SCREENING OF A HERBAL PREPARATION (PHEKO) FOR ANTI HIV-1 REPLICATION PROPERTIES

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ABSTRACT

Infection with Human Immunodeficiency Virus (HIV) is of global public health concern. Highly active antiretroviral therapy (HAART) significantly reduces morbidity and mortality due to AIDS, the outcome of HIV infection. However, due to the limitations of HAART, alternative forms of treatment, such as herbal therapies, are common particularly in developing communities. The current study investigated the anti-HIV effects of a herbal preparation (Pheko) for inhibitory effects against potential therapeutic targets of HIV.

Several extract types of Pheko were assayed for inhibitory effects against HIV-1 reverse transcriptase (RT) and integrase (IN) in a cell-free method. Additionally, the methanol extract was studied for anti-replicative properties against pseudoviruses of HIV-1 subtypes B and C of wild and resistant types. Viruses used included wild type and resistant mutants of NL4-3 (HIV-1 subtype B), wild type IndieC (HIV-1 subtype C).

The cytotoxicity of extracts against the human cell lines (U87.CD4.CCR5 and Vero T35) was also evaluated with the Lactate dehydrogenase (LDH) for cell death and MTT (3-[4, 5-Dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide) for cell viability. Methanol extract of Pheko showed the highest cell death with a CC_{50} of 0.5mg/ml using the LDH assay, and water extract showed the least cell death with the CC_{50} of 1mg/ml. Evaluation of cell viability using MTT assay showed that at the maximum concentration (2mg/ml), dichloromethane extract inhibited cell viability more than other extracts with 81% inhibition, and methanol inhibited 57% of cell viability.

The water extract of Pheko inhibited integrase with an IC_{50} of 0.125mg/ml. Dichloromethane extract was most inhibitory to RT with an IC_{50} of 0.003 mg/ml, and the water extract was the least inhibitory with an IC_{50} of 12.5 mg/ml. The methanol extract also showed excellent HIV-1 RT inhibition (IC_{50} of 0.02mg/m).

Transformation of competent cells were successfully generated and successfully used to prepare plasmids for HIV-1 subtype C-based pseudoviral packaging. In the pseudovirus assays, the methanol extract inhibited RT activity of the HIV-1 subtype C virus (IndieC) with an IC_{50} of 0.025 µg/µl. Of interest was the strong inhibition of the NRTI and NNRTI resistant strains of HIV-1 subtype B (NL4-3) by the methanol extract with IC_{50} values of 0.035 µg/µl and 0.021 µg/µl, respectively. Low selective indices were observed when U87.CD4.CCR5 cell line where used as target for the pseudoviruses (range 0.01-0.023).

In the current study only the methanol extract was investigated in the pseudovirus assay for anti-HIV replicative property. Future work will particularly look at the water extract. In conclusion, this preliminary data shows that the methanol extract of Pheko can potentially inhibit HIV-1 Subtype C and viruses resistant to nucleoside RTI and non-nucleoside RTI.