Study of Silymarin and Vitamin E Protective Effects on Silver Nanoparticle Toxicity on Mice Liver Primary Cell Culture

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Abstract- Nanotechnology is a most promising field for generating new applications in medicine, although, only few nano products are currently in use for medical purposes. A most prominent nanoproduct is nanosilver. Nano-silver has biological properties which are significant for consumer products, food technology, textiles, and medical applications (e.g. wound care products, implantable medical devices, in diagnosis, drug delivery, and imaging). For their antibacterial activity, silver nanoparticles (Ag NPs) are largely used in various commercially available products. The use of nano-silver is becoming more and more widespread in medicine and related applications, and due to its increasing exposure, toxicological and environmental issues need to be raised. Cytotoxicity induced by silver nanoparticles (AgNPs) and the role that oxidative stress plays in this process were demonstrated in human hepatoma cells AgNPs agglomerated in the cytoplasm and nuclei of treated cells, and they induced intracellular oxidative stress. AgNP reduced ATP content of the cell and caused damage to mitochondria and increased production of reactive oxygen species (ROS) in a dose-dependent manner. Silymarin was known as a hepatoprotective agent that is used in the treatment of hepatic diseases including viral hepatitis, alcoholic liver diseases, Amanita mushroom poisoning, liver cirrhosis, toxic and drug-induced liver diseases. It promotes protein synthesis, helps in regenerating liver tissue, controls inflammation, enhances glucuronidation, and protects against glutathione depletion. Vitamin E is a well-known antioxidant and has hepatoprotective effect in liver diseases. In this study, we investigated the cytotoxic effects of Ag NPs on primary liver cells of mice. Cell viability (cytotoxicity) was examined with MTT assay after primary liver cells of mice exposure to AgNPs at 1, 10, 50, 100, 150, 200, 400 ppm for 24h. AgNPs caused a concentration- dependent decrease of cell viability (IC50 value = 121.7 ppm or µg/ml). Then the hepatoprotective effect of silymarin and vitamin E were experimented on silver nanoparticle toxicity on mice liver primary cell culture. The results showed that silymarin at 600µg/ml and vitamin E at 2500µmol/l have protective effects on silver nanoparticle toxicity on mice liver primary cell culture. Viability percentage of the primary liver cell of the mouse were exposed to silver nanoparticles at 121.7ppm and co-treatment of silymarin, and vitamin E is more than viability percentage of the primary liver cell of the mouse were exposed to silver nanoparticles and silymarin or silver nanoparticles and vitamin E.

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Keywords: Silver nanoparticle; Silymarin; Vitamin E; MTT assay

Introduction

Nanotechnology and nanoparticles have great potential applications in medical healthcare and consumer products, aerospace engineering, nano-electronics, and environmental remediation (1-3).

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Nanoparticles are structures that have one dimension in the 1–100 nm range (4,5).

Nanosilver is one of the nano-materials most commonly used in consumer products (6). Surgical instruments, wound dressings, contraceptive devices, bandages, and bone prostheses all coated or embedded with nanosilver (7,8). Silver nanoparticles have been used in textiles for the manufacture of clothing, underwear, and socks (8,10,11,16). Other uses of Silver nanoparticles are in detergent, antibacterial sprays, respirators, household water filters, cosmetics, dietary supplements, cutting boards, shoes, cell phones, laptop, keyboards, and children's toys so the use of silver nanoparticles because of antimicrobial properties of silver nanomaterials is going to widespread in world (3,8,12,13,14).

More importantly is the potential for the application of Ag NP in the treatment of diseases that require maintenance of circulating drug concentration or targeting of specific cells or organs (3). For example, Ag NPs have been shown to interact with the HIV-1 virus and inhibit its ability to bind host cells in vitro (3). Therefore, exposure to nanosilver in the body is becoming increasingly widespread and intimate. So, silver in the form of nanoparticles has gained increasing access to tissues, cells, and biological molecules within the human body.

Their small size, high surface area per unit mass, chemical composition, and surface property effects may be important factors in NP-induced toxicity (18), and nonspecific oxidative damage is one of the greatest concerns (4,9,21).

Despite their widespread application of silver, comprehensive biologic and toxicological information is insufficient.

The cytotoxicity study in human macrophages indicates toxicity of silver nanoparticles (14). Oral toxicity, genotoxicity, and gender-related tissue distribution of AgNPs in the rat were investigated (17). Subchronic inhalation toxicity of AgNPs was also investigated which showed increases in lesions related to silver nanoparticle exposure (19,22). The toxicity of AgNPs has been investigated in some cell types that illustrated toxicity of silver nanoparticles, including BRL3A rat liver cells (4,23), PC-12 neuroendocrine cells (4,24), human alveolar epithelial cells (4,25) and germline stem cells (4,26).

In earlier studies, Takenaka *et al*., (7,15) reported that liver appears to be a major accumulation site of circulatory silver nanoparticles. A recent clinical report also described absorption of nano-silver into the circulation following the use of nano-silver coated dressings for burns (27). In such cases, primary cells isolated from target tissues are desirable for cytotoxicity testing to simulate the in vivo situation more closely. Further, primary cultured liver cells (rodent or human origin) also represent a useful tool for studying toxicity, drug metabolism and enzyme induction (7,28).

Therefore, the materials can use in cases to apply of Ag-nanoparticles on the human body that protect or decrease the toxicity of nanosilver in the body.

Milk thistle or Silybum marianum is a herb that have been used to treatment of hepatic diseases and gallbladder disorders for more than 2000 years (29, 30,31). Seeds of this herb contain silymarin. Silymarin useful for treatment of hepatitis, jaundice and cirrhosis (29,30,32-40,59,69,70). Silymarin has protectant effects against liver poisoning from Amanita phaloides (37,40,48,56,57), Ethanol (29,37,46,55,65,66,68,99), Paracetamol (29,30,36,54), carbontetrachloride (35,52,53,60) galactosamine (61,62,67,71) thioacetamide (45,47,50,51), halothane, (63,64,72) and snake bites (42,43,44,45).

This compound also protects hepatocytes from injury caused by radiation, ischemia, iron overload and viral hepatitis (29,33,36,37,39,40,58,75,80).

Silymarin has strong antioxidative properties and acts as a free radical scavenger reducing free radicals, ROS, and lipid peroxidation in patients with alcoholic cirrhosis (36,37,41,49,60,73,85,86,87,88).

Silymarin enhances levels of glutathione and superoxide dismutase, two primary antioxidants in the liver (35,89,90,91,92).

Vitamin E, which is a well-known antioxidant and cytoprotectant have been used for the treatment of liver damage in viral infections, cirrhosis and Amanita phalloides poisoning (43,74,76-79,84).

In this study, we investigated toxic effects of Ag-NPs on primary hepatocyte of mice that were exposed to Ag-NPs at different doses and for toxicity evaluations, cell viability using with MTT assay were assessed under exposed conditions and IC50 of silver nanoparticles on this cell cultures was calculated. Then, we studied silymarin and vitamin E effects on silver nanoparticle toxicity on mice liver primary cell culture and we calculated hepatoprotection doses of silymarin and vitamin E on silver nanoparticle toxicity on mice liver primary cell culture and was compared effect of only silymarin or only vitamin E in this case and too cotreatment effects of silymarin together with vitamin E were investigated on silver nanoparticles toxicity on mice liver primary cell culture.

Materials and Methods

Nanoparticle preparation

AgNPs (Nanocid L2000) were provided by Nanonasb Pars co. They were a clear colloidal aqueous suspension with a concentration of 4000PPM (or mg/l), and particles size was 20-40Nm. The particles could be stably diluted with distilled water (no color change and no precipitation), and Then, this solution was diluted with deionized water and sterilized with microfilter (0.22 micron).

Silymarin preparation

Silymarin powder was purchased from Sigma Chemical Co. Silymarin powder was dissolved in DMSO then were diluted with complete medium (DMEM/F12).

Vitamin E preparation

Vitamin E were purchased from Sigma Chemical Co. 230mg Vitamin E were diluted to complete medium (DMEM/F12) with 0.5% ethanol 98% that total volume of stock1 reach to 2ml then vitamin E were provided in 0, 50, 250, 500, 1000, 2500, 5000 µmol/l concentrations.

Isolation and culture of primary mouse hepatocytes

Swiss albino mice (7-10 day old) were euthanized with an intraperitoneal injection of ketamine and xylazine, and heparin was injected into them by intraperitoneal route; then mice were sterilized with 70% ethanol. For the isolation of liver cells, the excised liver was minced with a scalpel, and tissue fragments of liver were washed with Hanks buffer. Tissue particles were washed once with PBS(phosphate buffered saline) then tissue fragment of liver was transferred to a solution of Hanks with collagenase (17mg collagenase in 25ml hanks solution) in a flask, incubated and agitated at 37°C in shaker incubator for 2 hours. This solution of the cells was transferred to a 50ml centrifuge tube and then centrifuged for 10 minutes at 500rpm, the supernatant was removed, and the cells were re-suspended in complete medium (DMEM/F12) supplemented with 15% heat-inactivated fetal calf serum and 3% (v/v) penicillin-streptomycin. The viability of the hepatocytes was 95–100% as determined by Trypan Blue exclusion. Freshly isolated liver cells of mice were seeded at a density of 15000 cells/well in 96-well plates. Wells were already coated with 15 microliter poly-D-lysine. Cells were incubated in a humidified incubator at 37◦C containing 5% CO2 and 95% air for 24 hours.

MTT assay

Silver nanoparticles treatment stage

Cell viability was tested using MTT assay which is based on the cleavage of the tetrazolium salt (MTT) by metabolically active cells to form a formazan dye that is water-insoluble. The insoluble dye formed in MTT assay was solubilized using DMSO. The MTT assay was performed according to a modification of the method described by Mosmann (1983). Cells were seeded in 96-well tissue culture plates (15000cell/well in the 100µl culture medium, DMEM/F12) and incubated overnight at 37◦C and 5% CO2, and then morphology of cells was observed in invert microscope before exposure to silver nanoparticles. After overnight growth, supernatants in the culture plates were aspirated out and then 10µl silver nanoparticles solutions that diluted with deionized water were added in concentrations 1, 10, 50, 100, 150, 200, 400ppm to primary liver cells of mice in each well of 96-well tissue culture plates. To grow cells, 90µl of culture medium (DMEM/F12) to each well was added. Treated cells were incubated for 24 hours at 37◦C and 5% CO2. Morphology of cells was observed in invert microscope after exposure to different concentrations of silver nanoparticles. After overnight, supernatants were aspirated out and 10µl MTT solution (50mg/10ml PBS) was added to each well then 90µl of culture medium (DMEM/F12) to each well was added and the plates were incubated for 6h in primary liver cells of mice then supernatants were replaced by100µl DMSO and plates were shaken at 37◦C in shaker incubator for 15 minutes then absorbance at two wavelengths (570 nm and 650 nm) was recorded using ELISA reader. All absorbance values were corrected against blank wells which contained growth media alone. Each assay involved six wells per condition. After that, the IC50 of silver nanoparticles was calculated in primary liver cells of mice.

Silver nano-particles and silymarin treatment stage

Cells were seeded in 96-well tissue culture plates (15000cell/well in the 100µl culture medium, DMEM/F12) and incubated overnight at 37◦C and 5% CO2. After overnight growth, supernatants in the culture plates were aspirated out and then were added 10µl silver nanoparticles solution at 121.7 µg/ml (IC50 value of silver nanoparticles was calculated in primary liver cells of mice) and silymarin in concentrations 0, 50, 100, 200, 400, 600, 720 µg/ml to primary liver cells of mice in each well of 96-well tissue culture plates then culture medium (DMEM/F12) to each well was added that

volume of each well reaches to 100 µl . Treated cells were incubated for 24 hours at 37◦C and 5% CO2. After overnight, following procedures of MTT assay were performed as in above were described.

Silver nanoparticles and vitamin E treatment stage

Cells were seeded in 96-well tissue culture plates (15000cell/well in the 100µl culture medium, DMEM/F12) and incubated overnight at 37◦C and 5% CO2. After overnight growth, supernatants in the culture plates were aspirated out and then were added 10µl silver nanoparticles solution at 121.7 µg/ml (IC50 value of silver nanoparticles was calculated in primary liver cells of mice) and vitamin E in concentrations 0,50,250,500,1000,2500,5000 µmol/l to primary liver cells of mice in each well of 96-well tissue culture plates then culture medium (DMEM/F12) to each well was added that volume of each well reaches to 100 µl. Treated cells incubated for 24 hours at 37◦C and 5% CO2. After overnight, following procedures of MTT assay were performed as in above were described.

Silver nanoparticles and silymarin and vitamin E treatment stage

Cells were seeded in 96-well tissue culture plates (15000cell/well in the 100µl culture medium, DMEM/F12) and incubated overnight at 37°C and 5% CO2. After overnight growth, supernatants in the culture plates were aspirated out and then were added 10µl silver nano-particles solution at 121.7 µg/ml (IC50 value of silver nanoparticles was calculated in primary liver cells of mice) and silymarin in concentrations 0,50,100,200,400,600,720 µg/ml and vitamin E in concentrations 0, 50, 250, 500, 1000, 2500, 5000 µmol/l to primary liver cells of mice in each well of 96-well tissue culture plates then culture medium (DMEM/F12) to each well was added that volume of each well reaches to 100 µl . Treated cells incubated for 24 hours at 37◦C and 5% CO2. After overnight, following procedures of MTT assay were performed as in above were described.

Statistical analysis

All the experiments were carried out three times, independently. The data obtained were expressed in terms of 'mean ± standard deviation' values. Wherever appropriate, the data were also subjected to one-way ANOVA using with software GraphPad Prism 5. A value of $P<0.0001$was considered as significant.

Results

The result of MTT assays showed a dose-dependent decrease in viability percentage of primary liver cells of mice after 24h exposure to AgNPs (Figure1).

Viability percentage was measured by MTT assay on a primary liver cell of mice exposed to 0,1,10,50,100,150,200, 400ppm of AgNPs for 24h represents a dose-response pattern as is shown in Figure 2. Data were reported as mean ± SD of three independent experiments performed in quadruplicate. Using the dose-response curves, IC50 was calculated to be 121.7µg/ml (ppm) ($P< 0.0001$).

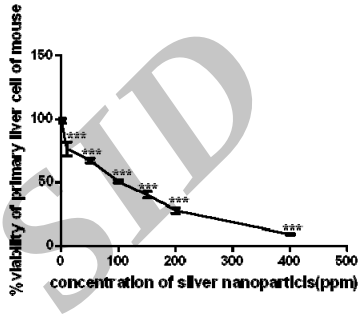

Figure 1. Viability percentage measured by MTT assay on a primary liver cell of mice exposed to 0, 1, 10, 50, 100, 150, 200, 400ppm of AgNPs for 24h. An OD value of control cells (unexposed cells) was taken as 100% viability (0% cytotoxicity). Data were reported as mean ± SD of three independent experiments performed in quadruplicate. The relative cell viability related to control was calculated by [OD] test/ [OD] control ×100. Using the dose-response curves, IC50 was calculated to be 121.7µg/ml (ppm).(*** $P < 0.0001$).

Viability percentage measured by MTT assay on a primary liver cell of mice exposed to 0,50,100,200,400,600,720 µg/ml of silymarin and 121.7µg/ml (ppm) of AgNPs for 24h is shown in Figure 2. An OD value of control cells (unexposed cells) was taken as 100% viability (0% cytotoxicity). Data were reported as mean ± SD of three independent experiments performed in quadruplicate. The relative cell viability related to control was calculated by [OD] test/ [OD] control ×100. Using the dose-response curves, a protective dose of silymarin on silver nanoparticle toxicity on mice liver primary cell culture at 121.7µg/ml (ppm) was calculated to be 600µg/ml. (*** $P < 0.0001$, ** P 0.0001 to 0.01).

Viability percentage measured by MTT assay on primary liver cells of mice exposed to vitamin E in concentrations 0,50,250,500,1000,2500,5000 µmol/l and 121.7µg/ml (ppm) of AgNPs for 24h is shown in Figure 3. An OD value of control cells (unexposed cells) was taken as 100% viability (0% cytotoxicity). Data

were reported as mean ± SD of three independent experiments performed in quadruplicate. The relative cell viability related to control was calculated by [OD] test/ [OD] control ×100. Using the dose-response curves, a protective dose of vitamin E on silver nanoparticle toxicity on mice liver primary cell culture at 121.7µg/ml (ppm) was calculated to be 2500 µmol/l (***P<0.0001).

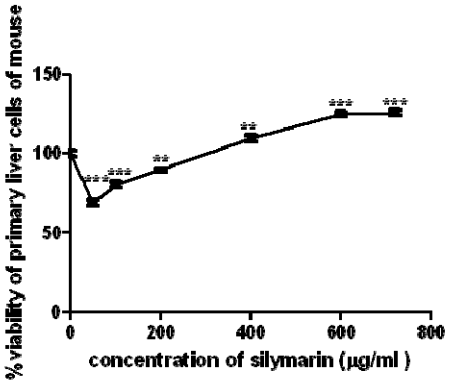

Figure 2. Viability percentage measured by MTT assay on a primary liver cell of mice exposed to 0, 50, 100, 200, 400, 600, 720 µg/ml of silymarin and 121.7µg/ml (ppm) of AgNPs for 24h. An OD value of control cells (unexposed cells) was taken as 100% viability (0% cytotoxicity). Data were reported as mean ± SD of three independent experiments performed in quadruplicate. The relative cell viability related to control was calculated by [OD] test/ [OD] control ×100. Using the dose-response curves, a protective dose of silymarin on silver nanoparticle toxicity on mice liver primary cell culture was calculated to be 600µg/m. (*** P < 0.0001, ** P 0.0001 to 0.01).

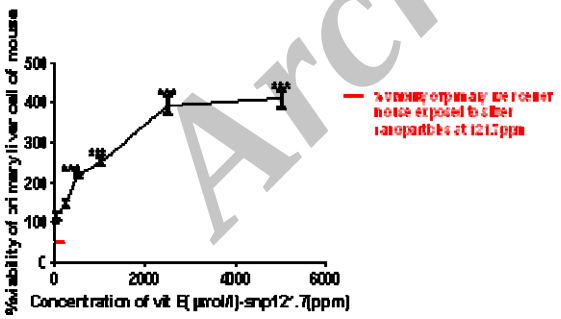

Figure 3. Viability percentage measured by MTT assay on a primary liver cell of mice exposed to vitamin E in concentrations 0, 50, 250, 500, 1000, 2500, 5000 µmol/l and 121.7µg/ml (ppm) of AgNPs for 24h. An OD value of control cells (unexposed cells) was taken as 100% viability (0% cytotoxicity). Data were reported as mean ± SD of three independent experiments performed in quadruplicate. The relative cell viability related to control was calculated by [OD] test/ [OD] control ×100. Using the dose-response curves, a protective dose of vitamin E on silver nanoparticle toxicity on mice liver primary cell culture at 121.7µg/ml (ppm) was calculated to be 2500 µmol/l (*** p<0.0001).

Viability percentage measured by MTT assay on a primary liver cell of mice exposed to vitamin E in concentrations 0,50,250,500,1000,2500,5000 µmol/l, silymarin at 600 µg/ml and 121.7µg/ml (ppm) of AgNPs for 24h is shown in Figure 4. An OD value of control cells (unexposed cells) was taken as 100% viability (0% cytotoxicity). Data were reported as mean ± SD of three independent experiments performed in quadruplicate. The relative cell viability related to control was calculated by [OD] test/ [OD] control ×100. Using the dose-response curves, a protective dose of vitamin E on silver nanoparticle toxicity on mice liver primary cell culture while silymarin at 600 µg/ml were used together with vitamin E to reduce silver nanoparticle toxicity at 121.7µg/ml (ppm) was calculated to be 2500µmol/l (*** P<0.0001, ** P 0.0001 to 0.01).

Figure 5 represents viability percentage measured by MTT assay on primary liver cell of mice exposed to vitamin E in concentrations 0, 50, 250, 500, 1000, 2500, 5000 µmol/l , silymarin at 600 µg/ml and 121.7µg/ml (ppm) of AgNPs for 24h(above curve) and viability percentage measured by MTT assay on primary liver cell of mice exposed to vitamin E in concentrations 0,50,250,500,1000,2500,5000 µmol/l and 121.7µg/ml (ppm) of AgNPs for 24h (below curve). An OD value of control cells (unexposed cells) was taken as 100% viability (0% cytotoxicity).

Data were reported as mean ± SD of three independent experiments performed in quadruplicate. The relative cell viability related to control was calculated by [OD] test/ [OD] control ×100. Using the dose-response curves, a protective dose of vitamin E on silver nanoparticle toxicity on mice liver primary cell culture while silymarin at 600 µg/ml were used with vitamin E to reduce silver nanoparticle toxicity at 121.7µg/ml (ppm) was calculated to be 2500µmol/l. Viability percentage in above curve is more than below curve (*** P < 0.0001, ** P 0.0001 to 0.01).

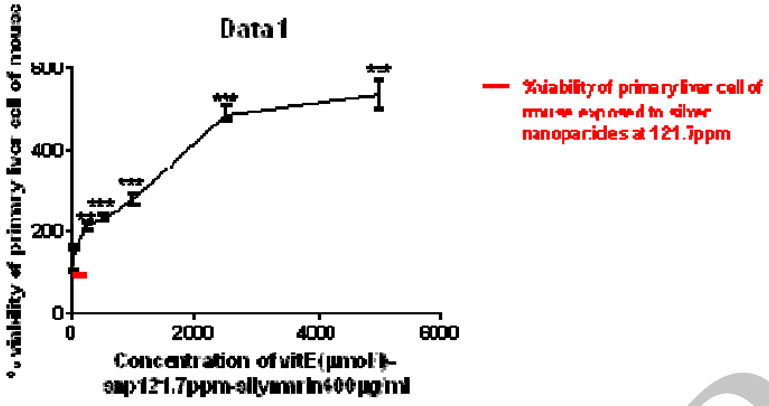

Figure 4.Viability percentage measured by MTT assay on a primary liver cell of mice exposed to vitamin E in concentrations 0, 50, 250, 500, 1000, 2500, 5000 µmol/l, silymarin at 600 µg/ml and 121.7µg/ml (ppm) of AgNPs for 24h. An OD value of control cells (unexposed cells) was taken as 100% viability (0% cytotoxicity). Data were reported as mean ± SD of three independent experiments performed in quadruplicate. The relative cell viability related to control was calculated by [OD] test/ [OD] control ×100. Using the dose-response curves, protective dose of vitamin E on silver nanoparticle toxicity on mice liver primary cell culture while silymarin at 600 µg/ml were used with vitamin E to reduce silver nanoparticle toxicity at 121.7µg/ml (ppm)) was calculated to be 2500µmol/l (*** $P < 0.0001$, ** P 0.0001 to 0.01).

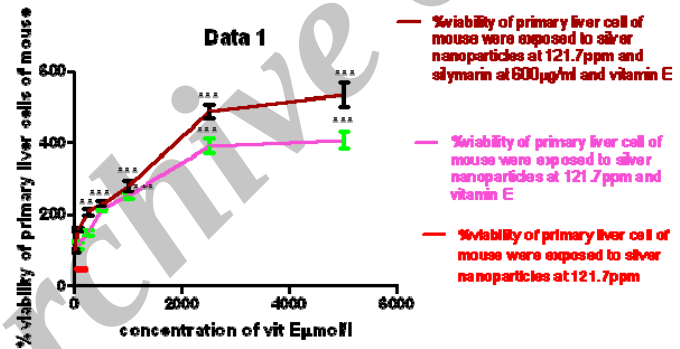

Figure 5. Viability percentage measured by MTT assay on primary liver cell of mice exposed to vitamin E in concentrations 0, 50, 250, 500, 1000, 2500, 5000 µmol/l , silymarin at 600 µg/ml and 121.7µg/ml (ppm) of AgNPs for 24h (above curve) and viability percentage measured by MTT assay on primary liver cell of mice exposed to vitamin E in concentrations 0, 50, 250, 500, 1000, 2500, 5000 µmol/l and 121.7µg/ml (ppm) of AgNPs for 24h (below curve). An OD value of control cells (unexposed cells) was taken as 100% viability (0% cytotoxicity). Data were reported as mean ± SD of three independent experiments performed in quadruplicate. The relative cell viability related to control was calculated by [OD] test/ [OD] control ×100. Using the dose-response curves, a protective dose of vitamin E on silver nanoparticle toxicity on mice liver primary cell culture while silymarin at 600 µg/ml were used together with vitamin E to reduce silver nanoparticle toxicity at 121.7µg/ml (ppm) was calculated to be 2500µmol/l. Viability percentage in above curve is more than below curve (***$P < 0.0001$, ** P 0.0001 to 0.01).

Effects of Ag NPs on cellular morphology

Figure 1 represents morphological changes of primary liver cells of mice exposed to AgNPs at 121.7 ppm for 24h. Changes in cell morphology were examined using inverted microscope. Compared to control cell morphology significant morphological changes of cell death, including cell shrinkage, restricted spreading patterns, and increased floating cells were observed in primary liver cells of mice exposed to AgNPs at 121.7 ppm.

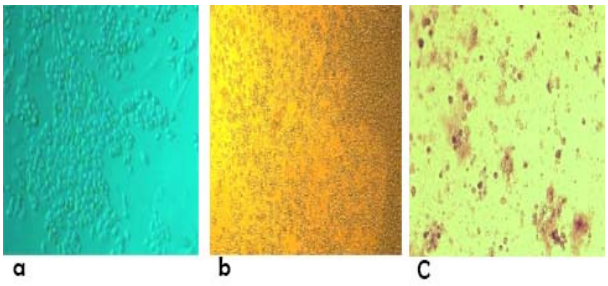

Figure 1. Morphological characterization of primary liver cells of mice: a: untreated primary liver cells of mice, b: untreated primary liver cells of mice with high confluency, c: treated primary liver cells of mice at121.7 ppm of AgNPs for 24h.

Discussion

Silver nanoparticles have great applications in medicine. Antibacterial activity is one of the important uses of silver nanoparticles. The present study indicated that silver nanoparticles have toxicity effects on primary cell culture of mice as shown in Figure 1 and can decrease the toxicity of silver nanoparticles in primary cell culture of mice by use silymarin and vitamin E as shown in Figures 2,3,4,5. Toxicity effects of silver nanoparticles already by another works have been illustrated in different cells, including: In vitro exposure of human peripheral blood mononuclear cells (PBMCs) to silver nanoparticles (1-2.5 nm, 72 h) resulted in inhibition of phytohemagglutinin (PHA) induced proliferation (at a concentration 15 ppm) (93). Hussain *et al*., (23) evaluated the in vitro toxicity of several nanoparticles, including nano-silver (15 and 100 nm) on a rat liver derived cell line (BRL 3A). Following 24h after exposure the mitochondrial function and membrane integrity (measured as LDH leakage) were significantly decreased (at 5 mg/ml and 10 mg/ml, respectively). LDH leakage was dose dependent and more severe for 100 nm than for 15 nm silver nanoparticles. The observed cytotoxicity was attributed to be mediated by oxidative stress, as indicated by the detection of GSH depletion, reduced mitochondrial potential, and increased reactive oxygen species (ROS) levels. A similar concentration-dependent cytotoxicity was observed when the effects of the same nano-silver particles on a mouse cell line with spermatogonial stem cell characteristics were studied (26). In HaCaT cells, 24h exposure to AgNPs caused reduction of mitochondrial function, as measured by the MTT assay, at the highest concentrations tested (11 and 36µg/ml). Similar results have been reported investigating the effect of AgNPs on different cell lines, such as human (0.5–3 µg/ml) and rat (10–50 µg/ml) liver cells, alveolar macrophages (10–75 µg/ml), mice dermal fibroblasts and liver cells (30 µg/ml) or mouse germline stem cells (10µg/ml) (4,7,23,26,94).

Silver nanoparticles were absorbed by the gastrointestinal tract; they will be transported to the liver by the portal vein. In general, the liver is able to actively remove compounds from the blood and transform them into chemical forms that can easily be excreted.

Silymarin was shown as a hepatoprotective compound. Cytoprotective effect of silymarin was observed in various studies (29,30,32,33,37,40,42,,44,46 ,49,57,59,61,69,70,75,80).

Hepatoprotective properties of silymarin: Antioxidant properties; silymarin has antioxidant effect, inhibition of lipid peroxidation, regeneration of intracellular gluthation content, (29,30,35,36,38,39,54,95,96,97), free radical scavenging including ROS(reactive oxygen species), O2- and H2O2, increasing of superoxide dismutase activity in liver cells (29,30,35,98,99,100).

Protein synthesis-inducing properties; silymarin increase production and synthesis of proteins in liver cells by inducing of activation of RNA polymerase I in the nuclei of hepatocytes, thus increase the synthesis rate of rRNA and stimulates liver regeneration (29,30,35,101).

Membrane-stabilizing properties; silymarin stabilizes cellular membranes and regulates membrane permeability that inhibits from the entrance of toxins into hepatocytes (29,30,35,82).

Antifibrogenic properties; silymarin inhibits fibrogenesis in liver by inhibition of stellate cell

Proliferation and its further transformation into myofibroblasts (29,30,35).

Vitamin E has cytoprotectant and antioxidative properties; scavenger of free radicals in the treatment of

liver damage from Amanita phalloides poisoning, viral infections, and cirrhosis (43,74,76,77,78,79,84).

The results of this study in Figure1. demonstrated that silver nanoparticle is a toxic agent at high doses (IC50=121.7µg/ml) on a primary liver cell of mice. The results are shown in Figures 2,3,4,5.

Table 1. Viability percentage measured by MTT assay on primary liver cell of mice exposed to vitamin E in concentrations 0, 50, 250, 500, 1000, 2500, 5000 µmol/l , silymarin at 600 µg/ml and 121.7µg/ml (ppm) of AgNPs for 24h and viability percentage measured by MTT assay on primary liver cell of mice exposed to vitamin E in concentrations 0, 50, 250, 500, 1000, 2500, 5000 µmol/l and 121.7µg/ml (ppm) of AgNPs for 24h.

Concentration of vitamin E (µmol/l)	0	50	250	500	1000	2500	5000
viability percentage on a primary liver cell of mice exposed to Vitamin E-silver nanoparticles	100	115.673	149.517	216.698	253.890	392.297	408.730
viability percentage on primary liver cell of mice exposed to VitaminE-silymarin-silver nanoparticles	100	158.022	207.565	230.272	279.735	489.448	535.392

Illustrated that silymarin and vitamin E increase viability percentage of primary liver cells of mice exposed to silver nanoparticles and they inhibit mortality of these cells.

viability percentage in primary liver cells of mice that treated to silymarin and vitamin E together is more than viability percentage in primary liver cells of mice that treated to only silymarin or only vitamin E as shown in Table1; therefore, use of only silymarin or only vitamin E or silymarin together with vitamin E while use of nano-silver on the body can decrease toxicity of silver nanoparticles and they can be hepatoprotectant agents against toxicity of silver nanoparticles in the body.

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