Original Article

Evaluation of genotoxicity and cytotoxicity of silver nanoparticles

Anastasia Evgenievna Pecherskaya[1], Daria Vladimirovna Andreeva[2], Kamilla Magomedovna Abdulazizova[3], Fariza Magometovna Sampieva[4], Madina Beslanovna Albogachieva[4], Anna Garikovna Babayan[5], Yana Ruslanovna Esenova[6*], Anastasia Gennadievna Lubentseva[4]

[1] State Budgetary Healthcare Institution "City Polyclinic No. 62", Moscow, Russia. [2]Department of Therapy, Faculty of Medicine, Kursk State Medical University, Kursk, Russia. [3]Department of Therapy, Faculty of Medicine, Institute of Clinical Medicine named after N.V. Sklifosovsky, First Moscow State Medical University named after I. M. Sechenov, Moscow, Russia. [4]Department of Therapy, Faculty of Pediatrics, Rostov State Medical University, Rostov-on-Don, Russia. [5]Department of Therapy, Faculty of Medicine, Stavropol State Medical University, Stavropol, Russia. [6]Department of Therapy, Faculty of Medicine, North Ossetian State Medical Academy, Vladikavkaz, Republic of North Ossetia-Alania, Russia.

Correspondence: Yana Ruslanovna Esenova, Department of Therapy, Faculty of Medicine, North Ossetian State Medical Academy, Vladikavkaz, Republic of North Ossetia-Alania, Russia. bucky99@yandex.ru

ABSTRACT

The toxicological and hygienic assessment of the safety of nanoscale colloidal silver produced on a large scale by the modern nano industry is an important task because of its employment medicine. This work aimed to evaluate the mutagenic and cytotoxic properties of silver nanoparticles stabilized with polyvinylpyrrolidone. Silver nanoparticles stabilized with polyvinylpyrrolidone were synthesized for the experiment. The silver particles had a size of about 300 nm, but some of them turned out to be slightly smaller, about 80 nm. The absorption spectrum of UV/Vis electromagnetic waves displayed an absorption peak of 409 nm, which is typical for silver nanoparticles. The exposure time was 10 minutes. Control cultures were placed in saline without glutamate for 10 minutes. Cultures were then returned to the original nutrient medium and placed in a CO2 incubator for 4.5 hours. The excess of β-galactosidase activity over the control in PVP was 0.9±0.03 and 1.2±0.03, and in AgNPs+PVP – 0.6±0.38 and 0.6±0.12. MCF7-test showed that in the presence of silver nanoparticles, the cells acquired a spherical shape and did not show proliferation. AgNPs+PVP activated cell proliferation, which can enhance the wound healing effect.

Keywords: Silver nanoparticle, Polyvinylpyrrolidone, Surgery, Wound healing, Toxicity

Introduction

Modern developments of new methods of treatment of infected wounds and inflammatory skin diseases are devoted to the use of silver nanoparticles, which have several advantages: multilevel antimicrobial effect (antibacterial, antifungal, antiviral), slow development of resistance, low level of toxicity [1-3]. Recent literature data indicate that the wound-healing effect of drugs with silver nanoparticles is due not only to antibacterial but also to anti-inflammatory, reparative properties (regulating the activity of several anti-inflammatory cytokines) [4-6]. At the same time, along with antibacterial and anti-inflammatory activity, silver nanoparticles can also have a toxic effect, expressed, among other things, by genotoxicity and cytotoxicity [7].

Compared to macro-sized silver, silver nanoparticles can potentially exhibit much greater toxicity. The mechanism of the toxic effect may be associated with oxidative stress, mitochondrial dysfunction and increased membrane permeability [8]. Experimental data obtained by various authors on the toxicity of silver nanoparticles are quite contradictory. There is no explanation of how, what time and what concentration of silver nanoparticles were injected into animals. Thus, some authors claim that the LD50 for mice should be 3000 mg/kg, others – 11600 mg/kg, others – 4500 mg/kg [9]. A number of researchers note that the experimental animals accumulated silver nanoparticles in the tissues of the body when receiving it

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Pecherskaya AE, Andreeva DV, Abdulazizova KM, Sampieva FM, Albogachieva MB, Babayan AG, et al. Evaluation of genotoxicity and cytotoxicity of silver nanoparticles. J Adv Pharm Educ Res. 2023;13(3):23-8. https://doi.org/10.51847/MAq20NSenF

with drinking water at doses of 20-50 mg/l [10]. Experiments involving mammalian test organisms, although they are able to bring us closer to understanding the effects of nanoparticles on human health, are currently shrouded in disputes about the ethics of methods. Therefore, the advantage of using primary cell cultures and continuous cell cultures of higher vertebrate organisms *in vitro* is obvious. Thus, when studying the cytotoxic effect of silver nanoparticles on primary fish cell cultures (hepatocytes) and continuous fish cell cultures (RTL-W1, RTH-149, RTG-2), it turned out that the latter are very sensitive indicators of their toxicity [11]. A similar effect for the same particles was demonstrated in a study on human mesenchymal stem cells and peripheral blood mononuclear cells, where silver nanoparticles showed toxicity to all test objects in a narrow concentration range from 12.5 to 50 mg/l [12]. Another study revealed cytotoxic effects of silver nanoparticles in primary gill cells and hepatocytes of rainbow trout [13]. As a result, it was noted that the indication of the level of glutathione and ROS can act as biomarkers of the toxicity of silver nanoparticles.

In addition, according to a number of authors, silver nanoparticles may exhibit some degree of toxicity to all organisms and cell cultures. It was found that nanosilver has almost the same negative effect in the concentration range from 1 to 100 mg/l, regardless of the choice of the test object (viruses, bacteria, microalgae, fungi, animal and human cells) [14]. The noted effect is probably related to their ability to easily form ions. Nanoparticles and their ions activate cell signaling pathways, penetrate through the cell wall and membrane, change the permeability of the latter, damage membrane proteins, nucleic acids and enzymes, inducing the formation of ROS in the cell [11].

In this regard, the toxicological and hygienic assessment of the safety of nanoscale colloidal silver produced on a large scale by the modern nano industry is an important task due to its presence in a large number of consumer products (including medicines, foodstuffs, and biologically active food additives) and the possibility of contamination of various environmental objects with it [10-12].

The work aimed to evaluate the mutagenic and cytotoxic properties of silver nanoparticles stabilized with polyvinylpyrrolidone to argue in favor of the safety of its use.

Materials and Methods

Synthesis of silver nanoparticles

Silver nanoparticles were also obtained using the polyvinylpyrrolidone 10000 cationic surfactant (PVP). The synthesis was carried out in two stages. In the first stage, aqueous solutions of silver nitrate and PVP 10000 were prepared. In 25 ml of distilled water, silver nitrate was dissolved in an amount of 4.24 grams. Further, 2 grams of PVP 10000 were dissolved in 200 ml of distilled water. In the second stage, 1 ml of an aqueous solution of silver nitrate and 0.03 ml of hydrazine hydrate were dissolved in 200 ml of an aqueous solution of PVP. The prepared solution was stirred for 20 minutes at room temperature until the color changed from transparent to dark orange [15].

Characterization of silver nanoparticles

The morphology and size of the obtained nanoobjects were studied using a Sigma Zeiss scanning electron microscope (Germany) with SiO2 plates as a substrate. The optical properties of the obtained nanoobjects were studied by UV spectroscopy on a UV-2600 spectrometer (Shimadzu, Japan). Spectral range: 1400-220 nm; spectral resolution: 0.01 nm.

Strains used

Salmonella typhimurium TA98 (hisD3052, rfa, uvr-, bio-, pkm101) and *Salmonella typhimurium TA1535/pSK1002* strains were used to test the mutagenicity of compounds. All bacterial strains were cultured in LB medium (trypton – 10 g/l; yeast extract – 5 g/l; NaCl – 5 g/l; pH 8.5) [16, 17].

Assessment of geno- and cytotoxicity

The mutagenicity of substances was evaluated in the Ames test. 5 ml of a night culture of *Salmonella typhimurium TA98* was transferred to 20 ml LB with ampicillin (25 mcg/ml) and incubated at 37 ° C with a swing for 2 hours until the culture reached an exponential growth phase. The cells were then collected by centrifugation for 15 min at 4000 rpm. The precipitate was resuspended in a single solution of a salt base. The concentration of the silver nanoparticle solution was 1, 10, and 100 mg/ml, which exceeds the planned pharmaceutical concentration by 10 times. Sodium azide (NaN_3) was used as a positive control. The tested substance was considered mutagenic if the number of revertant colonies in the experiment significantly exceeded that in the control (solvent) by more than 2 times. The DNA-damaging activity of the compounds was evaluated in an SOS chromatest on a strain of *S. Typhimurium TA1535/pSK1002*. The bacterial culture was diluted in LB medium 10 times. The resulting culture liquid was poured 3 ml into test tubes and grown for 4 hours in the presence of the studied compounds in the required concentrations. Then the cells were centrifuged and the activity of β-galactosidase in them was determined [18].

The cytotoxicity of the compounds was studied on the MCF7 line of human breast cancer cells. The cells were incubated in 96-well plates on DMEM medium supplemented with glutamine, FBS, penicillin, and streptomycin. Cell culture (4000 cells per well) was seeded into 96-well plastic plates (Cellstar Grenier bio-one No. 655 180) and the studied compounds were introduced. Then the cell culture was incubated for 24 hours at a temperature of 37°C and an MTS test was performed. This test is based on the suppression of mitochondrial dehydrogenase activity in the presence of a toxic compound and the ability to oxidize the substrate MTS (3-(4,5-dimethylt-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium) using phenazine methosulfate (PMS) as electron-binding reagent.

MTS is reduced by cells to a colored soluble product that can be measured photometrically [19].

Next, 100 µl of PMS solution was added in 1 ml of MTS immediately before addition to the culturally liquid-containing cells. Carefully mixed and 10 µl of the combined solution of MTS and PMS was added to each well of a 96-well analytical tablet containing 50 µl of cells in a culture medium. The tablet was incubated for 1-4 hours at 37℃ in a thermostat with a content of 5% CO2 until a brown color was formed in the control wells.

Results and Discussion

Characterization of silver nanoparticles

The silver particles had a size of about 300 nm, but some of them turned out to be slightly smaller, about 80 nm **(Figure 1a)**. The absorption spectrum of UV/Vis electromagnetic waves displays an absorption peak of 409 nm **(Figure 1b)**, which is typical for silver nanoparticles [20-22]. The shape of the silver particles tends to be spherical.

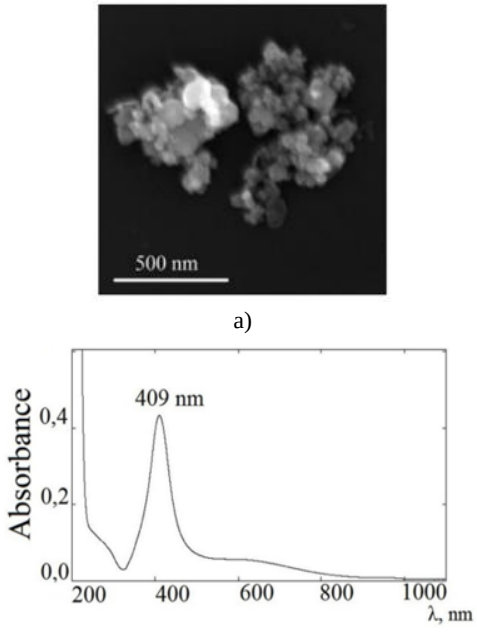

Figure 1. Characterization of silver nanoparticles: SEM micrographs (a) and absorption spectrum of UV-visible electromagnetic waves

Thus, the synthesis of silver particles in water allowed us to obtain nanoparticles whose size is less than 10 nm, while the composition of the particles strongly depends on the purity of the reagents used. The described process does not lead to the formation of one-dimensional particles [23].

Evaluation of the genotoxicity of silver nanoparticles

It was found that in the Ames test, the excess of the number of revertants over the control at concentrations of 10 and 100 µg/ml in PVP was 0.5±0.03 and 0.4±0.06, and in AgNPs+PVP – 0.9±0.12 and 1.1±0.09, which indicates the absence of mutagenic action. None of the compounds showed an increase in the SOS response in the DNA-damaging test. The excess of β-galactosidase activity over the control in PVP was 0.9±0.03 and 1.2±0.03, and in AgNPs+PVP – 0.6±0.38 and 0.6±0.12.

Evaluation of cytotoxicity of silver nanoparticles

The results of the MTS test are shown in **Table 1**. None of the compounds reduced the activity of mitochondrial dehydrogenase, which indicates the absence of their cytotoxicity. The data obtained are consistent with the results of Alsaleh *et al*. [24] and Tiwari [25].

Table 1. Results of cytotoxicity study

Substance	Cytotoxicity, %		
	1 mcg/ml	10 mcg/ml	100 mcg/ml
PVP	105±14	131±14	127±19
AgNPs+PVP	135±15	97±14	119±21

In addition to the MTS test, we investigated the effect of PVP and AgNPs+PVP on the morphology of MCF7 cells. The cells were examined by phase contrast microscopy on the 1st and 2nd days of incubation with the test substances **(Figure 2)**.

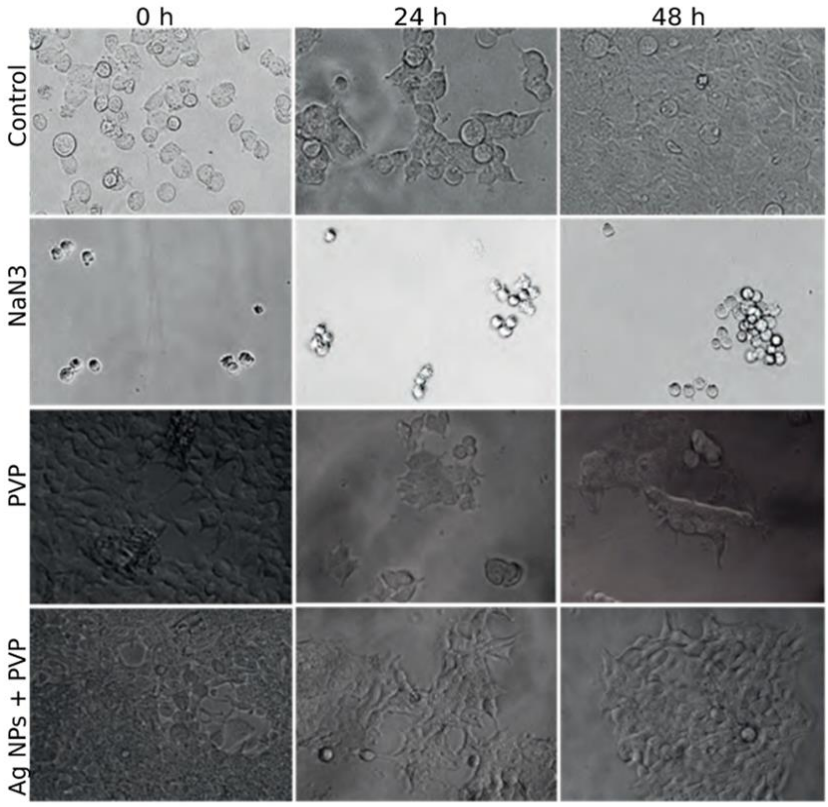

Figure 2. Morphology of MCF 7 cells in the absence of the studied compounds (control) and after proliferation in the presence of sodium azide, PVP, and AgNPs+PVP (10 mg/ml)

Sodium azide, a highly toxic substance, was used as a control **(Figure 2)**. As can be seen from the figure, in his presence, the cells acquired a spherical shape and did not show proliferation. AgNPs+PVP activated cell proliferation **(Figure 2)**, which can enhance the wound healing effect [21, 26-28].

Conclusion

It was found that in the Ames test, the excess of the number of revertants over the control at concentrations of 10 and 100 μg/ml in PVP was 0.5±0.03 and 0.4±0.06, and in AgNPs+PVP – 0.9±0.12 and 1.1±0.09, which indicates the absence of mutagenic action. None of the compounds showed an increase in the SOS response in the DNA-damaging test. The excess of β-galactosidase activity over the control in PVP was 0.9±0.03 and 1.2±0.03, and in AgNPs+PVP – 0.6±0.38 and 0.6±0.12. The results of the MTS test are shown in **Table 1**. None of the compounds reduced the activity of mitochondrial dehydrogenase, which indicates the absence of their cytotoxicity. MCF7-test showed that in the presence of silver nanoparticles, the cells acquired a spherical shape and did not show proliferation. AgNPs+PVP activated cell proliferation, which can enhance the wound healing effect. Thus, the assessment of the geno- and cytotoxicity of AgNPs+PVP did not reveal their toxicity and indicates the need for further research in connection with the possibility of application in the pharmaceutical industry, veterinary medicine, and medicine.

Acknowledgments: All the authors were involved in the conceptualization, methodology, formal analysis, writing, and editing of the manuscript.

Conflict of interest: None

Financial support: None

Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

References

1. Negut I, Grumezescu V, Grumezescu AM. Treatment Strategies for Infected Wounds. Molecules. 2018;23(9):2392. doi:10.3390/molecules23092392
2. Nqakala ZB, Sibuyi NRS, Fadaka AO, Meyer M, Onani MO, Madiehe AM. Advances in Nanotechnology towards Development of Silver Nanoparticle-Based Wound-Healing Agents. Int J Mol Sci. 2021;22(20):11272. doi:10.3390/ijms222011272
3. Khalil MA, El Maghraby GM, Sonbol FI, Allam NG, Ateya PS, Ali SS. Enhanced Efficacy of Some Antibiotics in Presence of Silver Nanoparticles Against Multidrug Resistant Pseudomonas aeruginosa Recovered From Burn

Wound Infections. Front Microbiol. 2021;12:648560. doi:10.3389/fmicb.2021.648560

4. Blinov AV, Kachanov MD, Gvozdenko AA, Nagdalian AA, Blinova AA, Rekhman ZA, et al. Synthesis and Characterization of Zinc Oxide Nanoparticles Stabilized with Biopolymers for Application in Wound-Healing Mixed Gels. Gels. 2023;9(1):57. doi:10.3390/gels9010057
5. Liu J, Liu Z, Pang Y, Zhou H. The interaction between nanoparticles and immune system: Application in the treatment of inflammatory diseases. J Nanobiotechnol. 2022;20(1):1-25. doi:10.1186/s12951-022-01343-7
6. Wang H, Zhou Y, Sun Q, Zhou C, Hu S, Lenahan C, et al. Update on Nanoparticle-Based Drug Delivery System for Anti-inflammatory Treatment. Front Bioeng Biotechnol. 2021;9:630352. doi:10.3389/fbioe.2021.630352
7. Verevkina M, Goncharov V, Nesmeyanov E, Kamalova O, Baklanov I, Pokhilko A, et al. Application of the Se NPs-Chitosan molecular complex for the correction of selenium deficiency in rats model. Potravinarstvo Slovak J Food Sci. 2023;17:455-66. doi:10.5219/1871
8. Wypij M, Jędrzejewski T, Ostrowski M, Trzcińska J, Rai M, Golińska P. Biogenic Silver Nanoparticles: Assessment of Their Cytotoxicity, Genotoxicity and Study of Capping Proteins. Molecules. 2020;25(13):3022. doi:10.3390/molecules25133022
9. Tufail MS, Liaqat I, Andleeb S, Naseem S, Zafar U, Sadiqa A, et al. Biogenic Synthesis, Characterization and Antibacterial Properties of Silver Nanoparticles against Human Pathogens. J Oleo Sci. 2022;71(2):257-65. doi:10.5650/jos.ess21291
10. Calderón-Jiménez B, Johnson ME, Montoro Bustos AR, Murphy KE, Winchester MR, Vega Baudrit JR. Silver Nanoparticles: Technological Advances, Societal Impacts, and Metrological Challenges. Front Chem. 2017;5:6. doi:10.3389/fchem.2017.00006
11. Haider A, Kang IK. Preparation of silver nanoparticles and their industrial and biomedical applications: a comprehensive review. Adv Mater Sci Eng. 2015;2015:1-6. doi:10.1155/2015/165257
12. Yaqoob AA, Umar K, Ibrahim MNM. Silver nanoparticles: various methods of synthesis, size affecting factors and their potential applications–a review. Appl Nanosci. 2020;10:1369-78. doi:10.1007/s13204-020-01318-w
13. Ostaszewska T, Śliwiński J, Kamaszewski M. Cytotoxicity of silver and copper nanoparticles on rainbow trout (Oncorhynchus mykiss) hepatocytes. Environ Sci Pollut Res. 2018;25:908-15. doi:10.1007/s11356-017-0494-0
14. Cameron SJ, Hosseinian F, Willmore WG. A Current Overview of the Biological and Cellular Effects of Nanosilver. Int J Mol Sci. 2018;19(7):2030. doi:10.3390/ijms19072030
15. Mirzaei A, Janghorban K, Hashemi B, Bonyani M, Leonardi SG, Neri G. Characterization and optical studies of PVP-capped silver nanoparticles. J Nanostruct Chem. 2017;7:37-46. doi:10.1007/s40097-016-0212-3
16. Wu K, Li H, Cui X, Feng R, Chen W, Jiang Y, et al. Mutagenesis and Resistance Development of Bacteria Challenged by Silver Nanoparticles. Antimicrob Agents Chemother. 2022;66(10):e0062822. doi:10.1128/aac.00628-22
17. Wu K, Li H, Wang Y, Liu D, Li H, Zhang Y, et al. Silver nanoparticles elevate mutagenesis of eukaryotic genomes. G3: Genes, Genomes, Genetics. 2023;13(3):jkad008. doi:10.1093/g3journal/jkad008
18. Rzhepakovsky IV, Areshidze DA, Avanesyan SS, Grimm WD, Filatova NV, Kalinin AV, et al. Phytochemical Characterization, Antioxidant Activity, and Cytotoxicity of Methanolic Leaf Extract of Chlorophytum Comosum (Green Type) (Thunb.) Jacq. Molecules. 2022;27(3):762. doi:10.3390/molecules27030762
19. Mussin J, Robles-Botero V, Casañas-Pimentel R, Rojas F, Angiolella L, San Martin-Martinez E, et al. Antimicrobial and cytotoxic activity of green synthesis silver nanoparticles targeting skin and soft tissue infectious agents. Sci Rep. 2021;11(1):14566.doi:10.1038/s41598-021-94012-y
20. Kamal T, Khan MS, Khan SB, Asiri AM, Chani MT, Ullah MW. Silver nanoparticles embedded in gelatin biopolymer hydrogel as catalyst for reductive degradation of pollutants. J Polym Environ. 2020;28:399-410. doi:10.1007/s10924-019-01615-8
21. Blinov AV, Nagdalian AA, Povetkin SN, Gvozdenko AA, Verevkina MN, Rzhepakovsky IV, et al. Surface-Oxidized Polymer-Stabilized Silver Nanoparticles as a Covering Component of Suture Materials. Micromachines (Basel). 2022;13(7):1105. doi:10.3390/mi13071105
22. Hamouda RA, Hussein MH, Abo-Elmagd RA, Bawazir SS. Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium Oscillatoria limnetica. Sci Rep. 2019;9(1):13071. doi:10.1038/s41598-019-49444-y
23. Ćurlin M, Barbir R, Dabelić S, Ljubojević M, Goessler W, Micek V, et al. Sex affects the response of Wistar rats to polyvinyl pyrrolidone (PVP)-coated silver nanoparticles in an oral 28 days repeated dose toxicity study. Part Fibre Toxicol. 2021;18:38. doi:10.1186/s12989-021-00425-y
24. Alsaleh NB, Minarchick VC, Mendoza RP, Sharma B, Podila R, Brown JM. Silver nanoparticle immunomodulatory potential in absence of direct cytotoxicity in RAW 264.7 macrophages and MPRO 2.1 neutrophils. J Immunotoxicol. 2019;16(1):63-73. doi:10.1080/1547691X.2019.1588928
25. Tiwari V, Tiwari M, Solanki V. Polyvinylpyrrolidone-Capped Silver Nanoparticle Inhibits Infection of Carbapenem-Resistant Strain of Acinetobacter baumannii in the Human Pulmonary Epithelial Cell. Front Immunol. 2017;8:973. doi:10.3389/fimmu.2017.00973

26. Banerjee SL, Das S, Bhattacharya K, Kundu M, Mandal M, Singha NK. Ag NPs incorporated self-healable thermoresponsive hydrogel using precise structural "Interlocking" complex of polyelectrolyte BCPs: A potential new wound healing material. Chem Eng J. 2021;405:126436. doi:10.1016/j.cej.2020.126436
27. Mezhidov BS, Belyaeva AA, Kh SM, Bimarzaev S, Bektashev A, Shekhshebekova AM, et al. Prospects for Creating 3D Models of Internal Organs Based on Computer and Magnetic Resonance Imaging Images in Emergency Surgery and Resuscitation. Pharmacophore. 2021;12(1):8-14. doi:10.51847/3TLcii4n42
28. Al Quwayd MN, Alluhaydan AS, Alsam AA, Alferaih AK, Oukda AH, Alagha BB, et al. An Overview on the Effect of Bariatric Surgery on Type 2 Diabetes Mellitus Patients: Literature Review. Int J Pharm Res Allied Sci. 2021;10(2):90-3. doi:10.51847/SLrvrjY9JN