The In Vitro Anti-Viral Activity of Honey on Type 1 Herpes Simplex Virus

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Abstract: Aims and Objectives: There are many reports on antimicrobial activities of honey. The aim of this study is to determine the in vitro anti-viral effect of honey on type 1 Herpes simplex virus (HSV-1) isolates.

Materials and Methods: Various concentrations of commercial honey were prepared and added to cell culture monolayer 1h prior and 1hr after infection with 100 PFU of the HSV-1 in Virology Laboratory of Shiraz University of Medical Sciences. Results: Honey at concentrations of 5% and higher had a complete inhibitory effect on HSV-1. Conclusion: It was found that honey at different concentrations could inhibit the growth of HSV1, so it can be considered as a therapeutic choice in folk medicine.

Key words: Honey, HSV-1, antiviral activity.

INTRODUCTION

The use of honey as a remedy has been reported only in folk medicine but now it is reborn in modern medicine too. It has been demonstrated that honey has important biological activities and therapeutic properties; its use in modern medicine has been evaluated more and more. It has been used for treatment of respiratory diseases, ulcers, wounds, eczema, psoriasis, and dan-druff. (Zaghloul, A.A. et al., 2001; Hazrati, M. Et 2010; Molan, P.C., 1999; Nejabat, M. Et al., 2009) reportedly; honey has an inhibitory effect on aerobic and anaerobic bacteria, yeast, fungi and viruses. (Al-Waili, N., 2004; Al-Jabri, A.A., Nzeako B. Al Mahrooqi, 2003; Lusby, P.E. et al., 2005; Al-Wailli, N.S., 2004; Basson, N.J., S.R. Grobler, 2008; Pooya, A.A. et al., 2003) Moreover, it can enhance antibody production against thymus-dependent and thymus-independent antigens (Al-Wailli, N.S., 2001).

Honey increases antioxidant agents, serum iron, blood indices and trace elements. It can decrease immunoglobulin E, liver and muscle enzymes and fasting blood sugar in healthy subjects. (Al-Waili, N.S., 2003) It can also lower the concentration of prostaglandins in the plasma of normal individuals, (Al-Wailli, N.S., N.S. Boni, 2003) lower C-reactive protein, homocysteine, blood lipids in healthy and hyperlipidemic subjects and cause less elevation of plasma glucose level in comparison with dextrose and sucrose in diabetic patients (Oshima, T. et al., 2000).

Herpes simplex virus (HSV) is a DNA virus that is present in 60->95% of certain populations infected to Herpes simplex virus type 1 (HSV-1), and 6-50% infected subjects to Herpes simplex virus type 2 (HSV-2). (Cunningham, A.L., Z. Mikloska, 2001) The frequency of HSV-seropositive males is significantly higher in populations infected to Human immunodeficiency virus (HIV). (Russell, D.B. et al., 2001) Moreover, sexually transmitted diseases like genital HSV increase the risk of HIV transmission. (Dickerson, M.C. et al., 1996) Acyclovir, an acyclic guanosine analog, is widely used to treat HSV infection. However, this “gold-standard” drug is less effective in recurrent HSV episodes and the development of acyclovir resistant strains has been observed and can be problematic. (Cohen, D.M., 1996) Thus, the development of novel anti-HSV agents is still an important area of research. The aim of the present study was to determine the in vitro antiviral effect of honey on HSV-1 isolates.
MATERIAL AND METHODS

Cell Culture:
Vero cell was grown in DMEM medium supplemented with 10% fetal bovine serum (GIBCO) containing 100 u/ml penicillin and 100 ug/ml streptomycin. For determination of honey cytotoxicity, uninfected cells were grown in the absence and presence of various concentration of honey for 1, 2, 3 and 4 days. Afterwards, the cells were exposed to trypanblue, loaded into hemocytometer, and the number of viable cells (unstained) was counted using low power of microscope. The number of cells per ml was then calculated (Mahy, B.W.J., H.O. Kangro, 1996).

Virus Stock and Titration:
Herpes simplex virus type 1 (HSV-1) was isolated from the lip lesion of patients and confirmed by PCR and neutralization test using guinea pig anti-HSV-1 serum (NIH USA) and monoclonal (D and G) anti-HSV-1 antibodies. (Moattari, A. et al., 2002) The virus titration was performed using cell monolayer in 24 micro well plates for 48 hrs under 5% Co2 at 36°C. The titer of virus was determined by plaque forming unit (PFU) method (Leary, J.J. et al., 2002).

Treatment With Honey:
Various concentrations of commercial honey were prepared in DMEM from 1% to 50% (v/v). One milliliter of each concentration was then added to cell culture monolayer 1h before and 1hr after and simultaneously with 100 PFU of the virus. Uninfected monolayer of cell line was incubated with different concentration of honey as negative controls.

Infectivity Assay:
Virus titration was carried out on 24 micro plates (NUNC), and was incubated for 3 days under 36°C and 5% Co2. The infectivity titer was determined by PFU. All experiments were performed three times in quadruplicates.

Statistical Analysis:
Statistical analysis was performed by SPSS software (SPSS for Windows, 11.0, 2001, SPSS Chicago, Illinois, USA) using Kruskal Wallis test.

RESULTS AND DISCUSSIONS

A complete viral inhibition was noticed using 5% and higher concentrations of honey added to cell culture simultaneously or one hour before infection with HSV-1 [Figure 1].

In another experiment when different concentrations of honey were added to the cell culture one hour after infection with HSV-1, viral inhibition was observed at 30% or higher concentrations [Figure 2]. Carbohydrate solution at the same concentration did not have any inhibitory effect on HSV-1.

Fig. 1: Growth of HSV-1 in the presence and absence of various concentrations of honey one hour before infection. Data from three experiments in quadruplicate are shown as Mean±SEM.
Fig. 2: Growth of HSV-1 in the presence and absence of various concentrations of honey one hour after infection. Data from three experiments in quadruplicate are shown as Mean±SEM.

Discussion:
The potential biologic and therapeutic effects of honey have been recognized since antiquity. However, few studies on its properties have been reported in modern medicine. (Molan P., 2000; McInerney F., 1990) Honey exhibits potent antimicrobial activities against pathogenic and non-pathogenic bacteria, yeast and fungi. (Zaghloul AA, et al., 2001; Ghabanchi J, et al., 2010) But, little investigation has been reported on antiviral activity of honey. Zeina et al. demonstrated the efficient anti-rubella activity of honey at varying concentrations (Zeina, B. et al., 1996).

According to our results, complete viral inhibition was achieved by 5% and higher concentrations of honey added to cell cultures simultaneously and one hour before infection with HSV-1. In another experiment, when various concentrations of honey were added to cell culture one hour after infection HSV-1, viral inhibition was only observed at 30% or higher concentrations. This may be consistent with the presence of glucose-oxidase that generates hydrogen peroxide and gluconic acid (Salomon, D. et al., 2010).

A therapeutic application of Canadian propolis against viral and bacterial infections has already been described. Propolis was reported to be effective against respiratory tract infection in children and genital infection by Herpes simplex virus type 2. (Cohen, H.A. et al., 2004; Vynograd, N. et al., 2000) Patients with recurrent genital Herpes infections were treated with C. propolis containing natural flavonoids and acyclovir ointments, in which the healing process of HSV-2 lesions was faster in C. propolis treated patients (Cohen, H.A. et al., 2004).

Schnitzler et al., (2010) demonstrated that a topical application of C. propolis for the treatment of oral herpetic infections with Herpes simplex virus appears promising, especially for those patients suffering from frequent recurrences. (Schnitzler, P. et al., 2010) In the present study, we showed that honey at different concentrations could inhibit the growth of HSV1, so it can be considered as a therapeutic choice in folk medicine.

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REFERENCES


