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Antiviral activity of *Momordica charantia*: A preliminary study on in vitro anti herpes simplex virus

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ABSTRACT

Praseno, Suparwoto S, and Ning Rintiswati - *Antiviral activity of Momordica charantia: A preliminary study on in vitro anti herpes simplex virus*

Traditional medication has been used by Indonesian people since long time ago and still continuing up to the present time. Many of them proved to be effective in curing various diseases. In this study an in vitro activity of *Momordica charantia* againts Herpes Simplex Virus type 2 has been evaluated by standard method of plaque reduction assay. The aim of this study is to determine whether traditional medicine we are searching has an antiviral activity. The results showed that total inhibition of plaque formation on HSV 2-infected Vero cell line was achieved at concentration of 8% v/v of crude extract, whereas the concentration of 1% v/v was capable of reducing the number of plaques by approximately 50% (inhibitory dose50 = ID50). These results were very interesting as even with only small amount of crude extract we could get ID50. We expect that much lower concentration will be required to obtain ID50 if we use purified extract in the assay. Further studies are needed to elucidate other properties of the extract, including its in vivo antiviral activity, possible effect on other viruses, and mechanism of action.

Key words*: *antiviral activity - herpes simplex virus - Momordica charantia - traditional medicine - plaque reduction assay

ABSTRAK

Praseno, Suparwoto S, and Ning Rintiswati - *Aktivitas antiviral Momordica charantia: suatu studi pendahuluan in vitro anti herpes simplex.*

Pengobatan tradisional telah digunakan oleh orang Indonesia sejak jaman dulu dan masih berlangsung sampai sekarang. Banyak di antara pengobatan tersebut terbukti efektif dalam menyembuhkan penyakit. Pada penelitian ini aktivitas in vitro *Momordica charantia* (pare) terhadap virus herpes simpleks tipe 2 telah dievaluasi dengan cara *plaque reduction assay*. Tujuan penelitian adalah membuktikan adanya efek antiviral obat tradisional. Hasil menunjukkan bahwa hambatan total terhadap pembentukan plak pada biakan sel Vero yang diinfeksi HSV 2 dicapai oleh ekstrak pare pada konsentrasi 8% v/v, sedangkan pada konsentrasi 1% v/v jumlah plak berkurang sampai kira-kira 50% (*Inhibitory Dose*$_{50}$ = ID$_{50}$). Hasil tersebut sangat menarik karena hanya dengan sejumlah kecil ekstrak dapat dicapai ID$_{50}$. Diperkirakan bahwa ID$_{50}$ akan dicapai dengan dosis yang jauh lebih kecil jika dipakai ekstrak yang dimurnikan. Diperlukan penelitian lebih lanjut untuk mengetahui sifat ekstrak yang lain, di antaranya aktivitas aktiviral in vivo, kemungkinan efek terhadap virus lain serta mekanisme kerja ekstrak.

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INTRODUCTION

Herpes Simplex Virus is the cause of various infection of mucocutaneous and visceral organs. Classically, herpes virus type 1 (HSV-1) is associated with infection of oropharyngeal area,

whereas herpes simplex virus type 2 (HBV-2) is implicated with infection of genital organ. However, because of changes in sexual behavior, both type of the virus can cause infection of both sites.[1] From these sites the virus can spread and reach internal organs, including liver and brain. Encephalitis due to HSV usually occurs in patients with devastating disease or in immunocompromised state[1,2]. HSV infection during pregnancy may cause spontaneous abortion, stillbirth, and congenital malformations[3].

So far, the drug of choice for HSV infection is acyclovir, although vidarabine and gancyclovir are also commonly used. However, several studies showed that some strains of HSV-2 were resistance to acyclovir and other antiviral drugs[4,5].

Indonesian people have been using traditional medication since a long time ago. Many traditional medicine proved to be effective in cure many kind of diseases, including metabolic diseases such as diabetes mellitus and hypercholesterolemia, and some infectious diseases. Even with the introduction of modern medicine, these practices are still continuing up to the present time. Furthermore in the recent years several centers for traditional medicine research and development have been established, such as "Pusat Pengembangan Obat Tradisional" (PPOT) at Gadjah Mada University.

Based on that traditional practice, we are now searching for an antiviral agent from herbs available in our environment. In this preliminary study we observe the possible antiviral effect of *Momordica charantia* againts Herpes Simplex virus type 2.

MATERIALS AND METHODS

Preparation of crude extract of *Momordica charantia*. Approximately 25 grams of the herb was finely ground and liquid from this material was transfered into a clean bottle. The liquid was then centrifuged to pellet small particles in it. Supernatant was filtered through a membrane of 0,45 μm pore size and stored in a refrigerator. This extract would be used in the next experiment.

Preparation of HSV-2 suspension. HSV-2-infected Vero cell line (provided by Dr. Norbert Ryan, Victorian Infectious Diseases Reference Laboratory, Victoria, Australia) was cultivated in 100 ml of Dulbeco's Modified Eagle Medium (DMEM) Gibco supplemented with 10% Fetal Calf Serum (FCS), gentamycin 10 μg/ml, and Fungizone (Squibb) 0,10 μg/ml using 800 ml-tissue culture flask (Nunc). Culture was incubated at 37°C in a humidified incubator with 5% CO_2 atmosphere. After seven days of incubation period culture supernatant was transferred into sterile bottle. The supernatant was added with 10% polyethylene glycol and 1 M NaCl (final concentration), and mixed by gently stirring for several hours to dissolve. The mixture was left overnight at 4°C to precipitate virus particles. The precipitate was pelleted by centrifugation and resuspended in borate-saline buffer and stored at -20°C.

Assay of antiviral activity by standard plaque reduction assay method. Normal Vero cell line was cultured in DMMEM supplemented with 10% FCS, gentamycin, and Fungizone in 50 ml-tissue culture flaks. Culture was incubated in a humidified incubator with 5% CO_2 atmosphere at 37°C. After confluent monolayer growth of the cell was achieved, culture supernatant was decanted and 2 ml of fresh medium was added into the flasks. Culture was inoculated with 0.2 ml of HSV-2 suspension and was incubated at 37°C for one hour to allow the virus to adsorb. Supernatant was decanted and replaced with 5 ml of fresh medium. 400 μl, 300 μl, 200 μl, 100μl, and 50μl and 25 μl of the extract were added into individual flask and the culture was incubated at 37°C. One flask of the culture was served as control.

After 3 days of incubation supernatant was decanted and the culture was stained with crystal violet stain to visualize the presence of plaques. The number of plaques in each flask was counted and compared to that of control.

RESULTS

Result of the experiment is summarized in TABLE 1.

Total inhibition of plaque formation was achieved by the extract with concentration of 8% v/v, whereas inhibition of 50% plaque formation (inhibitory dose = ID_{50}) was achieved by concentration of aproximately 1% v/v. There was no in-

hibition of the growth of Vero cell line. It seems that the extract has no "antiseptic-like" effect.

TABLE 1. - Inhibitory effect of momordica extract on plaque formation on HSV-2 infected Vero cell line culture.

Addition of extract (µl)	Final concentration of extract (% v/v)	Number of plaque	% inhibition*
400	8	0	100
300	6	5	94,68
200	4	8	91,49
100	2	23	75,53
50	1	49	47,87
25	0,5	87	7,44
0 (control)	0	94	-

*Percentage of inhibition is calculated by the following formula:

$$\frac{\text{No. of plaque in control - No. of plaque}}{\text{No. of plaque in control}} \times 100\%$$

DISCUSSION

Momordica charantia or spring cucumber has long been used for the treatment of hepatitis by Indonesian people although they do not actually know the cause of the disease. However, it seems that this medication has been effective in curing many cases of hepatitis. Based on this fact we predicted that spring cucumber may have antiviral activity as the most common cause of hepatitis is of viral origin.

Since hepatitis viruses are quite difficult to isolate or even it cannot be adapted in cell culture system, we used herpes simplex virus type 2 (HSV-2) to evaluate antiviral activity of *Momordica charantia*. In this study we used a crude instead of purified extract.

Total inhibition of plaque formation on HSV-2-infected Vero cell line culture was achieved by extract at the concentration of 8% v/v. Reduction of plaque number by 50% was achieved by the concentration of 1% v/v (TABLE 1). It means that inhibitory dose (ID_{50}) was approximately 10 µl/ml. It is estimated that much lower concentration would be required to get ID_{50} if purified extract was used.

Despite antiviral activity, the extract did not inhibit the growth of Vero cell line. It is likely that the extract had no toxic effect on the cell.

Purification of the extract and evaluation of its in vitro activity againts HSV-2 was not under investigation. Inevitably, we would also evaluate its in vitro antiviral activity and possible effect of the extract on other viruses as well as on other pharmacological aspects. Hopefully, the extract we are studying would be, to some extent, superior to already commercially available antiviral agents.

CONCLUSION

Based on the data obtained in this study it was concluded that crude extract of *M. charantia* has anti HSV-2 activity in vitro.

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