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Cytotoxic effect of silver nanoparticles biosynthesized from *Hirudo medicinalis* saliva on HepG2 cells

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Abstract

Primary hepatocellular carcinoma is a devastating type of liver cancer. Silver nanoparticles (AgNPs) have been assessed for a variety of purposes, including being tested as an anticancer agent. The aim of this study was to assess the cytotoxicity of AgNPs that were biosynthesized from leech saliva on HepG2 cells, through the undertaking of a simple MTT assay. HepG2 cells were obtained from the cell bank of the Pasteur Institute of Iran. In this study, AgNP-treated HepG2 cells were cultured at a density of 10^4 cells per well, and 100 µL of MTT at a concentration of 0.5 mg/mL were added to each well; the treated cells were then let to incubate for 4 h. Subsequently, a plate reader device operating at a wavelength of 570 nm was used in order to determine the concentration of the chemical dissolved in isopropanol. Representative images of the cells show remarkable changes in their morphology at AgNP concentrations of 25 and 50 µg/mL. At 48 h, the nanoparticle's IC_{50} value was 50 µg/mL. Our study shows that leech salivary extract-derived AgNPs are cytotoxic to HepG2 cells.

KEYWORDS
MTT assay, LSE-AgNPs, *Hirudo medicinalis*, HepG2 cells

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1. INTRODUCTION

The green synthesis of silver nanoparticles (AgNPs) has become one of the most promising methods of nanoparticle synthesis. Because of their strong antibacterial activity, AgNPs have been used in a wide range of applications, such as an anticancer agent and in wound dressing [1]. It is becoming necessary to obtain a better understanding of the toxicity of AgNPs and their potential mechanism of toxicity [2], as their biological method of synthesis is the least complicated, environmentally friendly, and commercially viable [3].

Hepatocellular carcinoma is the most common primary liver malignancy, with an increasing global incidence [4]. This is why we, herein, chose to assess the toxicity of AgNPs against HepG2 cells; a human hepatoma cell line widely used in hepatotoxicity studies. The toxicity mechanism of AgNPs is mainly attributed to the AgNPs' ability to release a large amount of reactive oxygen species (ROS)

that damage the cell membrane and lead to cell apoptosis [2].

2. MATERIALS AND METHODS

The production of AgNPs from leech salivary extract (LSE) was undertaken by using the method described by Jaganathan *et al*. [5]. Subsequently, different concentrations of LSE-AgNPs (0, 25, 50, and 100 µg/mL) were made using Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS), in order to study the toxicity of the nanoparticles and their impact on the growth and proliferation of the cells [6]. Finally, the MTT assay was performed as previous described in detail [7].

3. RESULTS

Characteristics of the produced LSE-AgNPs: The precipitates of these were dark brown in colour. The LSE-AgNPs' average size was measured by using a dynamic light scattering test and their zeta potential (measured at 649.1 nm) was found to be -0.060. Moreover, field emission scanning electron microscopy revealed that our samples included particles that were evenly scattered and nearly square in shape, ranging in size from about 20 to 720 nm with an average size of 600 nm.

Quantitative toxicity test (MTT assay): Cell death caused inhibition of the biochemical reaction and formation of purple formazan. The biochemical enzymatic cascade in live cells triggered a reduction of the substrate and changed the colour of the wells to purple (positive control). The MTT assay was performed on four determined nanoparticle concentrations (0, 25, 50, and 100 µg/mL) at three time different timepoints (24, 48, and 72 h). The viability of the HepG2 cells was found to be reduced after the interaction with various concentrations of the LSE-AgNPs. This reduction increased gradually with the increase of the concentration and the time of the cells' exposure to LSE-AgNPs. At 48 h, the nanoparticles' IC_{50} was 50 µg/mL (Table 1). These results reveal the significant differences in the % viability between non-treated HepG2 cells (control) and HepG2 cells treated with LSE-AgNPs, the significant differences in the % viability between the HepG2 cells that were exposed to the same concentration of LSE-AgNPs (25, 50, 100 µg/mL) between 24 and 48 h, and the absence of significant differences among the HepG2 cells between 48 and 72 h, and the absence of significant differences among HepG2 cells treated with different concentrations of LSE-AgNPs for the same duration.

Morphological changes in the HepG2 cells after an exposure to LSE-AgNPs: The HepG2 cells' morphology was observed under light microscopy after an incubation of 24, 48, and 72 h with the AgNPs at concentrations 0, 25, 50, and 100 µg/mL. Representative images reveal remarkable morphological changes, indicating the presence of unhealthy cells due to the exposure to AgNPs. As the AgNPs' concentration and exposure time increase, the exposed cells appeared to be clustered with a few cellular extensions, and cell spreading patterns were restricted with the observation of a few floating cells (as compared with the control cells). The morphology of cells between 24 and 48 h was greatly affected.

Table 1. The effect of different concentrations of leech salivary extract (LSE)-derived silver nanoparticles (AgNPs), that have been biosynthesized from *Hirudo medicinalis*, on the % viability of HepG2 cells.

	Period of exposure (B)		
Concentration of LSE-AgNPs (A)	**24 h**	**48 h**	**72 h**
Control	100±0.0	100±0.0	100±0.0
25 µg/mL	4.35±0.82	2.14±0.13	1.84±0.01
50 µg/mL	3.57±0.14	2.05±0.07	1.51±0.13
100 µg/mL	3.21±0.11	1.80±0.02	1.48±0.25

Note: values represent means ± standard deviations; LSD (p<0.05) (A*B)=1.217.

4. DISCUSSION

Hirudo medicinalis is one of the species that is most commonly used as a model in medicine. Although this organism secretes more than 100 different substances, only a small number of them exhibit potent anti-inflammatory, antibacterial, analgesic, anticoagulant, and anticancer activities [8]. The LSE is not pure and contains many factors [9]. The average size of the nanoparticles and par-

ticles, which ranged from 20 to 720 nm, was 600 nm. The leech saliva is abundant in protein, vitamins, amino acids, antioxidants, and other nutrients that have an important effect in lowering the amount of LSE-AgNPs produced [9].

The *in vitro* cytotoxic effects of the LSE-AgNPs on the HepG2 cells was assessed by the herein undertaken MTT assay. The IC_{50} of LSE-AgNPs was 50 µg/mL at 48 h. According to an earlier study [5], the earthworm-mediated AgNPs' IC_{50} was found to be 25.96 µg/mL, which is within the accepted range of activity. The active physicochemical interaction of silver atoms with the functional groups of intracellular proteins, nitrogen bases, and phosphate groups in DNA is what causes AgNPs to exert cytotoxic effects, the production of ROS, and the increase of intracellular oxidative stress, which in turn triggers cell death processes like apoptosis and necrosis [10]. According to Supraja *et al*. [10] three concentrations of AgNPs over three times, resulted in somewhat greater morphological changes in AgNP-treated cells. Our results indicate good cytotoxic activity against cancer cells. Some of the chemotherapy drugs that were authorized had serious side effects. Therefore, there is a critical need to create alternative medications to combat this devastating illness. The use of LSE-AgNPs, which seem to be efficient cytotoxic agents against HepG2 cells, could be a promising step forward.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interest.

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