Thai Herbal Formula 'Kerra Capsules' Effectively Inhibits H1N1 Influenza Virus Infection

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Abstract

The rising incidence of severe influenza infections presents a significant challenge to global health systems. Traditional Thai herbal medicines, particularly those based on the Takasila formula, have shown potential in treating infectious diseases. This study evaluates the efficacy of "Kerra Capsules," a registered Thai herbal medicine, in inhibiting H1N1 influenza virus infection. Laboratory tests demonstrated that Kerra Capsules inhibited H1N1 virus infection in MDCK cells showed an IC50 of 241.00±7.91 μg/mL and no toxicity. Additionally, a dose-dependent reduction in infection was observed with decreasing concentrations of

Kerra. Cell cytotoxicity, assessed using the MTT assay, yielded a CC50 of 315.4 ± 5.06 µg/mL. Neuraminidase inhibition assays also suggested that Kerra's antiviral action may be mediated through neuraminidase inhibition, with greater reduction in enzyme activity at 10 µg/mL. These findings support the potential of Kerra Capsules as an anti-influenza treatment and highlight the necessity for further research into its therapeutic applications.

Key words: Influenza, MDCK, Neuraminidase

Introduction

Herbal medicine is Thai wisdom that has been passed down from generation to generation for more than 2,000 years. According to the herbal plant database, Thailand has over 1,800 types of beneficial local herbs (Department of Academic Affairs, Ministry of Education, 1999) and has a long history of being used for treating diseases. The treatment of severe fevers according to the Takasila scriptures involves seven different herbal formulas, ranging from the first to the seventh formula. The first formula is "Ya Kaeo 5 Duang" (Five Roots Medicine), followed by the second formula, "Ya Prasa

Phiw Phai Nok" (External Skin Medicine), the third formula, "Ya Phon Phai Nok" (External Spray Medicine), the fourth formula, "Ya Phon and Ya Kin" (Spray and Oral Medicine), and the fifth formula, "Ya Prae Khai" (Fever Transforming Medicine), which includes 11 ingredients. The sixth formula is "Ya Phon Prae Phiw Phai Nok" (External Skin Transforming Spray Medicine) with 4 ingredients. The seventh formula, called "Ya Khrop Khai Takasila" (Takasila Fever Encompassing Medicine), consists of 14 ingredients: *Santalum album* (Sandalwood, Red sandalwood), *Styrax benzoin* (Benzoin resin), *Sauropus androgynus* (Leaf of Sauropus), *Pterocarpus indicus* (Pterocarpus), *Scilla sp.* (Bulb of Scilla), *Cissampelos pareira* (Gac root), *Aquilaria malaccensis* (Agarwood), *Cissus quadrangularis* (Veldt Grape, Cissus), *Pinus spp.* (Pine wood bark), *Cissus quadrangularis* (Cissus root), *Vatica spp. (*Swada, or Vatica), *Tinospora crispa* (Tinospora, Gulancha), *Punica granatum* (Pomegranate), *Chionanthus retusus* (White flower, Chinese fringe tree). The treatment involves regular consumption until recovery (Department of Academic Affairs, Ministry of Education, 1999). The "Ya Khrop Khai Takasila" or the seventh formula is widely used, especially during epidemics involving fever. Vetchakorn Osot has modified this

herbal formula by replacing some currently rare herbs such as agarwood, eaglewood, *Sauropus androgynus* leaves, Swada leaves, and *Cissus quadrangularis* with *Tinospora crispa*, which has properties to reduce fever, expel phlegm, prevent and treat infections, reduce inflammation, boost immunity, increase strength, and slow aging. Various parts of *Tinospora crispa* contain antioxidants. The formula is encapsulated to ensure comprehensive efficacy, high stability, and compliance with modern medical principles (Vetchakorn Osot, 2021). This KERRA has evidence against COVID-19 virus and HEPES virus. Therefore, it is interesting to investigate that it has effect against influenza too.

Material and Methods

1. Cell Cytotoxicity

MDCK cells were seeded in a 96-well plate at a concentration of 20,000 cells per well and incubated overnight. The cells were then treated with Kerra at concentrations ranging from 1,000 to 1.56 μg/mL for 72 hours. After the culture medium was added, it was changed to 100 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium

bromide (MTT) (Thermo Fisher Scientific, USA) solution (0.5 mg MTT in 1 mL culture medium) and incubated at 37 ℃ for 3 hours. After that, the medium was discarded and 50 μL of 100% DMSO was placed in each well for solubilization of formazan. The absorbance of each well was measured at 570 nm using a microplate spectrophotometer (BioTek Synergy HTX, USA)

2. Anti H1N1 virus assay

MDCK cells were seeded in a 96-well plate at a concentration of 20,000 cells per well and incubated overnight. The cells were then treated with Kerra at concentrations ranging from 1,000 to 1.56 μg/mL for 1 hour. After the incubation, H1N1 virus was added at an MOI of 100 and incubated for another hour. Following this, the virus was washed off, and 200 μL of cell culture medium were added. The cells were then cultured for 3 days. After the incubation, the cells were fixed with formalin and stained with crystal violet. Images were then recorded.

3. Anti neuraminidase assay

Neuraminidase assay was done according to neuraminidase assay kit from MERCK (catalogue number

MAK121). Prepare the master reaction mix as outlined in Table 1, with 80 μL allocated for each sample, blank, and standard reaction well. Added 80 μL of the respective reaction mix to each well and ensure thorough mixing, either by using a horizontal shaker or pipetting. Then, incubate at 37°C and protected from light, for 20 minutes. Measure the absorbance of samples and standards at 570 nm for colorimetric assays. For fluorometric assays, set the excitation wavelength (λex) to 530 nm and the emission wavelength (λem) to 585 nm, and measure at 20 minutes. Extend the incubation for an additional 30 minutes at 37°C before measuring absorbance again at the same wavelengths to determine at 50 min.

Table 1.

Reaction Mixes Reagent	Sample and Standards	Sample blank
Assay Buffer	30 μL	85 μL
Substrate	55 μL	–
Cofactors	1 μL	1 μL
Enzyme	1 μL	1 μL

Dye Reagent	0.5 μL	0.5 μL

Results

The tests showed that Kerra exhibited biological activity was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay is CC50 315.40 ± 5.06 μg/mL, as shown in Figure 1. Kerra, at a concentration of 1,000 μg/mL, was able to completely inhibit H1N1 influenza virus infection in MDCK cells, compared to untreated cells; however, it showed toxicity, The Kerra tested H1N1 virus infection in MDCK cells showed an IC50 of 241.00±7.91 μg/mL and 80% cell viability, indicating its potential as candidate inhibitor (Figure 2). The percentage of infection gradually increased as the concentration of Kerra decreased.

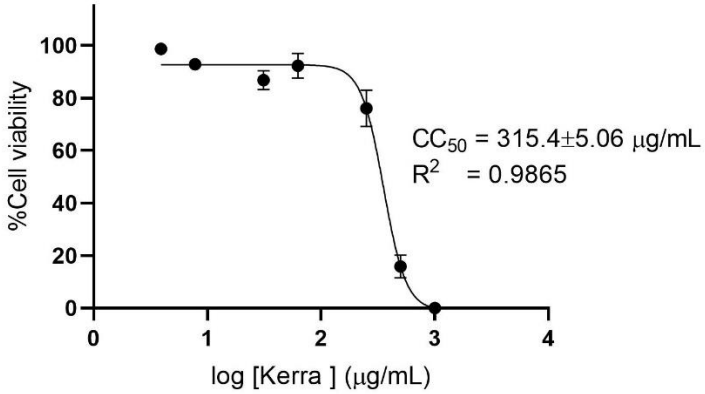

Figure 1 Cell viability of Kerra on MDCK cells line

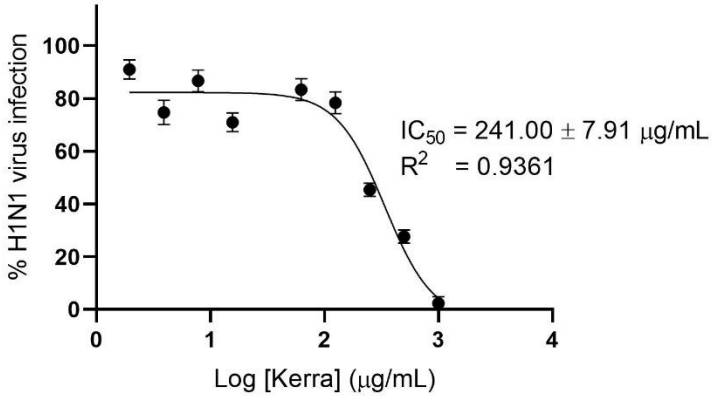

Figure 2 The percentage of H1N1 virus infection in MDCK cells treated with various concentrations of Kerra.

For mechanism of Kerra, the neuraminidase enzyme was tested against the crude extract. The result showed that Kerra might inhibit virus infection via inhibition of neuraminidase enzyme as shown in Figure 3. Some inhibition of the neuraminidase activity was observed, but the observed inhibition was not dose dependent.

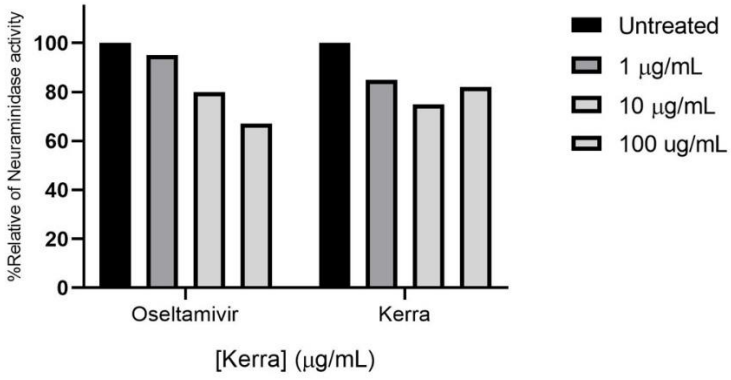

Figure 3 The activity of the neuraminidase enzyme at concentrations of 1-100 μg/mL of Kerra.

Conclusion

The experiment demonstrated that Kerra effectively inhibited H1N1 influenza virus infection in MDCK cells, with

an IC50 of 241.00±7.91 μg/mL and no observed toxicity. Furthermore, in the neuraminidase activity assay, Kerra at a concentration of 10 μg/mL was more effective in reducing the activity of the neuraminidase enzyme.

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