Cytotoxicity Concentration of Acyclovir and *Clinacanthus nutans* (Burm. f.) Lindau. Extract to Koi Fin Cell Line

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**Introduction**

Acyclovir has a direct viral replication inhibition effect to thymidine kinase gene of virus in herpesviridae family (2). It has been used for the treatment of human herpesvirus *Clinacanthus nutans* (Burm. f.) Lindau or Payayor is a herbal medicinal plant with antiviral activities. Clinical trials for the treatment of genital herpes and varicella zoster were reported (4). However, the anti-herpes virus activity of both Acyclovir and plant has not been investigated in fish herpesvirus. Therefore, in the present study, we applied various concentration of Acyclovir and *C. nutans* plant extract to observe their toxicity to KFC cell line as a primarily study for future application as an antiviral medicine against Koi herpesvirus which has been a global epizootic in common carp and koi.

**Materials and Methods**

Koi Fin cell line (KFC) used was developed by Dr. Peiyu Lee, Institute of Medical Biotechnology, Taiwan. Acyclovir stock solution was suspended by DMSO at 100 mg/ml while *C. nutans* plant extract stock solution was suspended in the cell culture medium at 0.01% concentration. Cell was prepared in 24-well plates at 24°C. After incubation for 24 hours, acyclovir was added at concentration of 100, 50, 25, 10, 5 and 1 µg/ml. DMSO at 0.1 and 0.01% as for diluents control. *C. nutans* plant extract stock solution was added at 0.01, 0.005, 0.001, 0.0005, 0.0001 and 0.00001% into 3 wells at each concentration. After 72 hours at 24°C incubation, cell was trypsinized and stained with 2% trypan blue for viable cell counting. The experiments were done in triplicates. The result was reported as 50% cytotoxicity concentration.

**Results and Discussion**

After 24 hours all cells exposed to the *C. nutans* plant extract in 0.01 and 0.005% wells died while others were normal compared to the control. After 72 hours, cells were harvested from each well for counting and calculating for % viability and % cytotoxicity. At concentration above 50% cytotoxicity is identified as non-toxic concentrations which were at 5 µg/ml and 0.001% for acyclovir and *C. nutans*, respectively. Both DMSO concentrations were not toxic to KFC.

The cytotoxicity results indicated that Acyclovir at the concentration of 10, 25, 50 and 100 µg/ml and *C. nutans* plant extract at 0.005% and 0.01% damaged KFC cells and had the cytotoxic effect to the cell in those concentrations. For non toxic concentration of acyclovir began at 5 µg/ml and *C. nutans* plant extract at 0.001%. This safety concentration are lower than these reported for human used (1, 3). The present results may be used as a minimum concentration for effective antiviral concentration against Koi herpes virus (KHV), which will be very useful for treating or preventing the disease and can be used as basic data for other future studies.

**References**