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Original Article

# *In vitro* antiviral activity of curcumin-loaded selenium nanoparticles against human herpes virus type 1

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**Abstract**

**Background and aims:** Herpes simplex type 1 (HSV-1) is one of the most common and contagious viruses in humans. Curcumin is a natural polyphenol that produces antiviral effects against various viruses, such as HSVs. Despite curcumin's numerous effects and benefits, its insolubility has reduced its clinical effectiveness. During recent decades, significant progress has been made in nanodrugs, which has helped expand new delivery systems. This study investigated the *in vitro* antiviral activity of curcumin-loaded, folic acid-chitosan-coated selenium nanoparticles (SeNP) against HSV-1.

**Methods:** Synthesized SeNPs loaded with curcumin and coated with folic acid-chitosan molecules were used in this experimental study. The studied groups comprised cells and the virus-containing curcumin, acyclovir, and NPs with or without curcumin. The cytotoxicity of the compounds was evaluated on Vero cells using the MTT assay. Antiviral activity was investigated using the MTT colorimetric assay, and the inhibitory effect on HSV-1 was studied using a 50% tissue culture infectious dose assay.

**Results:** The results of this research demonstrated that curcumin (50% inhibitory concentration [$IC_{50}$] = 5.64 µg/mL) and curcumin-loaded SeNPs ($IC_{50}$ = 1.15 µg/mL) exhibited satisfactory antiviral potential against HSV-1 *in vitro*, while curcumin-loaded, folic acid-chitosan-coated SeNPs produced no antiviral effect against HSV-1 due to increased cytotoxicity.

**Conclusion:** Based on the findings, the curcumin and curcumin-loaded SeNPs had acceptable antiviral potential against HSV-1. Loading curcumin with SeNPs makes the compound more active at a lower concentration, and therefore, lower doses can be administered to treat HSV-1 infection.

**Keywords:** Herpes simplex virus, Curcumin, Selenium nanoparticles, Antiviral activity, Folic acid-chitosan

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## Introduction

Herpes simplex type 1 (HSV-1) is one of the most prevalent and contagious viruses in humans (1). Today, the treatment of HSV infections with available antiviral drugs such as acyclovir is facing challenges because of the emergence of drug resistance due to mutations in the virus DNA (2). Accordingly, efforts are being made to use and produce more antiviral drugs, especially those derived from herbal origins (2). Curcumin, with the chemical name diferuloylmethane and the molecular formula $C_{21}H_{20}O_6$, is a hydrophobic polyphenol derived from the rhizome of turmeric (*Curcuma longa*). This compound has various biological and pharmacological properties. It is used in most countries, especially India and China, to treat skin diseases, urinary tract infections, asthma, and rheumatoid arthritis (3). In previous research, several properties have been reported for this compound, such as anticoagulant, antidepressant, antimicrobial, anticancer, anticonvulsant, pain-relieving, anti-inflammatory, hypocholesterolemic, rheumatoid arthritis-relieving, antioxidant, antiviral, antiproliferative, and anti-angiogenic properties (4). The *in vitro* antiviral effects of curcumin and its derivatives against various types of viruses, such as HSVs, adenoviruses, hepatitis viruses, and influenza viruses, have been reported in some studies (5-9). Despite the numerous effects and benefits of curcumin, its insolubility has reduced its clinical effectiveness. However, much research has been performed to address these limitations, given the countless therapeutic properties reported for curcumin. Several approaches have been adopted to overcome these limitations, including the discovery of natural curcumin analogs from turmeric, the synthesis of curcumin analogs, and the reformulation of curcumin with different oils and metabolism inhibitors. During recent decades, significant progress has been made in nanodrugs, which has helped expand new delivery systems (10). Among the various nanomaterials, selenium nanoparticles (SeNP) have attracted much attention and are used as therapeutic agents and drug carriers. These NPs also have antioxidant and antipathogenic effects. Since SeNPs produce antioxidant and antimicrobial effects, curcumin loading in these NPs is expected to result in satisfactory outcomes. This study investigated the *in vitro* antiviral activity of curcumin-loaded, folic acid-chitosan-coated SeNPs against HSV-1.

## Materials and Methods

### *Nanoparticle preparation*

In this experimental study, SeNPs were synthesized, loaded with curcumin, coated with folic acid-chitosan molecules, and prepared as per the procedure of a previous study. Then, its physical and chemical properties underwent investigation (11).

### *Cells and viruses*

The cells required for cultivating HSV-1 are from the Vero cell line, purchased from the Cell Bank of the Pasteur Institute of Iran. The cells were cultured in Dulbecco's modified Eagle medium containing 10% fetal bovine serum (FBS). The Virology Laboratory, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran, provided HSV-1 strain KOS, which was kept at -70 ˚C as the initial virus seed after cultivation in the cell monolayer medium.

### *Studied groups*

The studied groups consisted of the cells and the virus-containing cells treated with (*a*) no NPs and no drug, (*b*) drug-free SeNPs, (*c*) drug-free, Se chitosan NPs, (*d*) drug-free, Se chitosan-folic acid NPs, (*e*) Curcumin, (*f*) Se curcumin NPs, (*g*) Se curcumin chitosan NPs, (*h*) Se curcumin chitosan-folic acid NPs, and (*i*) acyclovir. The experiments were performed in triplicate, with two independent experiments.

### *Cytotoxicity assay*

Before investigating the antiviral activity in different groups, their toxicity on Vero cells was investigated in the absence of the virus, and a non-toxic concentration to the cell was determined in this regard. To this end, in 96-well plates containing a cell monolayer, after removing the culture medium on top of the cells and washing them with PBS buffer, different successive dilutions of the extract were prepared in Dulbecco's modified Eagle medium containing 2% FBS, added to the wells, and incubated at 37 ˚C with 5% $CO_2$ for two days. Then, cell viability was determined using the 3-(4,5-*dimethylthiazol*-2yl)-2,5*diphenyltetrazolium bromide* (MTT) colorimetric assay. The MTT assay was performed in triplicate for each compound. Next, cell viability was determined using the MTT assay, which was performed in triplicate for each compound. Subsequently, the 50% cytotoxic concentration ($CC_{50}$), namely, the drug concentration that kills 50% of the cultured cells, was calculated based on the dose-response curve using regression analysis in GraphPad software (12,13).

### *Determining viral titer using a 50% tissue culture infectious dose assay*

First, Vero cells were cultured in 96-well microplates. After forming a cell monolayer, one logarithmic dilution of the virus was added to the wells in a medium containing 2% FBS. The microplates were incubated at 37 ˚C with 5% $CO_2$ until the appearance of cytopathic effects. The results were collected and calculated using the Reed and Muench $TCID_{50}$ (14).

### *Determining antiviral activity using 3 - (4,5-dimethylthiazol - 2yl)-2,5 diphenyl tetrazolium bromide assay*

Antiviral activity was evaluated using the MTT assay. For this purpose, after the formation of a single layer of cells in the microplates of 96 wells, the culture medium was removed from the wells, and 100 µL of culture medium containing different concentrations of non-toxic (lower than $CC_{50}$) extracts and 100 $TCID_{50}$ of HSV-1 were added to all wells. After incubation at 37 ˚C with 5% $CO_2$ for 72 hours, the MTT assay was conducted according to the procedure described above, and the virus inhibition rate was calculated. Finally, after three repetitions, the $IC_{50}$ was calculated as the minimum concentration of plant compounds that inhibit 50% of the virus by creating a regression line. Negative control (without virus and herbal compounds), virus control (virus without herbal compounds), and drug-positive control (acyclovir) were also designed during each step. The selectivity index, a criterion for the eligibility of a compound to be considered a drug candidate, was obtained by dividing the $CC_{50}$ by the $IC_{50}$.

### *Measuring the antiviral activity using the 50% tissue culture infectious dose assay*

The inhibitory effect was investigated using the $TCID_{50}$ assay. For this purpose, after the formation of a single layer of cells in the microplates, the culture medium was removed from the wells, and 200 µL of the culture medium containing different concentrations of non-toxic compounds (concentrations lower than $CC_{50}$) and 100 $TCID_{50}$ of HSV were added to each well. After incubation at 37 ˚C with 5% $CO_2$ for 72 hours, the supernatant of the microplate was collected, and the viral titer was calculated for each well using the $TCID_{50}$ assay (as per the protocol described above).

## Results

### *The death rate of Vero cells at different concentrations*

The death rate of Vero cells at different concentrations in the studied groups is illustrated in Figure 1. According to the results, the percentage of cell death increased with increasing concentration. A concentration of different compounds that destroy 50% of Vero cells ($CC_{50}$) was estimated using probit analysis (Table 1, Figure 1).

### *Antiviral activity*

The $IC_{50}$ was calculated at 5.64, 1.15, and 0.3 µg/mL for curcumin, Se curcumin NPs, and acyclovir, respectively (Figure 2, Table 1).

The results demonstrated that curcumin and curcumin-loaded SeNPs with selectivity indices of 3.2 and 3.63, respectively, had acceptable antiviral potential against

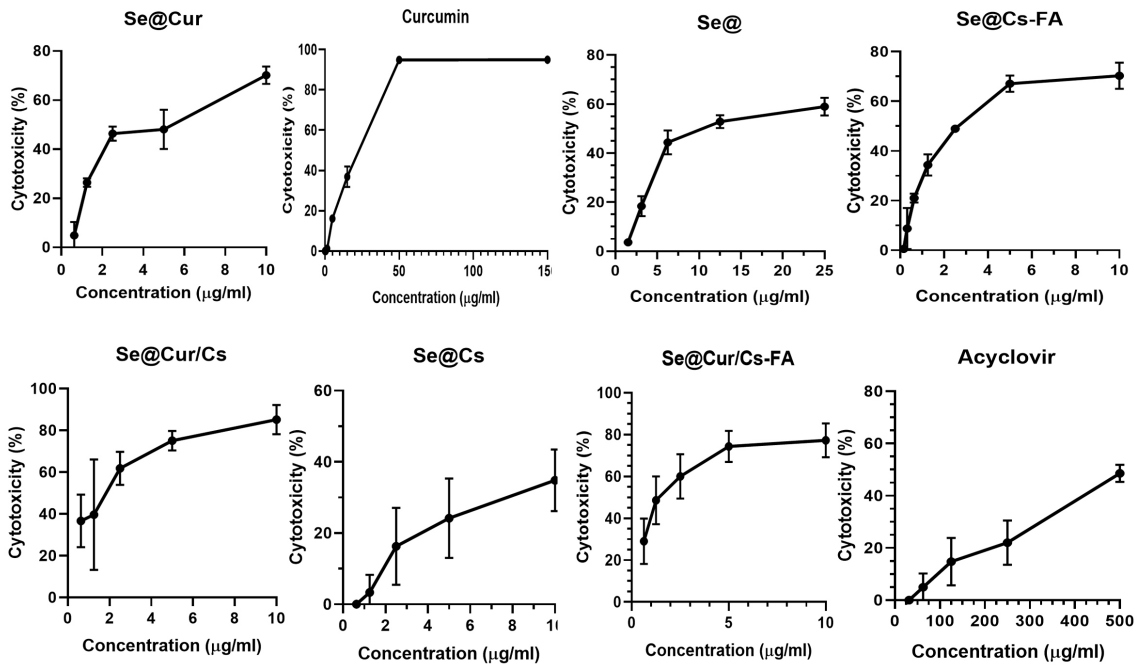


**Figure 1.** The cytotoxicity effect in the studied groups. *Note*. NP: Nanoparticle; MTT: 3-(4,5-*dimethylthiazol*-2yl)-2,5*diphenyltetrazolium bromide*. The death rate was calculated using the MTT assay. Se@Cur: Selenium curcumin NPs; Se@Cur/Cs: Selenium curcumin chitosan NPs; Se@Cur/Cs-FA: Selenium curcumin chitosan-folic acid NPs; Se@: Selenium NPs; Se@Cs: Selenium chitosan NPs; Se@Cs-FA: Selenium chitosan-folic acid NPs. The values are expressed as the means ± standard deviations of two independent experiments performed in triplicate

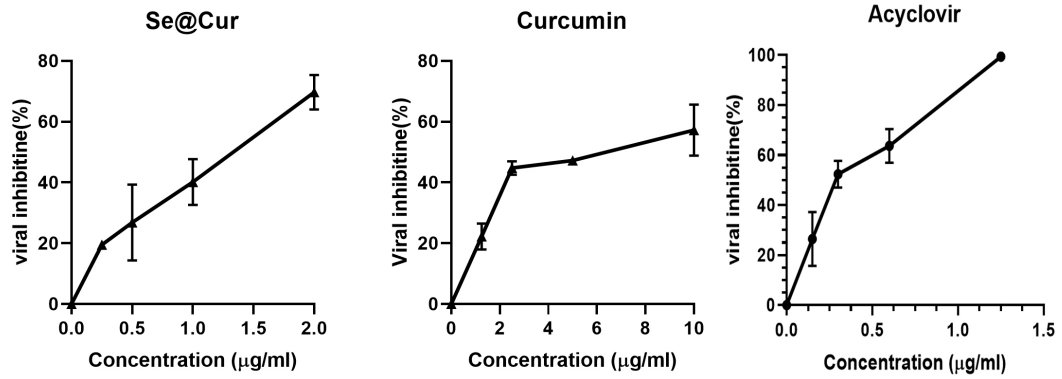


**Figure 2.** Anti-herpes simplex virus activity of curcumin, selenium curcumin nanoparticles, and acyclovir based on the MTT assay results. *Note*. MTT: 2,5-diphenyl-2H-tetrazolium bromide. The values are expressed as the means ± standard deviations of two independent experiments performed in triplicate. Se@Cur: Selenium curcumin nanoparticles

**Table 1.** Cytotoxicity, viral inhibition, and selection index in the studied groups

| Sample | $CC_{50}$ (95% CI) (µg/mL) | $IC_{50}$ (95% CI) (µg/mL) | Selectivity index |
|---|---|---|---|
| Curcumin | 18.07 (15.6-20.8) | 5.64 (3.8-10.9) | 3.2 |
| Se@Cur | 4.18 (3.33-5.4) | 1.15 (0.86-1.7) | 3.63 |
| Se@Cur/Cs | 1.5 (0.89-2.21) | >2 | - |
| Se@Cur/Cs-FA | 1.56 (1.1-2.1) | >2 | - |
| Se@ | 12.46 (9.8-16.7) | >12 | - |
| Se@Cs | 17.46 (17.5-43.1) | >17 | - |
| Se@Cs-FA | 2.8 (2.38-3.23) | >2.8 | - |
| Acyclovir | 537.7 (448.7-705.7) | 0. 3 (0.24-0.38) | >1,000 |

*Note*. Values are expressed as means and CI 95%; CI: Confidence interval; $CC_{50}$: 50% cytotoxic concentration; $IC_{50}$: 50% inhibitory concentration; NP: Nanoparticle; Acyclovir; Positive control. Se@Cur: Selenium curcumin NPs; Se@Cur/Cs: Selenium curcumin chitosan NPs; Se@Cur/Cs-FA: Selenium curcumin folic acid-chitosan NPs; Se@: Selenium NPs; Se@Cs: Selenium chitosan NPs; Se@Cs-FA: Selenium folic acid-chitosan NPs.

HSV-1 *in vitro*, while curcumin-loaded, folic acid-chitosan-coated SeNPs and drug-free NPs produced no antiviral effect on HSV-1 due to increased cytotoxicity. Based on the results of this study, the loading of curcumin with Se particles causes the plant compound to be more active on the virus at a lower concentration. Therefore, lower doses would be needed for administration.

### *Effects of curcumin and selenium curcumin nanoparticles on viral titers*

This study investigated the effects of curcumin and Se curcumin NPs on viral titers using the $TCID_{50}$ assay (Figure 3).

## Discussion

The results of this research showed that the curcumin and Se curcumin NPs exerted acceptable antiviral effects

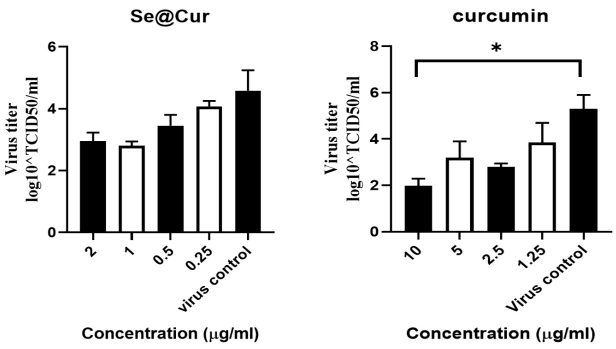


**Figure 3.** Comparison of viral titer at different concentrations of curcumin and selenium curcumin nanoparticles based on $TCID_{50}$ assay results. *Note*. $TCID_{50}$: 50% tissue culture infectious dose. The significance of the results was evaluated using the non-parametric Kruskal-Wallis and post-hoc Dunn's multiple comparisons tests. The values are expressed as the means ± standard deviations of three independent experiments. Se@Cur: Selenium curcumin nanoparticles

against HSV-1 *in vitro*. In contrast, Se curcumin folic acid-chitosan NPs exhibited no antiviral effect due to increased cytotoxicity. Consistent with our study, the antiviral activity of curcumin and its biocompounds has been reported against a variety of viruses, including parainfluenza virus, HSV-1 and HSV-2, respiratory syncytial virus, influenza, hepatitis B, hepatitis C, and human immunodeficiency virus (6). Curcumin has been recommended as a potent antiviral compound because of its inhibitory activity against the effect of inosine-5′-monophosphate dehydrogenase (15). In some studies, curcumin's *in vitro* antiviral effects and its derivatives against various viruses such as HSVs, adenoviruses, hepatitis viruses, and influenza viruses have been observed as well (5-9).

In the study of Kutluay et al, curcumin significantly affected HSV-1's immediate early gene expression, thereby reducing the capacity of the virus to initiate the lytic cycle of infection (16). Despite the numerous effects and benefits of curcumin, its insolubility challenges its clinical application. Significant progress has been made in nanodrugs in recent decades, contributing to the development of new delivery systems (10).

Based on the results of this study, loading curcumin with SeNPs makes the plant compound more active at a lower concentration and, therefore, leads to the administration of the drug at a lower concentration. Se is one of the rarest elements on earth, a powerful antioxidant, and a critical compound for human life (17). It has been established that SeNPs can be used as an antioxidant supplement with no side effects. They have also been reported to improve growth, contribute favorably to food productivity, and increase antioxidant capacity (18,19). Haggag *et al* investigated the effects of silver NPs loaded with *Lampranthus coccineus* and *Malephora lutea* extracts against hepatitis A, HSV-1, and Coxsackievirus and found that the NPs produced an antiviral effect (20).

The findings of de Souza E Silva et al demonstrated that using mesoporous silica NPs was a promising approach to controlling viral infection and could contribute to formulating potentially efficient strategies for managing viral infection (21). In the mentioned study, the interactions of NPs with the viral envelope were found to be the leading cause of the antiviral activity of mesoporous silica particles because stronger virus-mesoporous silica bonds disturbed the binding of cell receptors to the virus envelope. The ability of the virus to transmit represented a decrease.

Vonnemann et al have argued that the size of NPs varies depending on how these materials affect the virus, so larger gold NPs act as efficient cross-linkers between virions. In contrast, smaller gold NPs cover the surface of the virus particles (22). In addition, the increased immunogenicity of viral vaccines in combination with NPs has been reported in previous research (23).

Based on the results of this study, curcumin-loaded, folic acid-chitosan-coated SeNPs increased cytotoxicity and had no antiviral effects on HSV-1. Using folic acid-chitosan-coated NPs is one of the novel targeted treatment methods. Chitosan is an amino polysaccharide with various uses, including drug and gene delivery, magnetic resonance imaging, and tissue design. Chitosan is the most compatible polymer for multiple applications thanks to its biocompatibility, biodegradability, and antibacterial properties. This nanocompound's lack of antiviral activity in our study may be due to its inability to deliver drugs to virus-infected cells in a targeted manner. In contrast, increased drug delivery to healthy cells leads to the cytotoxicity of the compound to healthy cells. This argument still needs further investigation.

## Conclusion

Based on the results of this study, the curcumin- and curcumin-loaded SeNPs had acceptable antiviral potential against HSV-1. Loading curcumin with Se particles makes the plant compound active at a lower concentration and, as a result, leads to lower dose consumption. Therefore, it can be used as a source of medicine to control viral diseases.

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### Authors' Contribution

**Conceptualization:** Pegah Khosravian-Dehkordi and Mohammad-Taghi Moradi.
**Data curation:** Fatemeh Javadi-Farsani and Marzieh Akbari.
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**Methodology:** Fatemeh Javadi-Farsani and Marzieh Akbari.
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**Writing–original draft**: Pegah Khosravian-Dehkordi, Majid Asadi-Samani, Dhiya Altememy, and Mohammad-Taghi Moradi.
**Writing–review & editing:** Pegah Khosravian-Dehkordi, Majid Asadi-Samani, Dhiya Altememy, Fatemeh Javadi-Farsani, Marzieh Akbari, and Mohammad-Taghi Moradi.

## Competing Interests

The authors declare that there is no conflict of interests.

## Ethical Approval

Ethical considerations in this study included obtaining permission from the Ethics Committee of Shahrekord University of Medical Sciences (Ethical Code IR.SKUMS.REC.1401.070).

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