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Study of the antioxidant capacity of silver nanoparticles synthesized from *Laurencia caspica* seaweed on liver cancer cell line (HepG2)

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Abstract

Hepatocele carcinoma is a type of liver cancer that occurs most often after a person has been infected with viral hepatitis B and C or cirrhosis, which is most often due to alcohol consumption. Objective and importance of the research: Today, finding natural compounds from marine organisms, especially algae, as well as nanoparticles that have antibacterial, antiviral and anticancer effects is of particular importance around the world. The aim of this study was to investigate the antioxidant capacity of silver nanoparticles synthesized with *Laurencia caspica* seaweed on liver cancer cell line (HepG2). In this experimental (interventional) study, HepG2 cell lines were cultured in RPMI1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 110 u/m penicillin, 100 µg/ml, 0.1 mM non-essential amino acids and 1 mM sodium pyruvate. After collecting *L. caspica* samples from the southern coast of the Caspian Sea, the hydroalcoholic extract of the algae was obtained by percolation using 50% methanol solvent. The cells were exposed to silver nanoparticles and *L. caspica* algae extract at 24-hour intervals. This study showed that the combination of silver nanoparticles in *L. caspica* algal extract has a significant effect on the reduction of liver cancer cell line (HepG2). With increasing concentrations of the combination of silver nanoparticles with the extract, significant anticancer effects were observed in the studied combination.

Key words: Antioxidant, Silver nanoparticles, Algae extract, *Laurencia caspica*, Liver

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1. Introduction

The rapid development of nanoscience and nanotechnology in recent years has opened new horizons for many industries and sectors (Rather et al., 2011). The advancement of nanotechnology and the wide applications of nanoparticles in various industries have made it important to investigate the destructive effects of nanomaterials on organisms (Chambers et al., 1962). Among metal pollutants, silver ion is highly toxic, and its toxicity to a wide range of microorganisms as well as its low toxicity to humans have led to the development of a large number of silver-based products (Boenigk et al., 2014). Silver ion is widely used in healthcare to control microorganisms and, mainly, due to its physical and chemical properties, it is widely used in pharmaceutical and health applications (Gong et al., 2007). Silver nitrate is known as a generator of reactive oxygen species and damages cells through various mechanisms, including interaction with sulfhydryl groups of proteins and enzymes (Terbali, 2012). Silver nanoparticles disrupt the metabolism, proliferation, and respiration of microorganisms by producing reactive radicals such as oxygen and hydroxide ions, destroying organic structures, and strongly interacting with proteins and enzymes of the electron transport system, and are capable of killing 99.9% of more than 650 types of gram-positive and gram-negative bacteria resistant to common antibiotics in laboratory conditions (Mahdavi-Rad et al., 2014). The electrostatic interaction of nanoparticles has caused their attraction to target cells. Positively charged nanoparticles are attracted to cancer cells that have a high percentage of anionic phospholipids and certain groups of charged proteins and carbohydrates on their outer surface (Wang, 2004, Weisheng et al., 2009). So far, numerous biological compounds with a diverse range of applications such as antibiotic, antiviral, antifungal and anticancer effects have been identified and extracted from multicellular algae, and many of the primary or secondary metabolites of these organisms can be converted into bioactive substances used in the pharmaceutical industry (Tuney et al., 2006). Natural products of seaweeds have the potential to be used in anticancer drugs and anti-inflammatory drugs (Lee et al., 2013).

Hepatocellular carcinoma (HCC) is a type of liver cancer that occurs most often after a person has contracted viral hepatitis B and C or cirrhosis, which is most often due to alcohol consumption (Kumar et al, 2003). Like other cancers, hepatocellular carcinoma occurs when a mutation in the cellular machinery causes excessive cell proliferation or prevents cells from undergoing apoptosis. In the case of hepatitis B and C, the main cause of HCC is the attack of the person's own immune system on liver cells that are infected with the virus (Afshari et al, 2010). Today, finding natural compounds from marine organisms, especially algae, that have antibacterial, antiviral, and anticancer effects is of particular importance worldwide (Bahramian, 2009). Among these algae, one can mention the red algae branch, which have many practical uses (Sharma, 1986). Members of this group are multicellular and most of their cells have more than one nucleus. This group usually reproduces sexually (Kianmehr Diyar, 1992). Given the abundance of this species in some coastal areas of the Caspian Sea, the use of this algae along with anticancer compounds of silver nanoparticles on the liver cancer cell line (HepG2) is significant. In Iran, so far, studies have been conducted by Ghandhari et al. (2018) to investigate the antioxidant effects and toxicity of silver nanoparticles synthesized by a green method using an aqueous extract of the Rhubarb plant on liver cancer cells (HepG2) in comparison with normal dermal fibroblast (HDF) cells, Shali et al. (2018) to investigate the cytotoxic effects and antioxidant properties of silver nanoparticles synthesized by a green method from the root of the Anjbar plant on liver cancer cells (HepG2).

2.Materials and Methods

After collecting *L. caspica* samples from the southern coast of the Caspian Sea, the samples were transferred to the laboratory. There, after washing with distilled water, they were placed in the shade for a week to dry (Singaravelu et al., 2007). The dried samples were ground into powder by a grinder; to prepare the methanol extract, 10 g of dried algal powder was mixed with 40 cc of methanol and left to stand for 10 hours.

Then, the extracts were centrifuged twice for 25 minutes at 4000 rpm and the supernatant was filtered using Whatman 1.NO filter paper. A vacuum was used to remove the extract solvent from the operator. For the synthesis of silver nanoparticles, a silver nitrate solution was used as a receiver or precursor. 17 mg of silver nitrate was dissolved in 100 cc of distilled water. In order to reduce Ag+ ions, 10 cc of algae extract was added to 90 cc of 1 mM silver nitrate solution (Jegadeeswaran et al., 2012). Finally, UV-VIS, XRD, FT-IR and SEM spectrophotometric tests were used to determine the characteristics and ensure the production and quality of the synthesized silver nanoparticles.

2.1. Cell culture

This study used human liver cancer cell lines purchased from the Pasteur Institute of Tehran Cell Bank. Cells were cultured between passages 26 and 31 in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 mM sodium pyruvate, 1.5% sodium bicarbonate, and 1% penicillin-streptomycin antibiotics in a 2 CO incubator at 37°C with adequate humidity and 5% carbon dioxide. For various tests, when the cells reached at least 70% confluence, they were detached from the bottom of the flask by trypsin-ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1500 rpm for 5 minutes. The cell pellet was prepared in a suspension in one cc of culture medium. The percentage of cell viability in the suspension was determined by mixing equal amounts of trypan blue using a hemocytometer slide and examining with a light microscope. After ensuring that the cells were not contaminated, cells with a viability percentage of over 90% were used for testing. (Handler et al., 1980).

2.2. Evaluation of cellular toxicity using the MTT assay

In order to evaluate the effect of silver nanoparticles synthesized with seaweed on cancer cells, a colorimetric method was used (Mosmann, 1983). This method is a mitochondrial competitive metabolic test and is based on the breakdown of tetrazolium salt by the mitochondrial succinate dehydrogenase enzyme of living cells. In this method, 100 microliters of culture medium containing 104 cells was placed in each well of a 96-well plate. After 24 hours of incubation, concentrations of 25, 50, 100, 200 and 400 µg/ml of silver nanoparticles synthesized with seaweed were added to the cells and incubated for 24 hours, respectively. After the above-mentioned times, 20 µl of MMT at a concentration of 5 mg/ml was added to each well of the plate and incubated for another 4 hours in the dark at 37°C. After the required time, the culture medium was carefully removed and 1 µl of acidified isopropanol was added to each well of the plate to dissolve the purple formazan. After 15 minutes of incubation at room temperature, the absorbance of each well was read using an ELISA device at a wavelength of 570 nm against a reference wavelength of 690 nm. The results of the concentration that IC50 (resulting in cell survival rate and causing 50% inhibition of cell growth) were reported based on the concentration curve (micrograms per milliliter) (Arechabala et al., 1999).

2.3. Flow cytometry

Cells are cultured in six-well plates. They are then incubated with the nanoparticles. After 24 hours, cell viability is assessed by flow cytometry.

3. Results

As shown in Figure (1), the synthesized silver nanoparticles have 3 diffraction peaks in the numerical regions 38, 44 and 64. The above results are confirmed by comparing the location and intensity of the diffraction peaks with the location and relative intensities of the reference peak (Sharma et al., 2018). The synthesized silver nanoparticles are white in color, spherical in shape and 87 nm in size (Table 1). The toxicity of the nanoparticles on two cancer cell lines was evaluated in comparison to normal cells. The combination of silver nanoparticles with *L. caspica* algal extract has a significant effect on the reduction of the liver cancer cell line (HepG2). With increasing concentrations of the combination of silver nanoparticles with L. caspica algal extract, significant anticancer effects were observed in the studied combination (Figure 3). The rate of liver cancer cell viability (HepG2) in the range of concentrations greater than 50 µg/ml of the combination of silver nanoparticles with *L. caspica* algal extract shows a decreasing trend after 24 hours, showing a significant decrease compared to lower concentrations.

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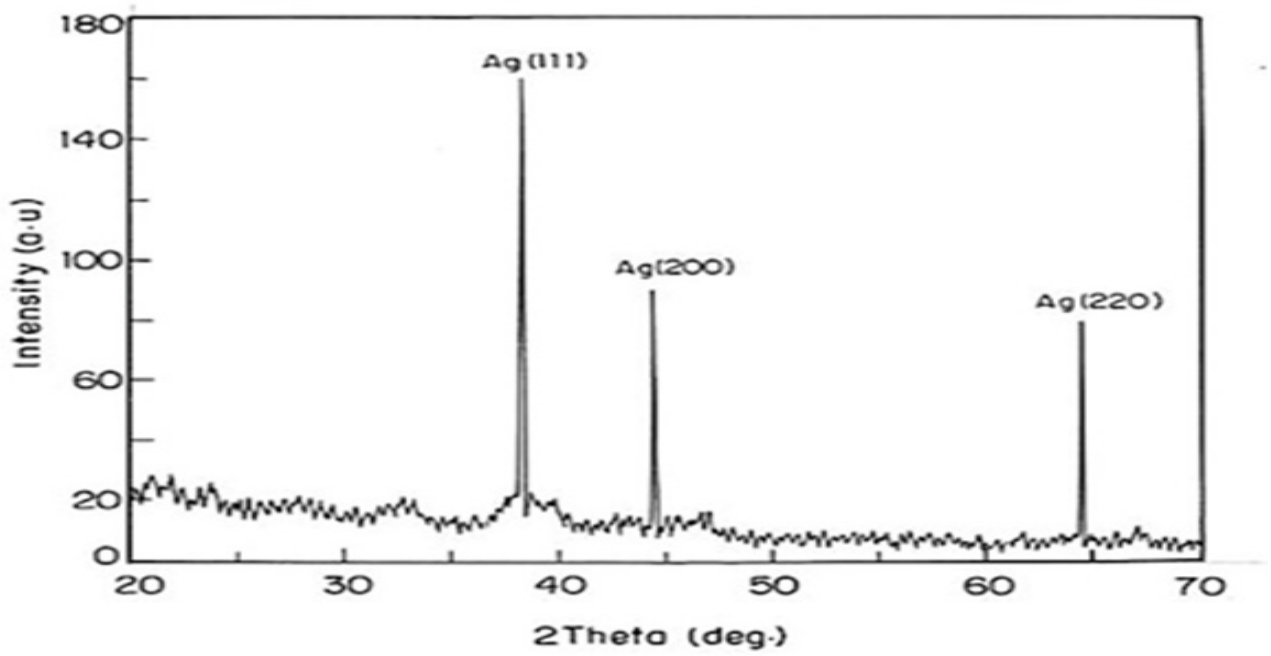

Figure 1. XRD spectrum of silver nanoparticles synthesized from the seaweed *Laurencia caspica*

Table 1. General characteristics of silver nanoparticles synthesized from the seaweed *Laurencia caspica*

Parameters	Purity percentage	Color	Particle shape	Particle size(nm)
Values	99>	White	Spherical	87

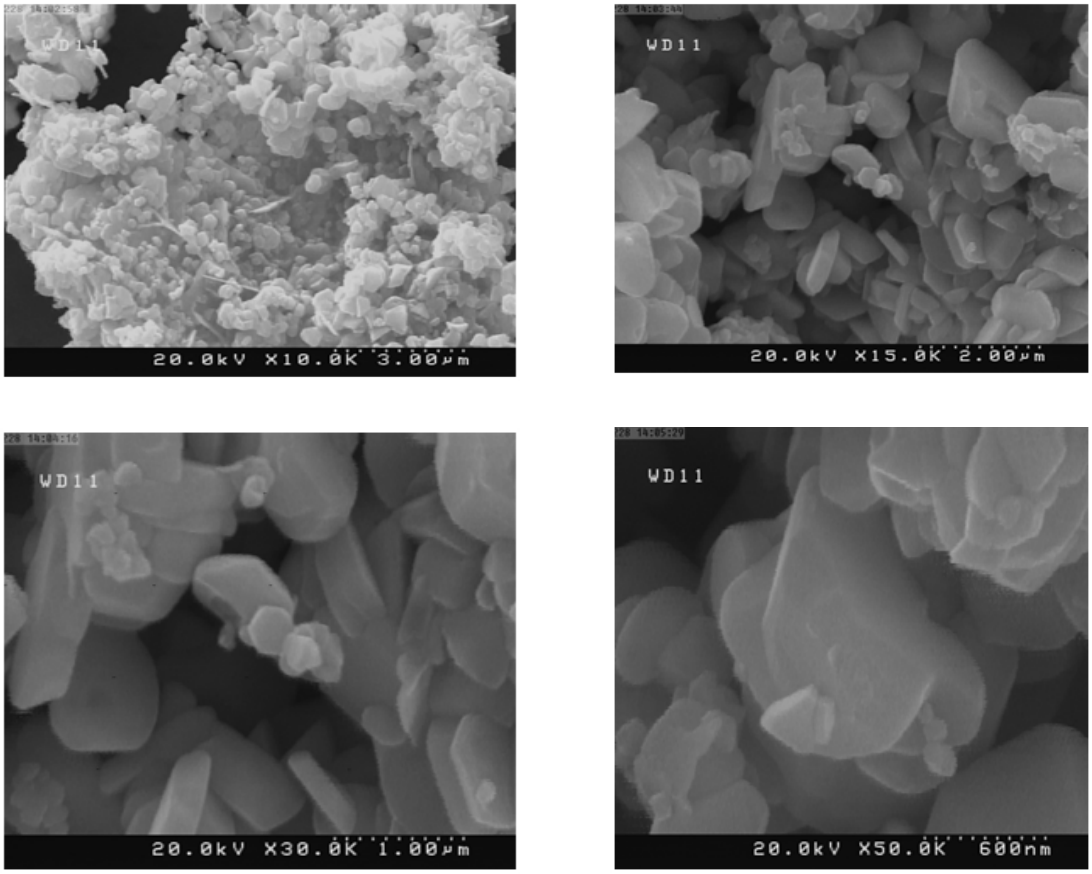

Figure 2. Scanning electron microscope (SEM) images of silver nanoparticles used in this research.

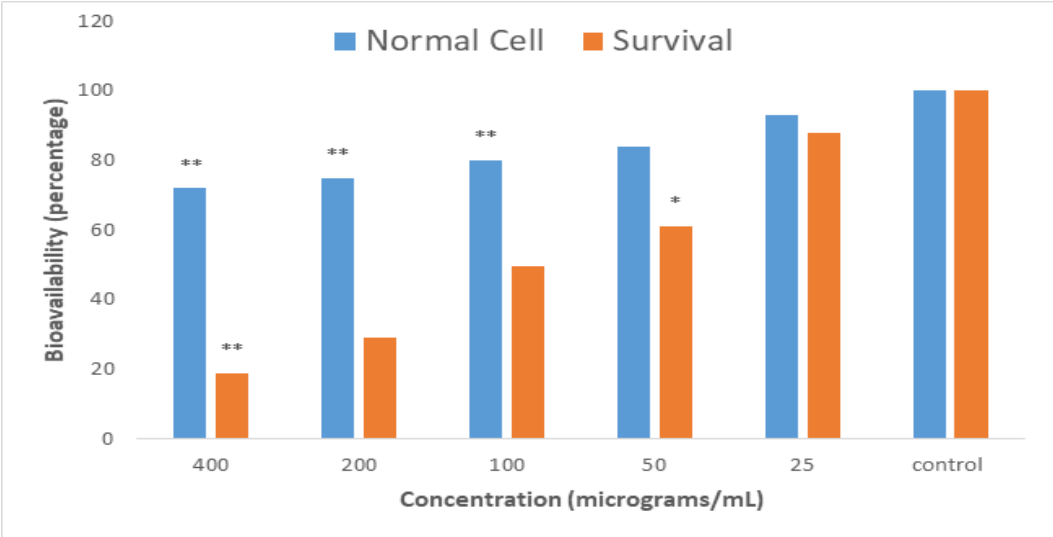

Figure 3. Percentage of survival of normal and cancer cells against the combination of silver nanohydrate concentration and *Laurencia caspica* algal extract over a period of 24 hours (significant difference at the level of *: $p<0.05$, **: $p<0.01$).

4. Discussion

So far, numerous biological compounds with diverse applications such as antibiotic, antiviral, antifungal and anticancer effects have been identified and extracted from multicellular algae, and many of the primary or secondary metabolites of these organisms can be converted into bioactive substances used in the pharmaceutical industry (Tuney et al., 2006). Natural products of seaweeds have the potential to be used in anticancer drugs and anti-inflammatory drugs (Lee et al., 2013).

The findings showed that the rate of liver cancer cell viability (HepG2) in the range of concentrations greater than 50 µg/ml of the combination of silver nanoparticles with L. caspica algal extract decreased after 24 hours and showed a significant decrease compared to lower concentrations. This decrease is proportional to the concentration of nanoparticles with algal extract, so that at high concentrations the rate of cell viability showed a significant decrease compared to lower concentrations. Zaleta et al. (2014) also showed that the compounds in this extract are able to inhibit the proliferation of gastric, breast, colon, ovarian, lung, pancreatic, prostate, skin and neuroblastoma cancer cells. In a study by Dellai et al. (2013) on the hydroalcoholic extract of Laurencia obtuse red algae isolated from the Mediterranean coast, they showed that this extract has antiproliferative activity on three cancer lines. MCF and 7 HCT15, A549 in humans. Shafaghi et al. (2016) investigated the anticancer effects of red algae extract L. caspica on breast cancer cell line (T47D) and showed that the algae extract reduces the growth of T47D cells. Also, studies conducted by Mahdavi Rad et al. (2014) entitled the anticancer effects of zinc oxide nanocomposite and silver nanocomposite with zinc oxide on malignant melanoma cancer cell line (A-375) showed that with increasing concentration, cell viability decreased significantly compared to lower concentrations. The findings of this study are consistent with the results of these researchers. In fact, the combination of silver nanoparticles with L. caspica algal extract has a significant effect on the reduction of liver cancer cell line (HepG2).

5. Conclusion

The results of this study show that the combination of silver nanoparticles with *L. caspica* algal extract has significant anticancer effects on the liver cancer cell line (HepG2). With increasing concentration of nanoparticles, the cell viability decreases significantly, which indicates the high potential of this combination in the treatment of liver cancers. Also, the evidence from previous studies indicates that biological compounds extracted from algae can be used as valuable sources for the development of new anticancer and anti-inflammatory drugs. Overall, these findings can contribute to scientific and clinical advances in the field of cancer treatment and emphasize the need for further research in this field.

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