Iranian Journal of Pharmaceutical Sciences
2014: 10 (2): 35- 46
www.ijps.ir

Original Article

Comparison of the Cytotoxic Effects of Nanosilver and Ancient Iranian and Pakistani Silver Kushtas on Primary Rat Hepatocytes and Human Cancer Cell Lines

Alireza Mozaffari Dehshiri[a], Maryam Hamzelou Moghaddam[a], Farzaneh Naghibi[a], Soheila Honary[b], Mahmoud Mosaddegh[a,*]

[a]*Department of Traditional pharmacy, Faculty of Traditional Medicine, Shahid Beheshti University of Medical sciences, Tehran, Iran,* [b]*Faculty of pharmacy, Mazandaran University of Medical sciences, Sari, Iran*

Abstract

Tumor growth is characterized by uncontrolled cell division. For centuries, silver kushta powder, composed of nano and submicro silver particles, has been used in traditional Iranian and Pakistani medicine for the treatment of melanoma and breast cancer. We have found that these nanocomposite particles are similar to silver nanoparticles (AgNps) in size and shape and that there are so differences in their physicochemical properties such as silver content.In the present study, a comparison of cytotoxic effects of nanosilver(AgNps) and two silver kushtas [Iranian silver kushta (IKAg) and Pakistan silver kushta (PKAg)]have been conductedindifferent concentrations against tumor cell lines (MCF-7, HepG2, A549) and a normal cell line (MDBK) using MTT andTrypan blue exclusion tests.At first, Particle size was analyzed using the Malvern Zetasizer. The Z average diameters of samples (AgNps, PKAg, and IKAg) were64.08, 51.72 and 190.4 nm, respectively.The result of MTTtest showed no toxicity of both silver kushtas (IKAg&PKAg) toward the cancer cell lines and MDBK cells. The IC_{50} values of AgNpsdetermined for A549, HPG2, MCF-7, and MDBK were 5.94, 1.41, 3.68, and, 1.9 ppm, respectively. According to trypan blue (0.2% w/v) exclusion test, the cytotoxicity of the silver materials toward primary rat hepatocytes followed the order AgNps (100%) >$AgNO_3$ (80.9%) >Pakistani silver kushta (48.35%)>Iranian silver kushta (45%).This result illustrated that the silver components [(IKAg) & (PKAg)] of the traditional kushtas do not penetrate the cancer cell membrane and do not show cytotoxicity. Therefore, kushtas are ineffective as anti-cancer agents. However, AgNps shows good anticancer properties.

***Keywords*:** Cancer; Cell line, Cytotoxcity, Kushtas, MTT assay, Particle size, Trypan blue.

Corresponding Author: Mahmoud Mosaddegh, Department of Traditional pharmacy, Faculty of Traditional Medicine, Shahid Beheshti University of Medical sciences, Tehran, Iran.
Tel: (+98)218773521-5
E-Mail: mmosaddegh@itmrc.org
Cite this article as: Mozaffari Dehshiri A, Hamzelou Moghaddam M, Naghibi F , Honary S, Mosaddegh M, Comparison of the Cytotoxic Effects of Nanosilver and Ancient Iranian and Pakistani Silver Kushtas on Primary Rat Hepatocytes and Human Cancer Cell Lines.Iranian Journal of Pharmaceutical Sciences, 2014, 10 (2): 35-46.

1. Introduction

The favorable physico-chemical and toxicological properties of nanoparticle have enabled their extensive use as drug delivery vehicles, particularly in cancer treatment. *In vitro* and *in vivo* studies have analyzed the

cytotoxic effects of Nanometals such as nanosilver and other silver materials [1].

Historically, silver kushtas were administered orally for leprosy, and is a key ingredient of the SalakhehMajoon (mixture) described in the Avicenna book [2]. Several studies have found that silver kushta could be used topically in Zanjafer and Hawarioun poultices (Zomad) for the treatment of melanoma and breast cancer [3, 4]. Available data indicates that in Africa and Asia in countries such as Egypt and Iran, one-third of the population opts to use alternative medicine [5,6]. They believe that usingtraditional medicine helps them to meet some of their health care needs [7]. The globalization of traditional medicine has important implication for toxicological studies, quality control, and standardization [8]. Thus, characterization of the physicochemical properties of these ancient traditional medicines has become critical.

Kushtas are calcinated metal preparations, obtained by the calcination of metals, minerals, and animal material. Typically, kushta is a mixture of metal, nonmetal, and metal oxide [5, 7, 9], and is commonly used in both traditional Iranian and Ayurvedic medicines [9-11].

Nanosilver, which has been extensively studied in the recent years, has been described in ancient and medieval pharmacopeias such as Canoon of Avicenna [10-14], Qarabadin of Aghili, and Alhavi of Rhazuz as useful for the treatment of melancholy, bronchitis, dementia, Alzheimer, leprosy, and heart diseases. These texts also described some key concepts regarding their preparation, including the high-temperature incineration (TadabirAladviyah). The Hakims (vaids) of Iranian traditional medicine believed that calcination (Altaklis), which produces fine particles, would be suitable for enhanced absorption [15]. It appears that the Unanikushta (Indian and Pakistani kushtas) and IKAg share similar therapeutic and toxicological properties.

Rhazus [11] and Sheikh Avicenna (10 century A.D.), Iranian physicians, have written extensively about the use of metals in therapeutic procedures [7,9,16]. In comprehensive Treaties (Qarabadin) of the Iranian and Indian medicine, Seyyed Mohammad HussainAqhiliKhorasani, a Hakim, has described kushtas [3, 14,16]. Pioneering work in the field of kushtas was done by Hakim Momen in 19th century [14].

It appears that for centuries, the vaids used the same method for preparing kushtas. Additionally, the drug safety was overlooked, likely because of the lack information and necessary laboratory equipment. During the Middle Ages, heavy metals, particularly mercury, entered the Ayurvedic armamentarium, and were used for the treatment of syphilis brought by the Portuguese. Mercury, lead, and arsenic have been detected in traditional Indian and Pakistani medicinal preparations such as Alkohl (Sormeh), which is used as a cosmetic product. That the main ingredient of Alkohl is lead sulfide suggested that users of this Ayurvedic preparation may be at risk of heavy metal toxicity [16-19]. Lack of information and awareness regarding the toxic effects of

heavy metals may have resulted in patients using such preparations and suffering from the deleterious effects of metallic components. Currently, the Helsinki rules and FDA regulations force the pharmacists and physicians to ascertain that heavy metals are not present in drugs [20].

Thus, toxicological studies are necessary before kushtas can be made commercially available for clinical use. The current literature shows that silver has several desirable properties to be used in nanomedicine. Additionally, it has been reported that nanosilver has anti-bacterial and antitumor effects [21].

Particles of size less than 100 nm are termed as nanoparticles. The large surface area per unit mass of nanoparticles enables greater reactivity and novel properties compared to bulk materials [22, 23]. AgNps shows therapeutic propertied of the precious metal silver, and is likely to replace it in traditional as well as modern medicine [24, 25]. Recent studies have shown that AgNps offer desirable anti-cancer properties. It appears that AgNps may be a cost-effective alternative for the treatment of cancer and angiogenesis-related disorders [26, 27].

Although the potential applications and benefits of using AgNps in cancer treatment have been extensively discussed, the cytotoxicity and anti-tumor effects of IKAg and PKAg have not been adequately characterized. In the study reported here, we compared the cytotoxic effects of AgNps and traditional silver kushtas (IKAg&PKAg) on primary rat hepatocytes,cancerous cell lines:HepG2, A549, MCF-7,and normal cell line (MDBK cells), and determined the IC_{50}values.

In an earlier study, we examined the properties of silver kushta and showed that Iranian silver kushta (IKAg), Pakistani silver kushta (PKAg), and silver nanoparticles (AgNps) formed suspensions with similar particle size. Here we report that there are similarities between AgNps and kushtas—their particle size was less than 200 nm.

2. Materials and Methods

2.1. Materials

PKAg was purchased from Hamdard Co. Pakistan. Sodium chloride, $AgNO_3$, and $NaBH_4$ were all purchased from Merck, Germany. Silver powder was bought from Sigma-Aldrich, USA. High molecular weight (MW) chitosan (100 and 600 KDa, respectively) were purchased from Fluka (Switzerland) and Biochemia (Japan), respectively.

Chemicals: N-(2-hydoxyethyl) piperazin-N-(2-ethane sulfonic acid (HEPES), Trypanblue, and heparin were purchased from sigma Aldrich Co (Tauftkrichen-Germany). All other chemicals were of the highest commercial grade available.

2.2. Methods

2.2.1. Preparation of IKAg

Silver kushtas and AgNpswere produced through calcination procedure and chemical reduction method using $AgNO_3$ and $NaBH_4$ as

a chemical reducer, respectively. To obtain modified IKAg the process was conducted based on Aghili's manuscripts, Qarabadin [3-13]. Totally, 8 gr silver fine powder and 750 mg of sulfur were added to 135 mL of 10% sodium chloride solution in a dry round-bottomed closed cylindrical Tin worked stainless steel container. The apparatus then heated at 960ºC for 20 minutes. Finally, the obtained component was pounded and grinded in a pestle and mortar after trituration of component.

2.2.2. Preparation of Silver Nanoparticle Dispersion

First, a stock solution of chitosan (2 mg/mL) was prepared by dissolving 5 mg chitosan in 10 mL acetic solution acid solution (1%, V/V) at 25ºC (room temperature). The mixture was stirred at room temperature for 24 hours on a magnetic stirrer. The resulting solution was filtered through a 0.22μmMilipore filter to remove any undissolved material.

In the second step, silver nanoparticles were prepared by the chemical reduction of $AgNO_3$ in dark at 4ºC using $NaBH_4$, which yielded zero-valence silver as nanosilver. Spherical and rod-shaped magnets, and various speeds (400, 500, 700, 800 rpm) were employed in the reactions. Typical reaction mixture consisted of 35 μL of a solution of $NaBH_4$ and 35 μL of $AgNO_3$ solution. To insure complete reduction, the ratio of molar concentrations of $NaBH_4$ (0.2 mol/L) and $AgNO_3$ (0.02 mol/L) was maintained at 10:1 [28].

The AgNps thus synthesized had a concentration of 780 ppm (or mg/L) and a mean size of 64.08 nm. This stock suspension of nanoparticles could be stably diluted with distilled water. The solution was diluted with deionized water and sterilized by filtering through a 0.22μm filter. The silver kushtas formed unstable suspensions, but were also diluted and sterilized in similar manner. For measuring the particle size in IKAg and PKAg suspensions, the respective suspension was filtered through a 0.45μm filter and the filtrate was analyzed using a Malvern Zetasizer.

2.2.3. Cell culture

The cell lines HepG2 (human hepatocellular livercarcinoma), MCF-7 (human breastadenocarcinoma), A549 (human lungadenocarcinoma) and MDBK (bovine kidneycells) were obtained from the national center for cell science, Pasteur institute of Iran (Tehran). The cells were cultured in RPMI1640 or DMEM/F12 medium supplemented with 100 mg/L streptomycin, 100000 U/L penicillin G and 10% (v/v) heat-inactivated fetal bovine serum (FBS). The cells were maintained under a humidified atmosphere of 5% CO_2 at 37ºC, and were routinely passaged by trypsinization when they reached 80% confluence.

Four different cultures were examined under an inverted microscope for confluence and the presence of bacterial and fungal contaminants. The cells were washed with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and dislodged by incubating for 2–10 min with Trypsin/EDTA. After examining the cells under an inverted microscope to

ensure that all the cells were separated, the cells were resuspended in a small volume of fresh growth medium. Then, 100 µL of the cell suspension was removed and stained with Trypan Blue. Cell counting was performed with the help of a hemocytometer under a light microscope. Desired number of cells was transferred to round-bottomed 96-well plates containing 90 µL of culture medium. The plate was incubated overnight at 37°C and 5 % CO_2, and the cell viability was measured using the (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) MTT(Sigma Aldrich) assay [28].

2.2.4. Evaluationof AgNpsCytotoxicity

The inhibitory concentration value (IC_{50}) of AgNpsand silver kushtas were evaluated on MCF-7, HepG2, A549, and MDBK cells using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were incubated with 0.243, 0.487, 0.975, 1.98, 3.9, and 7.8 µg/mL concentrations of AgNps (in distilled water as nanocolloids). While serial dilution for PKAgand IKAg were 25, 50, 100, 125, 200, 250, 400, 500 and 1000ng/mL.

For cytotoxicity measurements, 90 µLof cells were seeded in 96-well plates at a density of 1×10^4 cells per well and incubated overnight. After treating the cells for 72 h with 10µLof the prepared serial dilutions, 20 µL of a solution containing 0.5 mg/mL MTT in PBS was added into each well and the plates were incubated at 37°C for 4 hours. Following this, the medium was discarded and the formazan blue formed was dissolved by incubating with 100µL dimethyl sulphoxide (DMSO) at 37°C on a shaker (ELISA reader) for 20 minutes. The absorbance of the resulting solution at 570 nm was measured using a microplate reader (Anthos-Austria).

2.2.5.Statistical Analysis, Cell Viability, and IC_{50}

The results were expressed as IC_{50} values and percentage viability, which is directly proportional to the metabolically active cell number. The cell viability was calculated as a percentage of the control value. IC_{50} was defined as the concentration of the material required to inhibit the cell growth by 50% (IC_{50}±SD). All experiments were conducted in triplicate (SD less than 0.0001). The OD values were used to calculate the percentage of viability using the following formula:

$$\%\ \text{of cell viability} = \frac{\text{OD value of experimental sample (treated cells)}}{\text{OD value of experimental cxontrol (untreated cells)}} \times 100$$

The $IC_{50}$was determined from a plot of log [AgNPs] versus cell viability. A line parallel to the X-axis drawn from a point in the Y-axis corresponding to 50% cell viability meets the curve at point (X, Y). The antilog of this concentration X produces the IC_{50}value, which is a measure of the toxicity of the material. The data were expressed as mean ± SD. Whenever appropriate, the data were also analyzed using the one-way analysis of variance (ANOVA). Statistical analyses were performed with the help of the Magellan

software. P<0.0001 was considered statistically significant.

2.2.6. Isolation and Culture of Primary Rat Hepatocytes

Male Sprague-Dawley rats (weighing 250–300 gr) were purchased from the Pasteur Institute of Iran (Tehran, Iran). The rats were housed in individual cages maintained at 20–25ºC and 50–60% humidity under a 12h light/dark cycle, and were fed with a standard chow diet and water. All experiments were conducted in accordance with the recommended ethical standards and protocols approved by the Committee of Animal Experimentation of ShahidBeheshti University of Medical Sciences, Tehran, Iran. All efforts were made to minimize the number of animals used and their suffering.

The rats were euthanized with an intraperitoneal injection of Ketamine and Xylazine. Heparin was administered intraperitoneally. The rats were sterilized with 70% ethanol. For the isolation of hepatocytes, the excised liver was minced with a scalpel and the tissue fragments were washed with Hanks buffer. The tissue fragments were then transferred to Hanks solution and incubated with collagenase (17 mg collagenase in 25 mL hanks solution) at 37ºC in a shaker incubator for 1–2 h. The cell suspension was then transferred to a 50 mL centrifuge tube and centrifuged at 500 rpm for 10 minutes.

Hepatocytes were obtained by collagenase perfusion of the liver and their viability was assessed by the Trypan blue (0.2 w/v) exclusion test [29]. The cells were suspended in Krebs Henseleit buffer (pH=7.4) containing 12.5 mM HEPES in a round-bottomed flask at a density of 10^6 cells/mL and were incubated under an atmosphere of 10% O_2, 85% N_2, and 5% CO_2 in a rotating water bath maintained at 37ºC.

The viability of isolated hepatocytes was assessed by the Trypan blue (0.2% w/v) exclusion test. The rat hepatocytes were treated with three different concentrations of the silver materials. These solutions were prepared by serially diluting solutions of 8.5, 10.61, 8, and 1 mg of $AgNO_3$, IKAg, PKAg, and AgNps, respectively in 10 mL of distilled water. Aliquots of the hepatocyte cultures were withdrawn at specific intervals during the 2-hour incubation period, 10 µL of Trypan blue solution, and the viable cells were counted under an inverted microscope and hematologic lamella [29].

3. Results and Discussion

3.1. Particle size determination

Size, shape and chemical composition have been deemedimportant properties when investigating NP-mediated toxicity[30]. In the present study the size ofAgNps, PKAg and IKAgparticles had an average diameter of 64.08nm, 51.72 nm and 190.4 nm, respectively. Figure 1 shows the particle size of AgNps, PKAg, and IKAg after ultra-sonication. The development of new zeta sizers and other advanced technologies have enabled a more complete characterization of the physico-chemical properties of the particles.

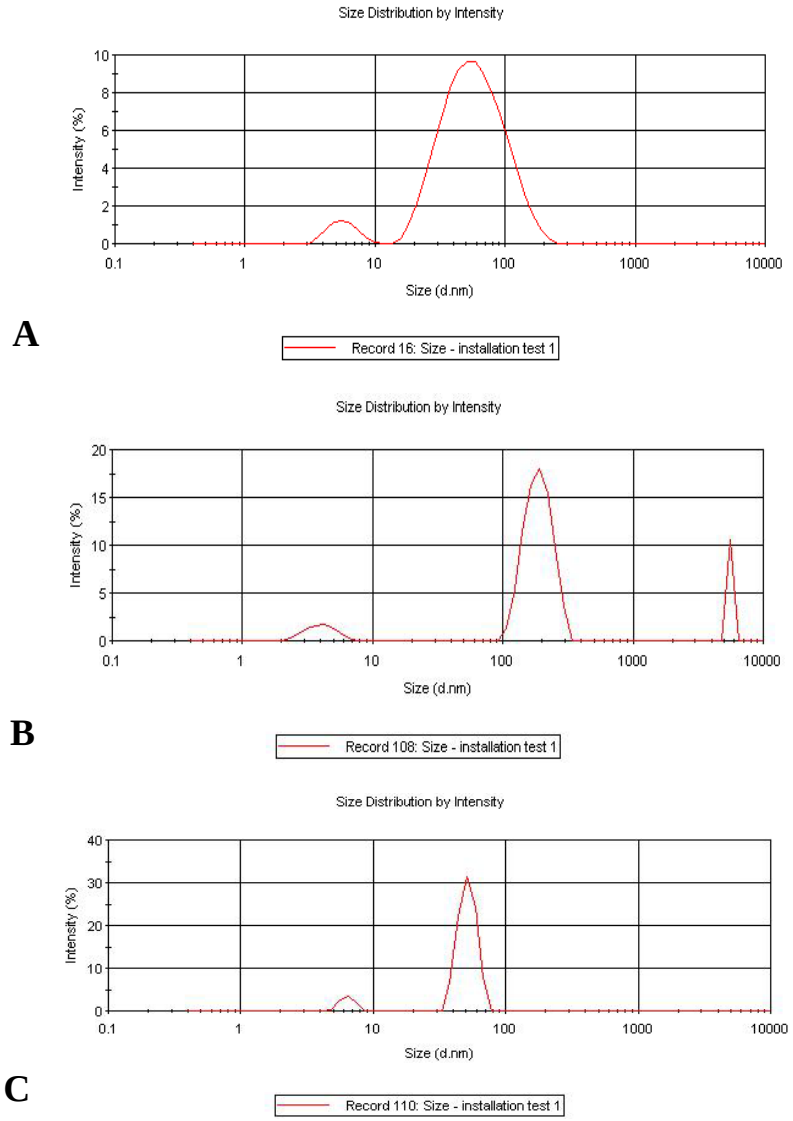
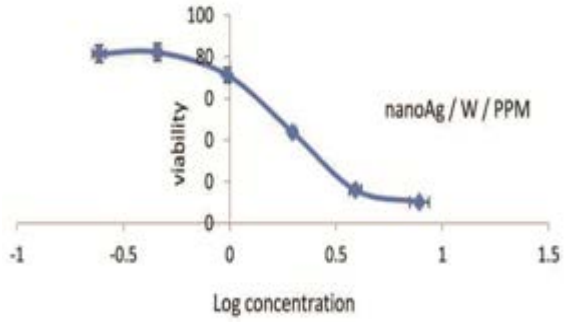


Figure 1. IKAg (Aqili method) after optimization and sonication show maximum peak of particle size at 190.4 nm (A). PKAg after optimization and sonication show maximum peak of particle size at 51.72 nm (B). AgNPs after optimization and sonication show maximum peak of particle size at 64.08 nm, PDI = 0.22 (C).

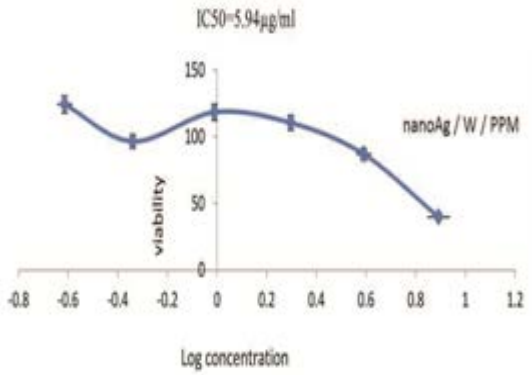
The calcination methods narrated in Iranian traditional manuscripts are not optimal for the fabrication of nanomaterials. However, the milling, sieving, and high-temperature calcination method appears to be valuable.

3.2. Cell Viability and MTT Test Results

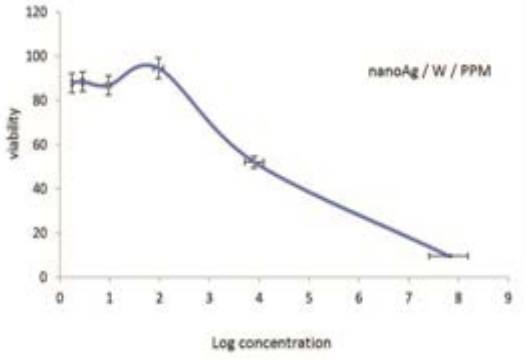
Cytotoxic effects of AgNpsand silver Kushtas on three human cancer cell lines and one normal cell line (MDBK) were assessed in different concentrations of the materialsby the decrease in mitochondrial activity using the MTT assay. Based on this results, the highest anticancer activity revealed inAgNpsagainst HepG2 (1.41 µg/mL), MCF-7 (3.68 µg/mL) and A549 (5.94 µg/mL), respectively.This results indicated that the IC_{50} for A549 and MCF-7 were 3.12 and 1.93-fold higher than that for MDBK (1.90 µg/mL), respectively. Our findings from the MTT assay provide



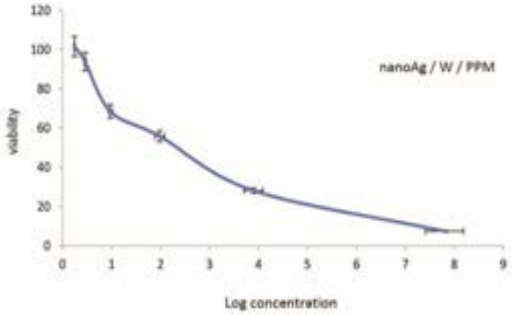
A: HepG2; IC50 = 1.41 µg/ml



B: A549; IC50 = 5.94 µg/ml



C: MCF-7; IC_{50} = 3.68 µg/ml



E: MDBK; IC_{50} = 1.90 µg/ml

Figure 2. AgNPs cytotoxicity against normal and cancer cell lines. Concentration response curve drafts using the mentioned Magellan software.

convincing evidence for the cyototoxic effects of AgNps in HepG2 cells at lower concentrations rather than in normal cells. These results suggested that AgNps exerted significant greater inhibitory effect on the liver carcinoma cell line HepG2 than on MDBK, MCF-7, and A549 cells. Notably, the present results show that none of the silver kushtas investigated can penetrate the normal and cancer cells (Figure 3 and 4). This results shows that AgNps are highly toxic to the cancer cells (Figure2).

Together, the present results show that AgNps can penetrate and diffuse into cancer cells, and exert antitumor effects. The toxic effects of Ag NPs could theoretically berelated to the release of free silver ions. However, two recent studies tested the content of free silver ions in AgNp solutions and found low levels of Ag^+ (0–1%). Furthermore, both studies concluded that the toxicity of AgNp exposure could not be explained solely by the presence of Ag+ in the NP solution [31, 32] suggested that AgNps induce higher levels of ROS than Ag^+, and ICP data on the AgNps only show negligible levels of metal contaminants. As a Consequence, it is not clear whether AgNps are inherently toxic or whether they act in a Trojan-horse like mode that enables uptake of

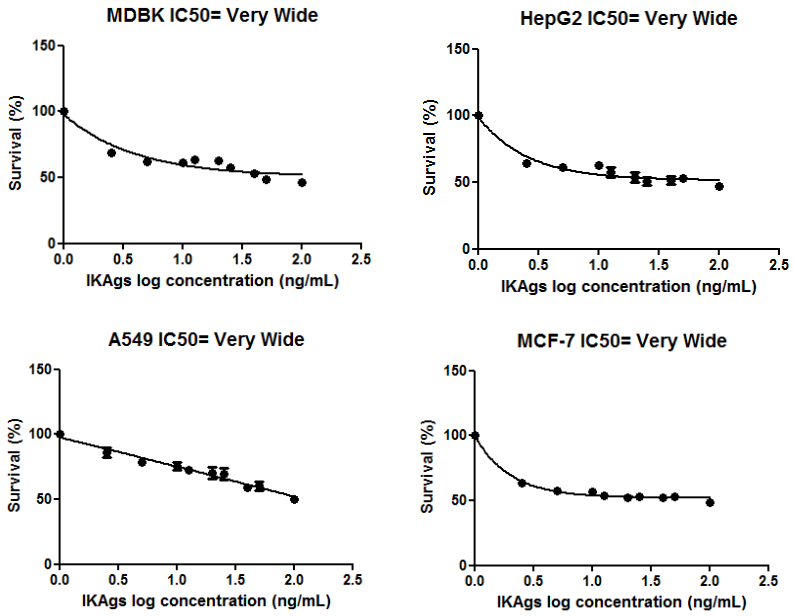


Figure 3. IKAgs cytotoxicity against normal and cancer cell lines. Concentration response curve drafts using PRISM software.

the NPs and subsequent liberation of ions inside the cell [33, 34].

3.3. Cytotoxic Effects of theSilver Materials onPrimary Rat Hepatocytes

The results of the trypan blue (0.2% w/v) exclusion test showed that the cytotoxicity of the silver materials toward primary rat hepatocytes followed the order AgNps (100%)>$AgNO_3$ (80.9%)>Pakistani silver kushta (48.35%)>Iranian silver kushta (45%) (Figure 5). Thus, compared with AgNps, the

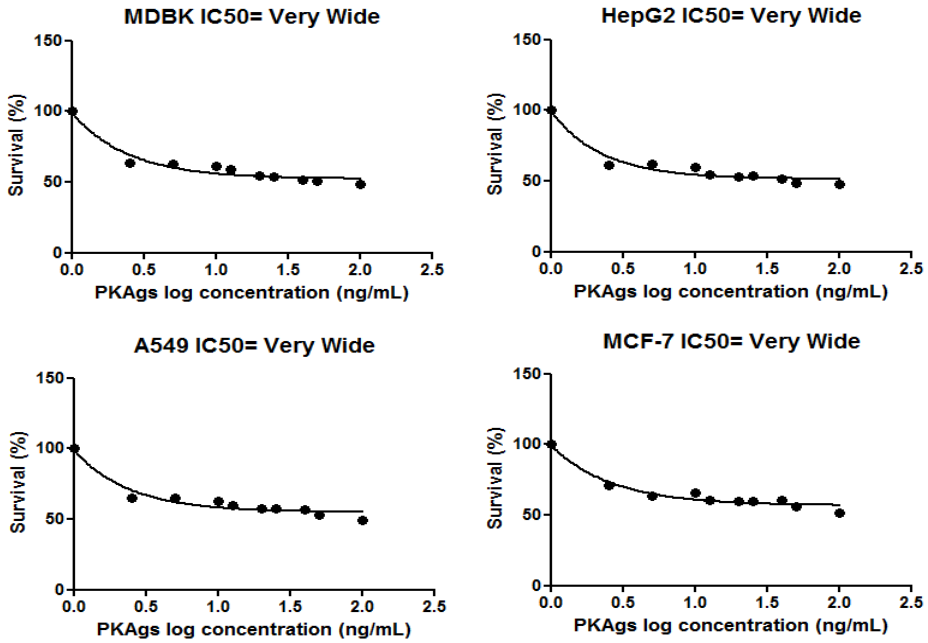


Figure 4. PKAgs cytotoxicity against normal and cancer cell lines. Concentration response curve drafts using PRISM software.

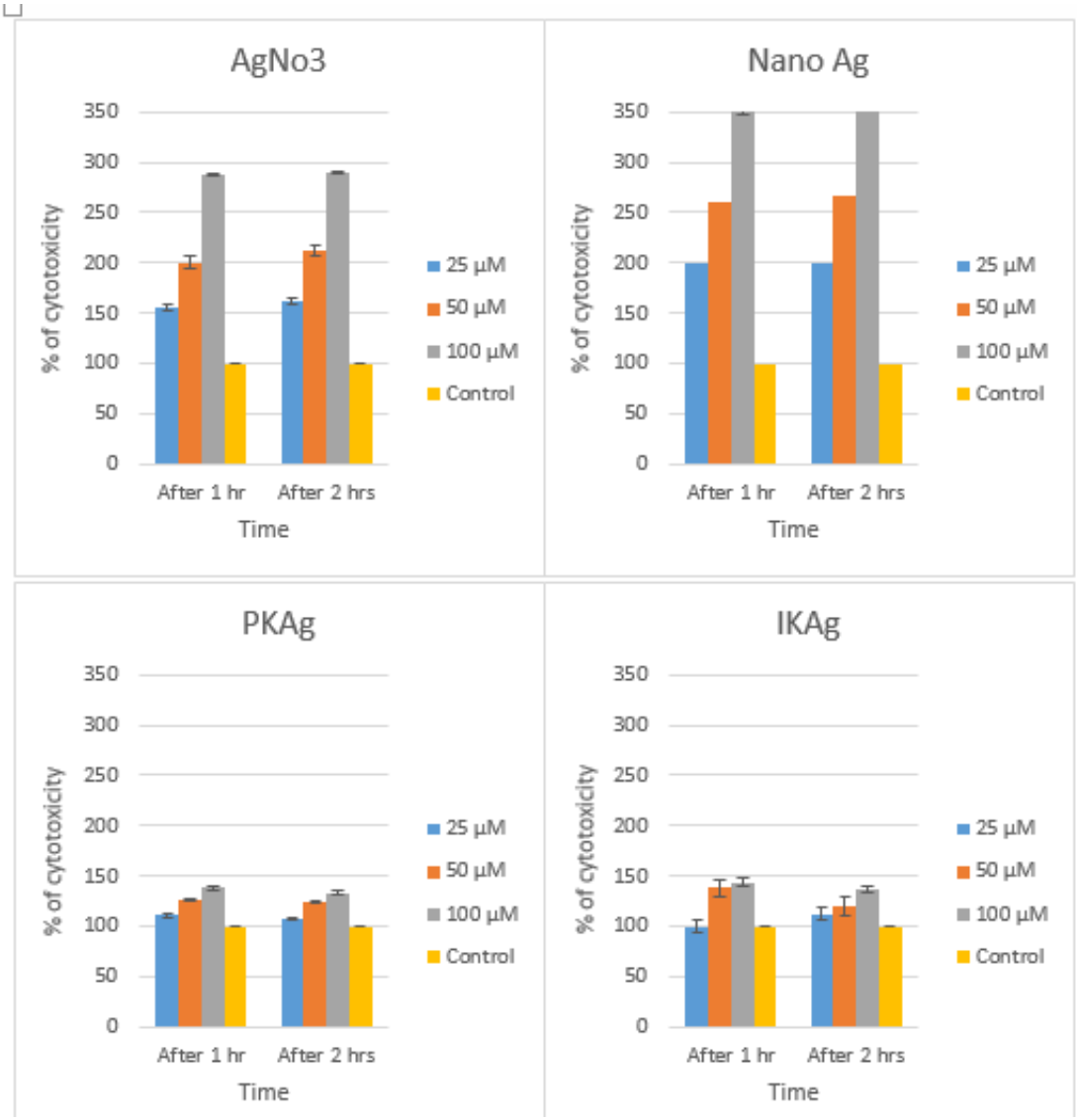


Figure 5. Cytotoxic effects of the Silver Materials on Primary Rat Hepatocytes. The results of the trypan blue (0.2% w/v) exclusion test showed that the cytotoxicity of the silver materials toward primary rat hepatocytes followed the order AgNps (100%)>$AgNO_3$ (80.9%)>Pakistani silver kushta.(48.35%)>Iranian silver kushta (45%).

silver kushtas showed no toxicity toward primary rat hepatocytes. Majority of the particles in kushtas were micro-sized, with a small portion falling in the category of nanoparticles. However, AgNps was significantly toxic to both normal cells and cancer cell lines. The IC_{50} determined for the cancer cell lines were less than 10 ppm.

4. Conclusion

The present results showed that silver kushtas exerted no toxic effects on cancer cell lines. Majority of the particles in kushtas were micro-sized, with a small portion falling in the category of nanoparticles. Additionally, the results of the Trypan Blue test showed that silver kushtas exerted no significant toxic effect on primary rat hepatocytes. However,

AgNps was significantly toxic to both normal cells and cancer cell lines except HT29. The IC_{50}determined for the cancer cell lines were less than 10 ppm.

The development of new zeta sizers and other advanced technologies have enabled a more complete characterization of the physico-chemical properties of the particles. Contrary to the expectations of the Iranian traditional Hakims, silver kushtas prepared using the procedures described in the ancient texts showed no or limited toxicity, likely because the particle size was not optimal. Future studies in this field may help us to find new traditional medicine for cancer treatment.

Acknowledgments

This work was supported by a grant (No.119) from School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran and contains some parts of Ph.D thesis of Alireza Mozaffari Dehshiri in traditional pharmacy.

References

[1]Arora S, Rajwade JM, Paknikar KM: Nanotoxicology and in vitro studies: the need of the hour. *ToxicolAppl Pharm*(2012)258:151-165.

[2]Avicenna. The Canon of Medicine. Trans. SharafkandiA. 7th Pub. (2008)6:410.

[3]AghiliAlaviKhorasaniShirazi SMH. Makhzanul-Advia [In Persian]. The Institue of Medical history, Islamic and complementary medicine studies: Tehran (2008):439.

[4]MomenTonekaboni M. Tohfat al Momenin.Rahimi, R., (Eds.) MoaseseNashreShahr. Tehran (2008): 279.

[5] Galabuzi C, Agea J, Fungo B, Kamoga R. Traditional medicine as an alternative form of health care system: a preliminary case study of Nangabo sub-county, central Uganda. *Afr J Tradit Complement Altern Med* (2010): 7.

[6] Kayne S. Introduction to traditional medicine. *Traditional medicine: A global perspective* (2010):1-24.

[7] Bodeker G: Lessons on integration from the developing world's experience. *BMJ: British Medical Journal* (2001)322:164.

[8] Keen R, Deacon A, Delves H, Moreton J, Frost P. Indian herbal remedies for diabetes as a cause of lead poisoning. *Postgrad Med J* (1994)70:113-114.

[9] Vohora SB, Athar M.Mineral drugs used in Ayurveda and Unani medicine.*Narosa Publishing House* (2008).

[10] Avicenna. The Canon of Medicine. Trans. SharafkandiA. 7th Pub. (2008)5:89, 265.

[11] Avicenna. The Canon of Medicine. Trans. SharafkandiA. 7th Pub. (2008)6:408, 409, 386, 457.

[12] Avicenna. The Canon of Medicine. Trans. SharafkandiA. 7th Pub. (2008)7:175, 185, 249, 279, 287.

[13] Avicenna. The Canon of Medicine. Trans. SharafkandiA. 7th Pub. (2008)2:273, 294.

[14] Hakim Abdolvahid and Hakim Sediqi.A survey of Drug, India (1985):7-18.

[15] Abobakr Mohammad IbnZakaria.A brief of Alhavi.Rev. Tabatabaei SM, Mashhad University of Medial Sciences Pub.(2011)3:236, 273, 277, 287.

[16] Glazier A. A landmark in the history of Ayurveda. *Lancet*(2000)356:1119.

[17] Kew J, Morris C, Aihie A, Fysh R, Jones S, Brooks D. Arsenic and mercury intoxication due to Indian ethnic remedies. *Bmj* (1993)306:506-507.

[18] Keen R, Deacon A, Delves H, Moreton J, Frost P. Indian herbal remedies for diabetes as a cause of lead poisoning. *Postgrad Med J* (1994)70:113-114.

[19] Aslam M, Davis S, Healy M. Heavy metals in some Asian medicines and cosmetics. *Public Health* (1979)93:274-284.

[20] Saper RB, Phillips RS, Sehgal A, Khouri N,

Davis RB, Paquin J, Thuppil V, Kales SN. Lead, mercury, and arsenic in US-and Indian-manufactured Ayurvedic medicines sold via the Internet. *Jama* (2008)300:915-923.

[21] ChristopherGB,BrownL, Whitehouse MW, Agrawal DS, TupeSG, PaknikarKM,TiekinkERT. Nanogold pharmaceutics the use of colloidal gold to treat experimentally-induced arthritis in rat models; Characterization of the gold in SwarnaBhasma, a microparticulate used in traditional Indian medicine.*Gold Bulletin*(2007)40:6.

[22] Kreuter J. Nanoparticles-a historical perspective. *Int J Pharm*(2007)331:1-10.

[23] Chaloupka K, Malam Y, Seifalian AM. Nanosilver as a new generation of nanoproduct in biomedical applications. *Trends Biotechnol* (2010)28:580-588.

[24] Arora S, Jain J, Rajwade J, Paknikar K. Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. *Toxicol Appl Pharm* (2009)236:310-318.

[25] Chen X, Schluesener H. Nanosilver: a nanoproduct in medical application. *ToxicolLett*(2008)176:1-12.

[26] Nowrouzi et al. Cytotoxicity of subtoxic AgNpin human hepatoma cell line (HepG2) after long-term exposure. *Iran Biomed J*(2010)14:23-32.

[27] Sriram MI, Kanth SBM, Kalishwaralal K, Gurunathan S. Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model. *Int J Nanomedicine*(2010)5:753.

[28] Guzmán MG, Dille J, Godet S. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. *Int J Chem Biomol Eng (*2009)2:104-111.

[29] Hosseini M-J, Seyedrazi N, Shahraki J, Pourahmad J. Vanadium induces liver toxicity through reductive activation by glutathione and mitochondrial dysfunction. *AdvBiosciBiotechnol* (2012)3(8):1096-1103.

[30] Auffan M, Rose J, Bottero JY, Lowry GV, Jolivet JP, Wiesner MR. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nat Nanotechnol* (2009)4(10):634–641.

[31] Kim S, Choi JE, Choi J, Chung K-H, Park K, Yi J, Ryu D-Y. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. *Toxicol In vitro* (2009)23:1076-1084.

[32] Navarro et al. Toxicity of silver nanoparticles to Chlamydomonas reinhardtii. *Environ Sci Technol* (2008)42:8959-8964.

[33] Foldbjerg R, Dang DA, Autrup H. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. *Arch Toxicol* (2011)85:743-750.

[34] Limbach LK, Wick P, Manser P, Grass RN, Bruinink A, Stark WJ. Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress. *Environ SciTechnol* (2007)41:4158-4163.