

EFFECT OF SOME ANTIVIRAL SUBSTANCES ON SOME FOOD-BORNE VIRUSES

By

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ABSTRACT

Naglaa Abozied Seif EL-Alfy: Effect of some Antiviral Substances on some Food-Borne Viruses. Unpublished Ph.D. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2019.

The antiviral role of *Moringa Oleifera* leaves extracts ((chloroform (CL), Ethyl acetate (E.A), methanol 80% (M) and n.butanol (n.b)) to control Coxsackie B (COX-BV) and Hepatitis A (HAV) viral infections were monitored in vitro and in vivo compared with human interferon alpha (IFN α -2a) in order to evaluate the antiviral activity of *Moringa* leaves extracts. The total phenolic content of CL, E.A, M and n.b extracts of *Moringa* leaves extracts were estimated using spectrophotometry, it was found that, the E.A extract contained the highest ratio of phenol followed by n.b extract. In vitro, cytotoxicity was determined for *Moringa* leaves extracts on viability of HEP-2 and Vero cells using MTT assay. It was found that, The 97.7 μ g/ml concentrations of four extracts were considerably non-toxic for HEP-2 and Vero cell lines culture. Antiviral activity of four *Moringa* leaves extracts compared with IFN α -2a to HEP-2 cell lines against COX-BV and Vero cell lines against HAV viral infection was determined by assessment of the COX-BV and HAV viruses infectivity titer declining rate and relative residual living cell count using End Point Assay, using three ways pre-treatment, co-treatment and post-treatment cell line was treated with *Moringa* extracts. It was found that, the extract E.A and n.b were better as antiviral activity of COX-BV in pre-treatment and HAV in co-treatment respectively compared with IFN α -2a. In vivo, toxicity of *Moringa* extracted by E.A and n.b were evaluated using male albino Wistar rats, the E.A and n.b safe concentration extracts 97.7 and 390 μ g/ml toxic concentration were given oral gavage administered daily for 28 days. It was found that, the toxic concentrations of E.A and n.b *Moringa* extracts at 390 μ g/ml were toxic on both the rats and the cell culture and the nontoxic concentration of E.A and n.b extracts

at 97.7 µg/ml were safe on the rats as well as the cell culture (HEP-2 and Vero cells). Antiviral experiment was performed using nontoxic conc. of E.A and n.b extracts comparing with the interferon on rats infected with COX-BV or HAV. The COX-BV and HAV titers in the infected intestinal of rats and treated rat with E.A, n.b and IFN- α 2a at 5 weeks were determined by infecting a particular HEP-2 and Vero cell line respectively and determined the highest dilution producing cytopathic effect in 50% of the inoculated cells. It was found that, The COX-BV and HAV infectivity titer 10^3 TCID₅₀ as in post treatment rat with E.A, n.b and IFN- α 2a showed antiviral activity in addition both antiviral showed an undetected virus infectivity titre on the 3rd week post infection. Ab level in blood collected from treated rat with E.A, n.b and IFN- α 2a and infected with COX-BV virus and HAV was measured at the end of each week for 5 weeks using ELISA technique. The immunity against COX-BV and HAV it was noticed that, antiviral E.A and n.b extracts showed decreases level of ab at 4th and 3rd week respectively, compared with rats infected with COX-BV and HAV only as positive control, no treated rats as negative control and rats treated with IFN- α 2a. Cytokines (Interlukin-6 (IL-6) and Interferon gamma (IFN- γ)) levels were measured in the infected treated rats with E.A and n.b extracts, infected treated rats with IFN- α 2a and rat infected with COX-BV or HAV virus only on the 3rd, 7th and 15th days post viral infection using DAS ELISA technique. Regarding IL-6, data showed that the highest level of IL-6 was reported in infected rats with virus only on the 3rd day. While infected treated rats with E.A and n.b extracts and IFN- α 2a showed a decreased IL-6 level at the same time. Regarding IFN- γ , data showed that, the highest level of IFN- γ was reported in infected treated rats with E.A and n.b extracts on the 3rd day post treatment then infected treated rats with FN- α 2a then infected rats with virus only. MX gene expression values were estimated in HEP-2 and Vero cells (in vitro) and intestinal tissue of rats (in vivo) treated with non-toxic conc. E.A, n.b extracts and IFN- α 2a that stimulate the induction of MX-mRNA as a marker of antiviral activity using specific primers by rt-

RT-PCR. It was found that, in vitro, the highest value for MX gene expression was in HEP-2 and Vero cells treated with IFN- α 2a then HEP-2 and Vero cells treated with E.A and n.b extracts compared to control cells. In vivo, the highest value for MX gene expression was in intestinal tissue of rats treated with E.A extract, then intestinal tissue of rats treated with IFN- α 2a, then intestinal tissue of rats treated with n.b extract compared to control intestinal tissues. Total Phenolic conc. (TPC) in rats serum were determined for serum of rats treated with E.A and n.b extracts and non-treated rats (control) using spectrophotometer. The result showed that, the TPC in serum of rat treated with E.A extract was higher than its conc. in serum of rat treated with n.b extract compared to control. The components present in the E.A and n.b extracts of Moringa leaf were identified by GC-MS analysis. It was found that, active compounds mutual between E.A and n.b leaves extracts of Moringa were: Phytol; (-)-Loliolide; 1,2-Benzenedicarboxylic Acid,3-Nitro; Methyl 15-Acetylhydroxypalmitate; Z,Z-8,10-Hexadecadien-1-ol; 2,6,10-Dodecatrien-1-ol,3,7,11-Trimethyl. Antiviral activity in Moringa extracts may be attributed to phenolic compounds and / or as a result of stimulation of COX-BV-sensitive HEP-2 cells and HAV-sensitive Vero cells or intestinal tissue of rats treated with E.A and n.b extracts to express MX gene protein. It was concluded that *Moringa* leaves extracts have antiviral activity against COX-BV and HAV.

Key Words.

Moringa Oleifera leaves extracts, phenol compound, IC50, Cytotoxicity, Antiviral activity, COX-BV and HAV Ab, IL-6, IFN- γ , MX gene, GC-MS.

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