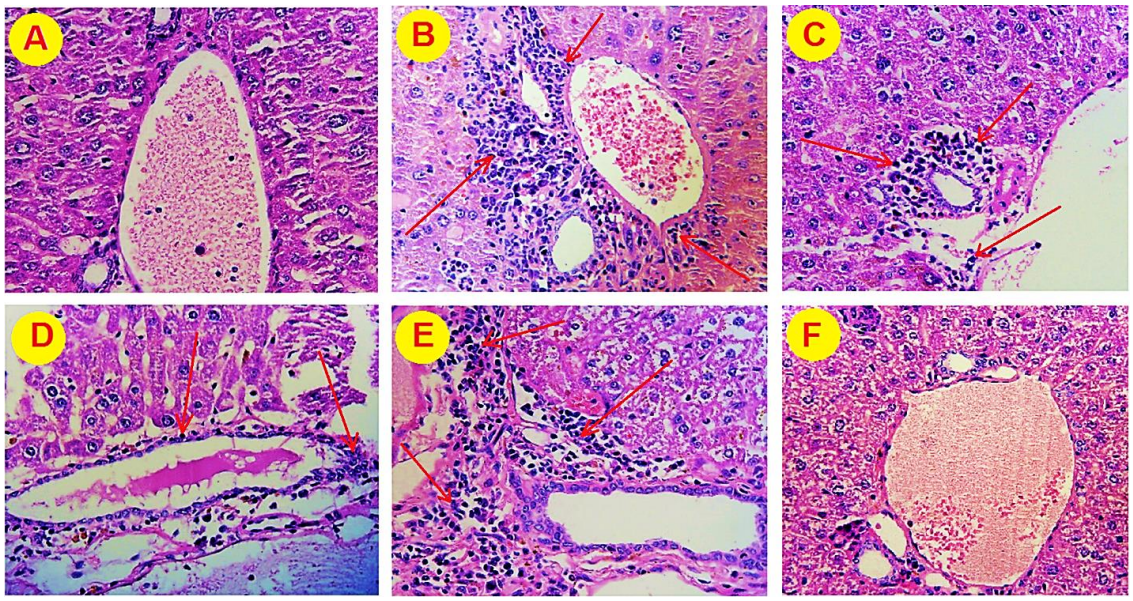

Fig. 4. Liver histopathology (400). Liver sections of: (A) control group mice liver; treated with (B) Ag^{+} (2 mg per kg per day); (C) MC-AgNPs (50 mg per kg per day); (D) MC-AgNPs (100 mg per kg per day); (E) MC-AgNPs (200 mg per kg per day); (F) MCLE (200 mg per kg per day), (90 days).

Histopathology of H&E-stained liver sections exhibited the normal architecture of hepatic lobules in the control group. Each lobule consists of a main vein in the center and radially dispersed hepatocytes (Fig. 4A). Examination of the liver sections of the animals treated with Ag^{+} revealed the presence of necrotic liver cells and the structure of the disintegrated liver lobule. At the same time, proliferation of fibrous tissues, bile duct proliferation, and widespread portal and peritoneal inflammation were detected in liver tissue. Furthermore, the manifest fatty degeneracy of liver cells and diffuse lymphocytes has been observed inside the lobules and severe inflammation was observed in the portal and per portal spaces (Fig. 4B). Three months of exposure did not show any visible impact on liver cyto-architecture in the low-dose silver nanoparticle receptor group and had a very similar control structure. Nevertheless, some lymphocytes were detected inside the lobules. Mild inflammation was seen in the port space (Fig. 4C). In groups 4 and 5, mild and moderate inflammation were observed in port and pre-portal space, respectively (Fig. 4D, E). In the group treated with the plant extract only, the lobular structure of the liver was normal and no inflammation was seen (Fig. 4F).

CONCLUSION

To sum up, in vivo and intraperitoneally exposure to the silver nanoparticles synthesized the use of *Myrtus communis* was once in a position to limit the damaging outcomes of silver nanoparticles on liver feature to shield wholesome tissues and decrease the toxicity of AgNPs. Our findings confirm the use of tannin compounds as a preventative agent to synthesize silver nanoparticles to decrease its toxicity.

Histopathological findings suggest only minor changes after 90 days of treatment that are consistent with the blood and biochemical profiles of laboratory mice, indicating that AgNPs can be safe even for long-term use in a therapeutic period if hybridized with active biomolecules. The consequences endorsed that AgNPs should be properly tolerated in mice when given intraperitoneally at noticeably high-dose (200 mg kg^{-1}) with no mortality. According to our results, the longtime intraperitoneal contact of AgNPs displayed little poisonous or unfavorable fitness results in mice. The presence of tannin molecules along with AgNPs could make the difference and reduce the toxicity of these hybrid nanoparticles (nano Ag + biomolecule) despite a longer treatment period.

ACKNOWLEDGMENT

The authors would like to thank the Vice Chancellor for Research and Technology of Mazandaran University of Medical Sciences (Sari, Iran) for their assistance in conducting this paper (No. 1250). The authors also thank the colleagues of the Cell-Molecular Research Center of Medical School (Sari, Iran), "Avicenna" hospital (Sari, Iran) and all individuals contributing to this study.

CONFLICT OF INTEREST

Potential conflicts of interest were not disclosed.

REFERENCES

1. Nafiujjaman M, Khan HA, Lee Y-k. Peptide-Influenced Graphene Quantum Dots on Iron Oxide Nanoparticles for Dual Imaging of Lung Cancer Cells. Journal of Nanoscience and Nanotechnology. 2017;17(3):1704-11.
2. Nurunnabi M, Parvez K, Nafiujjaman M, Revuri V, Khan HA, Feng X, et al. Bioapplication of graphene oxide derivatives: drug/gene delivery, imaging, polymeric modification, toxicology, therapeutics and challenges. RSC Advances. 2015;5(52):42141-61.
3. E. Ibrahim K, O. Bakhiet A, Khan A, A. Khan H. Recent Trends in Biomedical Applications of Nanomaterials. Biosciences, Biotechnology Research Asia. 2018;15(2):235-43.
4. Al-Harbi NS, Alrashood ST, Siddiqi NJ, Arafah MM, Ekhzaimy A, Khan HA. Effect of naked and PEG-coated gold nanoparticles on histopathology and cytokines expression in rat liver and kidneys. Nanomedicine. 2020;15(3):289-302.
5. Mosa IF, Youssef M, Shalaby T, Mosa OF. The Protective Role of Tannic Acid Against Possible Hepato-Nephrotoxicity Induced by Silver Nanoparticles on Male Rats. SANAMED. 2019;14(2):131.
6. Ebrahimzadeh MA, Tafazoli A, Akhtari J, Biparva P, Eslami S. Engineered Silver Nanoparticles, A New Nanoweapon Against Cancer. Anti-Cancer Agents in Medicinal Chemistry. 2019;18(14):1962-9.
7. Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases. 2007;2(4):MR17-MR71.
8. Kim YS, Kim JS, Cho HS, Rha DS, Kim JM, Park JD, et al. Twenty-Eight-Day Oral Toxicity, Genotoxicity, and Gender-Related Tissue Distribution of Silver Nanoparticles in Sprague-Dawley Rats. Inhalation Toxicology. 2008;20(6):575-83.
9. Kim YS, Song MY, Park JD, Song KS, Ryu HR, Chung YH, et al. Subchronic oral toxicity of silver nanoparticles. Particle and Fibre Toxicology. 2010;7(1):20.
10. Jun E-A, Lim K-M, Kim K, Bae O-N, Noh J-Y, Chung K-H, et al. Silver nanoparticles enhance thrombus formation through increased platelet aggregation and procoagulant activity. Nanotoxicology. 2010;5(2):157-67.
11. Chen X, Schluesener HJ. Nanosilver: A nanoproduct in medical application. Toxicology Letters. 2008;176(1):1-12.
12. Delves PJ, Martin SJ, Burton DR, Roitt IM. Roitt's essential immunology: John Wiley & Sons; 2017.
13. Elsabahy M, Wooley KL. Cytokines as biomarkers of nanoparticle immunotoxicity. Chemical Society Reviews. 2013;42(12):5552.
14. Yah CS. The toxicity of Gold Nanoparticles in relation to their physiochemical properties. 2013.
15. Dykman LA, Khlebtsov NG. Immunological properties of gold nanoparticles. Chemical Science. 2017;8(3):1719-35.
16. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. Natural Product Reports. 2009;26(8):1001.
17. Nićiforović N, Mihailović V, Mašković P, Solujić S, Stojković A, Muratspahić DP. Antioxidant activity of selected plant species; potential new sources of natural antioxidants. Food and Chemical Toxicology. 2010;48(11):3125-30.
18. Saha K, Agasti SS, Kim C, Li X, Rotello VM. Gold Nanoparticles in Chemical and Biological Sensing. Chemical Reviews. 2012;112(5):2739-79.
19. Ma X, Hartmann R, Jimenez de Aberasturi D, Yang F, Soenen SJ, Manshian BB, Franz J, Valdeperez D, Pelaz B, Feliu N. Colloidal gold nanoparticles induce changes in cellular and subcellular morphology. ACS nano, 2017;11 (8):7807-7820.
20. Eslami S, Ebrahimzadeh MA, Biparva P. Green synthesis of safe zero valent iron nanoparticles by Myrtus communis leaf extract as an effective agent for reducing excessive iron in iron-overloaded mice, a thalassemia model. RSC Advances. 2018;8(46):26144-55.
21. Rezaei A, Farzinpour A, Vaziry A, Jalili A. Effects of Silver Nanoparticles on Hematological Parameters and Hepatorenal Functions in Laying Japanese Quails. Biological Trace Element Research. 2018;185(2):475-85.
22. Yoshimura M, Amakura Y, Tokuhara M, Yoshida T. Polyphenolic compounds isolated from the leaves of Myrtus communis. Journal of Natural Medicines. 2008;62(3):366-8.
23. Aidi Wannes W, Mhamdi B, Sriti J, Ben Jemia M, Ouchikh O, Hamdaoui G, et al. Antioxidant activities of the essential oils and methanol extracts from myrtle (Myrtus communis var. italica L.) leaf, stem and flower. Food and Chemical Toxicology. 2010;48(5):1362-70.
24. Aidi Wannes W, Marzouk B. Differences between Myrtle Fruit Parts (Myrtus communisvar.italica) in Phenolics and Antioxidant Contents. Journal of Food Biochemistry. 2012:n/a-n/a.
25. Alipour G, Dashti S, Hosseinzadeh H. Review of Pharmacological Effects ofMyrtus communisL. and its Active Constituents. Phytotherapy Research. 2014;28(8):1125-36.
26. Ebrahimzadeh MA, Biparva P, Mohammadi H, Tavakoli S, Rafiei A, Kardan M, et al. Highly Concentrated Multifunctional Silver Nanoparticle Fabrication through Green Reduction of Silver Ions in Terms of Mechanics and Therapeutic Potentials. Anti-Cancer Agents in Medicinal Chemistry. 2020;19(17):2140-53.
27. Shittu KO, Ihebunna O. Purification of simulated waste water using green synthesized silver nanoparticles of Piliostigma thonningii aqueous leave extract. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2017;8(4):045003.
28. Maleki H, Naghibzadeh M, Amani A, Adabi M, Khosravani M. Preparation of Paclitaxel and Etoposide Co-loaded mPEG-PLGA Nanoparticles: an Investigation with Artificial Neural Network. Journal of Pharmaceutical Innovation. 2019.
29. Teng B, Zhang T, Gong Y, Chen W. Molecular weights and tanning properties of tannin fractions from the Acacia

mangium bark. African Journal of Agricultural Research, 2013;8 (47):5996-6001.
30. Khalili M, Ebrahimzadeh MA, Kosaryan M, Abbasi A, Azadbakht M. Iron chelation and liver disease healing activity of edible mushroom (Cantharellus cibarius), in vitro and in vivo assays. RSC Advances. 2015;5(7):4804-10.
31. Kazaryan S, Petrosyan M, Rshtuni L, Dabaghyan V, Hovhannisyan A. Effects of Green Silver Nanoparticles on CCl4 Injured Albino Rats' Liver. IFMBE Proceedings: Springer International Publishing; 2019. p. 127-31.
32. Gaiser BK, Hirn S, Kermanizadeh A, Kanase N, Fytianos K, Wenk A, et al. Effects of Silver Nanoparticles on the Liver and Hepatocytes In Vitro. Toxicological Sciences. 2012;131(2):537-47.
33. Rai M, Paralikar P, Jogee P, Agarkar G, Ingle AP, Derita M, et al. Synergistic antimicrobial potential of essential oils in combination with nanoparticles: Emerging trends and future perspectives. International Journal of Pharmaceutics. 2017;519(1-2):67-78.
34. Dkhil MA, Abdel-Gaber R, Alojayri G, Al-Shaebi EM, Qasem MAA, Murshed M, et al. Biosynthesized silver nanoparticles protect against hepatic injury induced by murine blood-stage malaria infection. Environmental Science and Pollution Research. 2020;27(15):17762-9.
35. Ansari MA, Khan HM, Khan AA, Alzohairy MA, Waseem M, Ahmad MK, et al. Biochemical, histopathological, and transmission electron microscopic ultrastructural changes in mice after exposure to silver nanoparticles. Environmental Toxicology. 2015;31(8):945-56.
36. Clogston JD, Patri AK. Zeta Potential Measurement. Methods in Molecular Biology: Humana Press; 2010. p. 63-70.
37. Goodman CM, McCusker CD, Yilmaz T, Rotello VM. Toxicity of Gold Nanoparticles Functionalized with Cationic and Anionic Side Chains. Bioconjugate Chemistry. 2004;15(4):897-900.