An inhibitory effect of Kerra™, KS™, and Minoza™ on herpes simplex virus wild-type and mutant strain

Method

Cytotoxicity

Vero cells at a density of 10^4 cells/ml were seeded into a 96-well plate per well and maintained for 24 hours. Each extract was added to the cells at various concentrations and incubated for 48 hours. The absorbance at 540 nm of formazan dissolved in DMSO was measured after 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (Sigma-Aldrich, St Louis, MO, USA) treatment for 4 hours. Cell viability was calculated using (OD_{sample} x 100)/OD_{DMSO}.

Plaque assay

Pre-entry step

Vero cells at a density of 10^5 cells/ml were seeded into a 24-well plate per well and maintained for 24 hours. The virus at multiplicity of infection at 0.002 (MOI 0.002) was mixed with each extract and then incubated for 1 hour at 37ºC. The mixture was subjected to the cell and then incubated for 2 hours. After removing the unbound virus, the fresh medium containing 0.2% CMC was added to the cells and continuously maintained for 48 hours. The cytopathic effect was observed under the microscope and then was fixed and stained with 10% formaldehyde and crystal violet. The number of plaque formations were counted by the naked eye. The percentage of viral inhibition was calculated using 100 subtracting the percentage of viral infection.

Post-entry step

Vero cells at a density of 10^5 cells/ml were seeded into a 24-well plate per well and maintained for 24 hours. The virus at multiplicity of infection at MOI 0.002 was infected in the cells for 2 hours at 37ºC. After washing the cells to remove unbound virus, each extract at various concentrations was prepared in a fresh medium containing 0.2% CMC subjected to the cells and continuously cultured for 48 hours. The percentage of viral inhibition was performed as mentioned above.

Result

Kerra™, KS™, and Minoza™ completely inhibited HSV-1 KOS infection in pre-entry step

To investigate the ability of each extract to prevent viral binding with cell receptor through direct interaction between virion and extract, each extract was pre-incubated with HSV-1 KOS. If the extract directly interacts with a viral molecule particularly glycoprotein located in a viral envelope, the virus cannot infect the cells. From the result, all of the extracts at 100 and 200 μg/ml significantly inhibited HSV-1 infection in the pre-entry step (Figure 1).

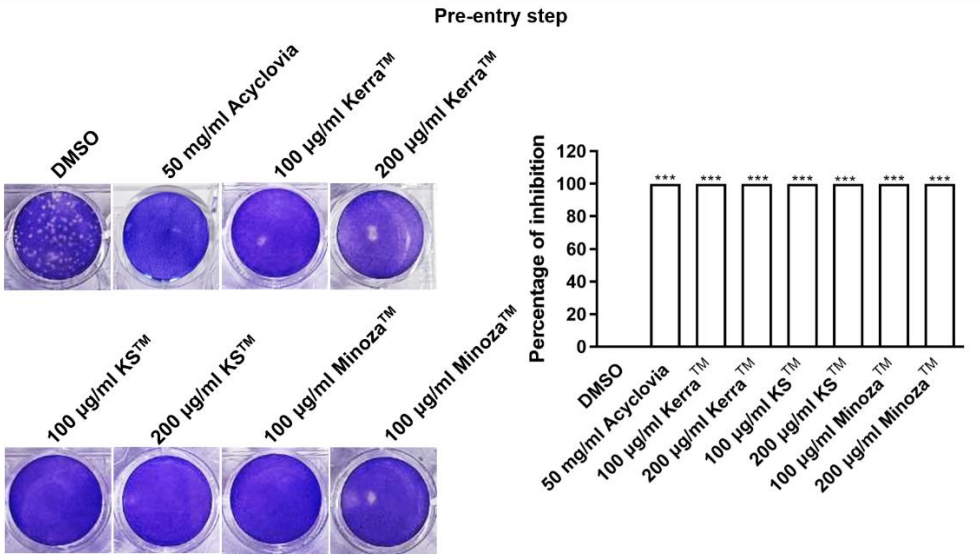

Figure 1. Kerra™, KS™, and Minoza™ completely inhibited HSV-1 KOS infection in the pre-entry step. Kerra™, KS™, and Minoza™ were pre-incubated with HSV-1 at MOI0.002 before infecting Vero cells. Plaque formation was stained with crystal violet and counted to calculate the percentage of inhibition. The symbol *** indicates the significant difference ($P < 0.001$) between extract and DMSO-treated cells.

Kerra™, KS™, and Minoza™ significantly inhibited HSV-1 infection in the post-entry step

To investigate the ability of each extract to reduce plaque formation after viral internalization, each extract was treated with HSV-1-infected cells. If the extract can reduce the number of plaques, it might target various mechanisms not only direct binding to viral molecules. In addition, the extracts reduce the size of plaque compared with DMSO. The result showed that all extracts particularly 200 µg/ml significantly inhibited HSV-1 infection in the post-entry step in a dose-dependent manner (Figure 2).

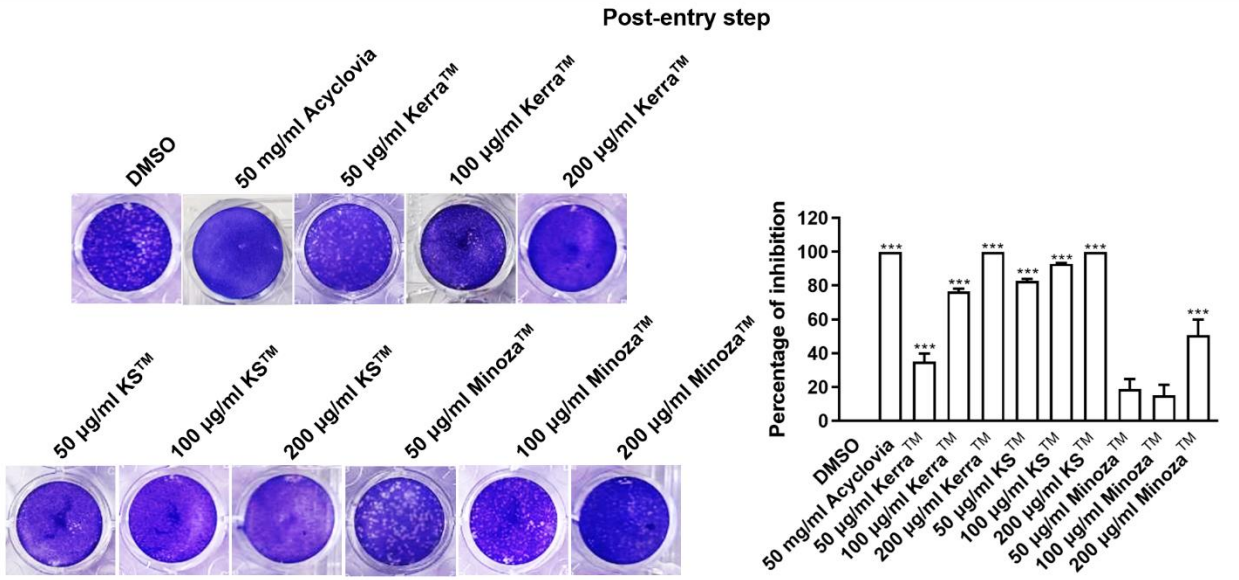

Figure 2. Kerra™, KS™, and Minoza™ significantly inhibited HSV-1 KOS infection in the post-entry step. HSV-1 at MOI0.002 was infected in Vero cells for 2 hours before adding the extract. Plaque formation was determined after Kerra™, KS™, and Minoza™ treatment for 48 hours, and counted to determine the percentage of inhibition. The symbol ** and *** indicates the significant difference ($P < 0.01$ and 0.001 respectively) between extract and DMSO-treated cells.

Cytotoxicity of Kerra™, KS™, and Minoza™ in Vero cell

Table 1 represents the ability of Kerra™, KS™, and Minoza™ on the potential HSV-1 treatment. KS™ (SI = 0.0922) is the most efficient drug and safe for HSV-1 treatment and is followed by Minoza™ (SI = 0.062), and Kerra™ (SI = 0.0427) respectively.

Table 1. The cytotoxic concentration at 50% (CC), inhibitory concentration at 50% (IC50), and selective index of Kerra™, KS™, and Minoza™ in Vero cells and anti-HSV-1

Extract	CC50 (μg/ml)	IC50 at 50%	SI
Kerra™	2.846 ± 0.025	66.543±4.759	0.0427
KS™	2.349 ± 0.026	25.476±3.430	0.0922
Minoza™	11.670 ± 1.804	187.807±39.869	0.062

Conclusion

According to SI value, KS is the best for HSV-1 treatment. The possible mechanism of Kerra™, KS™, and Minoza™ is directly bound to viral molecules because they can reduce the size of plaque (Figure 2). However, these extracts may alter the cellular biological activity which needs to be further investigated.

Further experiment

1. Plaque assay in HSV-1 dxpIII (phosphonoacetic acid and phosphonoformate resistance) and HSV-2
2. *GAPDH*, *UL30*, *gD* and *IFN*-α mRNA expression
3. Beta-actin and gB protein expression