INVESTIGATION OF THE ANTIVIRAL ACTIVITY OF *Ficus carica* L. LATEX AGAINST HSV-2

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Ficus carica L. has been widely used in the treatment of many diseases in folk medicine for centuries. It has been reported to have many pharmacological properties. In this study, we aimed to investigate the antiviral activities of *Ficus carica* L. latex against HSV-2 and its synergistic effects with acyclovir. In order to determine the presence of antiviral activity of *Ficus carica* latex samples, different concentrations of latex (1024, 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/mL) were added into the culture medium. HSV-2 proliferation was detected by real-time PCR method. Acyclovir was selected as the control drug. Compared with acyclovir, *Ficus carica* effectively inhibits viral replication of HSV-2, although the antiviral activity of *Ficus carica* is statistically significantly lower than that of acyclovir. It has been determined that the antiviral activity of Ficus carica is increased due to the polysaccharide. The activity of *Ficus carica* against HSV-2 was confirmed by a significant decrease in the number of viral copies. It was determined that *Ficus carica* L. samples have important antiviral effects compared with acyclovir. In particular, the synergy produced by antiviral activity of *Ficus carica* and acyclovir combined had a stronger effect against HSV-2 than acyclovir alone.

Keywords: *Ficus carica* L. HSV-2, antiviral, acyclovir, synergistic

INTRODUCTION

Ficus carica L. has been widely used in the treatment of many diseases in folk medicine for centuries. It has been reported to have many pharmacological properties (Ronsted *et al*., 2008; Duenas *et al*., 2008; Jeong and Lachance 2001).

Herpes simplex virus (HSV) infections are a common clinical problem all over the world. Especially in immunocompromised patients, the infection is severe and progressive and leads to significant morbidity and mortality. Herpes simplex viruses (HSV) are the most common viral agents in humans (Jerome and Ashley, 2003).

Infection caused by HSV can occur in a wide spectrum ranging from asymptomatic infections to disseminated diseases resulting in death. Recurrent HSV infections lead to severe clinical manifestations, such as meningoencephalitis, pneumonia and hepatitis, especially in newborns and immunocompromised patients (Whitley and Roizman, 2001).

Increased acyclovir resistance is particularly noteworthy in herpes simplex viruses, especially in immunocompromised individuals. For this reason, effective new drug research in herpesviruses is extremely important. Fig is one of the most important fruit of the Mediterranean region with many pharmacological features.

In this study, we aimed to investigate the antiviral activities of *Ficus carica* L. latex against HSV-2 and its synergistic effects with acyclovir.

MATERIALS AND METHODS

In this study, viral cultures were carried out in HEp-2 cell line. Al proliferation experiments were performed in flat bottom microplates. We inoculated $1x10^5$ cells per ml and RPMI 1640 medium with 10% fetal bovine serum into each culture plate. Firstly, the non-cytotoxic concentration of Ficus carica latex in the study was

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determined in the HEp-2 cell line. In order to determine the presence of antiviral activity of *Ficus carica* latex samples, different concentrations of latex (1024, 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/mL) were added into the culture medium. HSV-2 proliferation was detected by real-time PCR method. Acyclovir was selected as the control drug.

Ficus carica Latex

Ficus carica latex was obtained drop-by-drop through cutting young leaves and fruits of fig tree in Hatay district. Different concentrations of fig latex were provided including 12.5, 25, 50, 100, 200, 400 and 800 µg/ml. To determine the cytotoxicity of Ficus carica latex, HEp-2 cell line was used. For this purpose, the cell density was adjusted to. $2x10^4$ cells cultured with RMPI 1640 containing 10% fetal bovine serum and antibiotics (100 U/L penicillin and streptomycin). The culture plates were incubated at 37°C, with a saturated humidity and 5% CO_2.

DMSO

The cell number and viability of the cells were determined by trypan blue exclusion method with a hemocytometer. In the experiments DMSO (Sigma, USA) was used to dissolve the Ficus carica latex. To determine the non-toxic concentration of DMSO in the cells, HEp-2 cells were inoculated at a density of 1×10^5 cells/ml. Then, different concentrations of DMSO (4 %, 2 %, 1 %, and 0.5 %) were added to the cell cultures. After 72 hours of incubation, viable cells ratios were calculated.

Virus

The HEp-2 cell line was used for cytotoxicity tests. And also, all viral culture tests were carried out on HEp-2 cell lines. The cultivation of cell cultures was carried out in RPMI-1640 medium with 10% fetal bovine serum. The virus strains (HSV-2 virus) were obtained from Ankara University. Three different titers of HSV-2 were used in the experiments (1, 10, and 100x$TCID_{50}$; Tissue Culture of Infectious Dose). Incubation of all culture plates was performed in 5% carbon dioxide atmosphere at 37°C. Acyclovir (Sigma, USA) was selected as a control drug. Acyclovir was dissolved in bi-distillated water before use (1 mg/ml). Various concentrations of *Ficus carica* L. ranging from 1 to 1024 µg/ml were used in the experiments.

Viral quantification by real-time PCR

The real time PCR assay was performed according to the method of Readand and Kurtz, in 1999.

RESULTS

In the study, it was shown that 1% of DMSO did not affect cell proliferation, both by microscopic evaluation and by cell viability (Figure 1). *Ficus carica* L. was found to be toxic to HEp-2 cells at the level of 256 µg/ml (Figure 2). For this reason, antiviral effect studies were investigated at concentrations below 256 µg/ml. Compared with acyclovir; *Ficus carica* effectively inhibits viral replication of HSV-2, although the antiviral activity of *Ficus carica* is statistically significantly lower than that of acyclovir

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(Figure 3). It has been determined that the antiviral activity of Ficus carica is increased due to the polysaccharide (Figure 4). The activity of *Ficus carica* against HSV-2 was confirmed by a significant decrease in the number of viral copies (Figure 5).

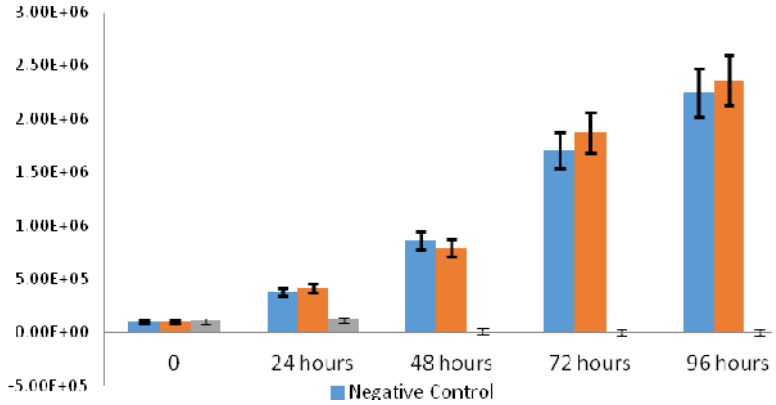

Figure 1. The effects on cell viability of the DMSO and Acyclovir compared with the control group

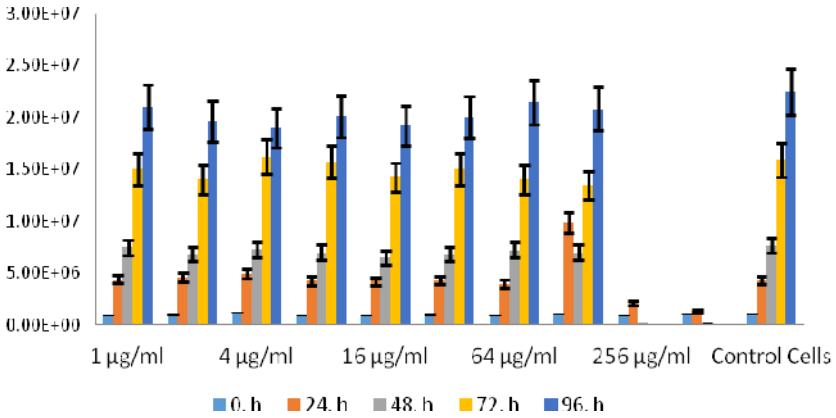

Figure 2. The effects of *Ficus carica* L. latex on cell viability of HEp-2 cells compared with control group cells

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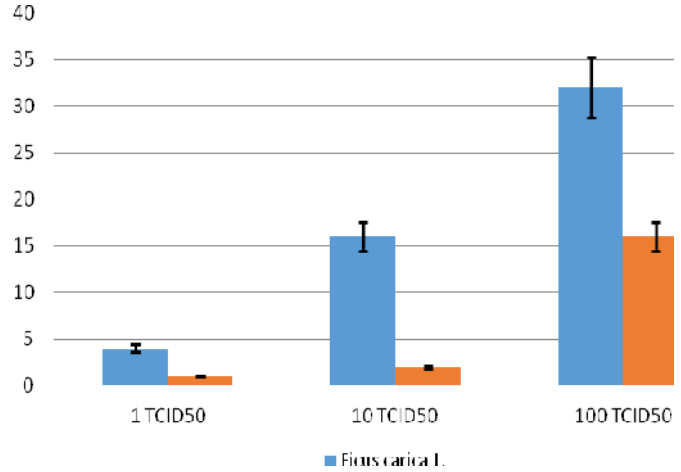

Figure 3. MIC values of *Ficus carica* L. and Acyclovir against some Herpes simplex virus type-2

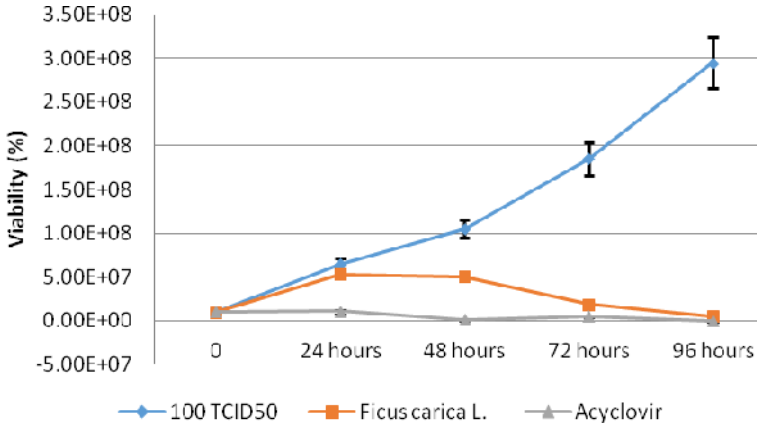

Figure 4. The effects of *Ficus carica* L. on HSV-2 replication as compared to the Acyclovir

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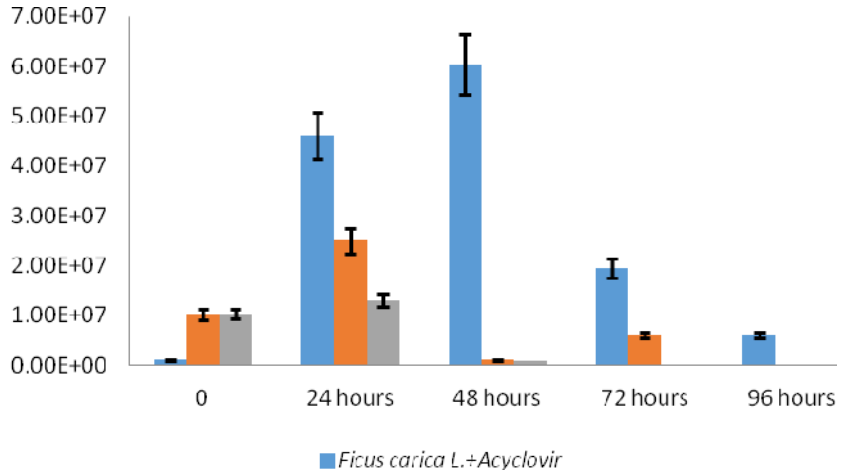

Figure 5. The synergistic effects of Ficus carica L. and Acyclovir on HSV-2 replication as compared to the Acyclovir

DISCUSSION AND CONCLUSION

It was determined that *Ficus carica* L. samples have important antiviral effects compared with acyclovir. In particular, the synergy produced by antiviral activity of *Ficus carica* and acyclovir combined had a stronger effect against HSV-2 than acyclovir alone.

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