

of special medium. The extracts were clarified by centrifugation, and determined their virucidal activity. Influenza virus A/Hong Kong/1/68 (H3N2) was diluted with a special medium containing (experiment) and not containing the calluses' extract (control) to a concentration of 10,000 LD₅₀. Control and experimental samples were incubated at 4 °C for 20 h and then at a temperature of 37 °C for 4 h. Number of infectious virus in the samples was determined by titration on fragments of chorio-allantoic membranes of 12–14 days old chick embryos.

Results: Held on 3 experiments with each of the extracts. LD₅₀ in control was 41g. The highest levels of the virucidal activity had calluses' extracts from *Nicotiana suaveolens* and *Nicotiana alata* (distinctions from control were 3.2 and 2.31g LD₅₀ accordingly). Calluses' extracts from *Nicotiana pauciflora* and *Nicotiana good-speedii* did not demonstrated virucidal activity. Virucidal activities of calluses' extracts from *Nicotiana exelsior*, *Nicotiana rustica* and *Nicotiana trigonophylla* were highly expressed (distinctions from control were from 1.5 to 1.91g LD₅₀).

Conclusion: Calluses' extracts from different tobacco-plants have wide spectrum of virucidal activities levels.

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Impact of HIV Coinfection on State of Immunology of Patients with Chronic HCV-Infection

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Aim of study: to evaluate features of T-cellular immune response and associated cytokine profile in HIV/HCV-infected patients according to clinical and immunological stage of HIV-infection.

Materials and methods: Serum levels of the cytokines (interleukin-2 (IL-2), IL-4, IL-8, IL-6, tumor necrosis factor α (TNF- α), γ -interferon (IFN- γ), IL-10) and CD4+, CD8+ α -lymphocytes of blood have been investigated by ELISA method (DRG International, Inc., USA) and flow cytometry (Becton Dickinson facs caliber, USA) in 3 groups of patients. 1 group – 33 patients with HIV/HCV coinfection on 1 and 2 clinical stages of HIV-infection (WHO clinical classification, 2006); 2 – 10 patients with HIV/HCV on the 3 and 4 stages of HIV-infection 3 group – 25 patients with HCV-infection. Control group included 20 health people.

Results: We have detected that in the first group of patients hyperstimulation of immunity system took place: these patients had significantly higher level of IL-8 (medians, Mann-Whitney *U*-Test) (170 pg/ml vs. 110 pg/ml, $p < 0.02$), IL-2 (2.4 and 1.15 U/ml, $p < 0.02$), INF- γ (1.5 IU/ml vs. 1.15 IU/ml, $p < 0.05$), IL-4 (125.4 pg/ml vs. 85 pg/ml, $p < 0.05$) and CD8+ lymphocytes number (926 ± 579.8 vs. 450.3 ± 264.7 , $p < 0.01$) in comparison with HCV-infected patients. Progression of immunosuppression and development of 3rd and 4th stages of HIV-infection has been associated with fast decreasing of cellular immunity response, shift to Th2-type immunity response, activation of systemic inflammatory response because of manifestation of opportunistic infections.

Conclusion: On early stages of HIV-infection immunity control under HCV in HIV/HCV coinfecting patients is more strong in comparison with mono HCV-infection. Study of immunopathogenesis of HCV/HIV coinfection may be a key for new approaches in elaboration of antiviral treatment of HCV-infection.

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Styrylpyrone Derivative of *Goniiothalamus umbrosus* Inhibit HSV-1 Infection During Viral Early Replication Cycle

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We have observed a potent HSV-1 antiviral activity by Styrylpyrone derivative (SPD) extracted from the root of *Goniiothalamus umbrosus*. In the virus yield reduction assay, we observed reduction of virus yield after 48 h of treatment with SPD, with effective concentration (EC₅₀) were determined at 2.290 μ M in post-treatment study. Significant 100-fold reduction of virus plaque forming unit were observed when infected cell treated with SPD at the concentration of 12.5 μ M. We investigated the possibility of antiviral activity being retained with lower concentrations of SPD. We found 16-fold and 4-fold reduction when infected Vero cell were treated with 7.5 μ M and 5 μ M respectively. In plaque reduction assay, more than 95 percent of HSV-1 plaque successfully reduced with treatment of 12.5 μ M of SPD, confirming the antiviral activity exhibited by SPD. Furthermore, in the time dependent study, more than 75 percent reduction observed when SPD were administered at 2 h post-infection and the reduction percentage then dropped with the delay of the treatment time. The time-dependent activity may have suggested inhibition of viral early replication cycle. However, time removal experiment showed that 75 percent of reduction could only be observed after 10 hours post-treatment with SPD (results not shown). Thus, this might indicate longer time is needed for the adsorption of SPD into the cell before it can react. In this regards, the earlier treatment being administered to infected cells, the higher the chances for SPD to react at the intended HSV-1 cycle during infection. On the other hand, SPD mode of action might actually target the later time-point in HSV-1 virus cycle.

Keywords: HSV-1; Styrylpyrone derivative; Viral inhibitory activity

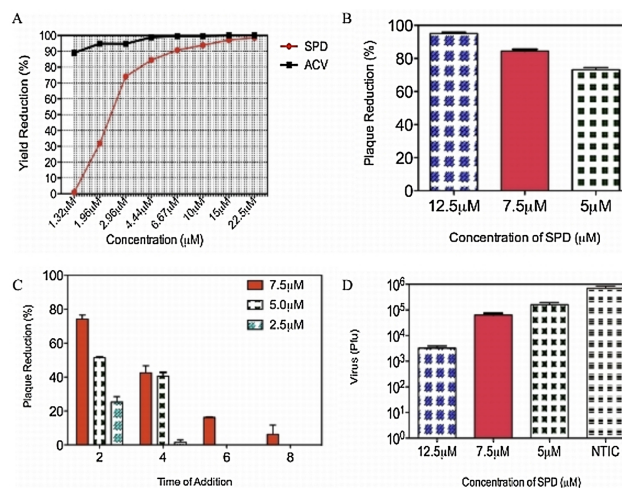


Figure 1. (A) Determination of SPD 50 percent effective concentration (EC₅₀) in yield reduction assay at 2.290 μ M. (B) Determination of SPD antiviral activity in virus plaque reduction assay. (C) Determination on effect of different time of treatment additions on HSV-1 plaque reduction at different SPD concentrations. (D) Titration of virus yield expressed as plaque forming unit (pfu) when infected cells were treated at different doses compared to non-treated infected cells (NTIC).

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