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Short Communication

Antiviral activity of silymarin against Mayaro virus and protective effect in virus-induced oxidative stress

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ABSTRACT

Mayaro virus (MAYV) is a neglected arbovirus belonging to the family *Togaviridae*. Its infection leads to Mayaro fever, with clinical manifestations such as fever, myalgia, headache, rash, arthralgia, vomiting, and diarrhea. The most prominent complaint from infected person is the long-lasting arthritis/arthralgia. The treatment for Mayaro fever is mainly symptom-based and there are no vaccines or antiviral drugs currently available, thus, natural products with anti-MAYV activity may provide a potential alternative. Recent evidences suggest that oxidative stress plays an important role in MAYV infection and compounds capable of modulating oxidative stress could represent a novel therapeutic approach in modulating MAYV-associated oxidative cellular damage. Silymarin is a complex extracted of *Silybum marianum*, or milk thistle, and its major active compound is silybin, which has a remarkable biological effect. Its antioxidant and antiviral effects, including its antiviral activity against the Chikungunya virus (CHIKV), prompted us to think whether silymarin could also reduce the replication of the MAYV and restore the pro-oxidant/antioxidant balance in the context of MAYV infection, leading to reduced cellular oxidative stress. We assessed the antiviral activity and protective effect of silymarin against oxidative stress in MAYV-infected HepG2 cells. Cytopathic effect inhibition, viral replication, and plaque reduction assays were used to determine the anti-MAYV activity of silymarin. Additionally, we determined whether silymarin could reduce MAYV-induced oxidative cell damage. Briefly, silymarin exhibited potent antiviral activity against MAYV and reduced MAYV-induced ROS formation and levels of malondialdehyde (MDA) and carbonyl protein, which are biomarkers of oxidative stress. In conclusion, the ability of silymarin to inhibit MAYV replication and attenuate MAYV-induce oxidative stress warrants further investigation of this compound as a novel therapeutic approach to Mayaro fever disease.

Mayaro virus (MAYV) is an arthropod-borne virus belonging to the *Togaviridae* family (*Alphavirus* genus), first isolated during the 1950s from febrile patients in Trinidad (Anderson et al., 1957). Since then, sporadic cases, outbreaks and small epidemics were reported in several countries of South and Central America, including Brazil, Peru, Suriname, French Guiana, Guyana, Venezuela, Colombia, Ecuador, Panama, and Bolivia (Pinheiro and LeDuc, 1988; Mota et al., 2015). After the bite of an arthropod vector (mainly *Haemogogus* mosquitoes), the virus causes Mayaro fever, which presents a variety of symptoms closely resembling the alphavirus infection, including fever, rash, and severe and prolonged arthralgia (Tesh et al., 1999; Hotez and Murray, 2017). Mayaro fever is still considered a neglected disease as it reaches mainly the poorest areas of the country and receives no attention from governmental programs. Due to the extensive urbanization of forest regions and the high chance of MAYV be transmitted by *Aedes aegypti* vector have expanded the exposure to the virus and its emergence in urban areas (Long et al., 2011). In Brazil, dengue, chikungunya and zika viruses have a profound impact on the public health system, and in addition to these viruses, one of the most recent concerns is the Mayaro virus emergence (Esposito and Fonseca, 2017). According to data from

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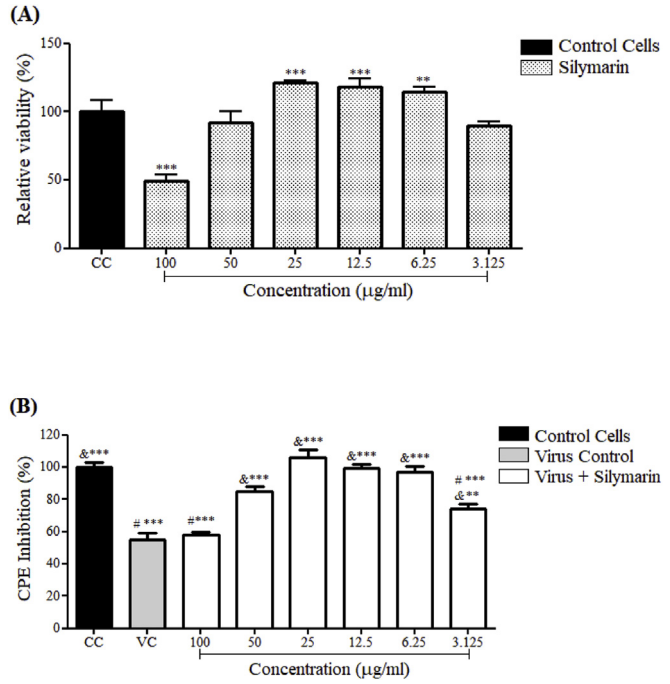

Fig. 1. Silymarin cytotoxicity in HepG2 cells and inhibitory effect on MAYV induced cytopathic effect. (A) Cells were cultured in the presence of different concentrations of silymarin and MTT assay was performed after 48 h. Results are reported as % over control cells, with $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with control cells. **(B)** CPE inhibition was carried out using HepG2 cells infected with 5 MOI of MAYV. Cells were treated with different concentrations of silymarin and assayed 48 h post treatment. Results are reported as % over control cell, where # and & indicate significant difference in relation to control (CC) and infected (VC) cells, respectively, with $^{**}p < 0.01$ and $^{***}p < 0.001$. All data are expressed as the mean ± standard deviation of three independent experiments with six replicates each and were plotted and analyzed using one-way ANOVA and Tukey's post-test.

the Brazilian Ministry of Health, from December 2014 until January 2016, a total of 343 suspicious human cases of MAYV infection were reported in 11 states distributed in the North, Northeast and Central West regions, notably in Goiás State (Ministério da Saúde, Brazil, 2016). To date, there are no available antiviral drugs or vaccines against MAYV infection; therefore, the search for compounds with anti-MAYV activity may provide a potential alternative.

Recent studies have demonstrated that oxidative stress in virus infections can contribute to several aspects of viral disease pathogenesis, including the inflammatory response and viral replication (Reshi et al., 2014; Camini et al., 2017a). Oxidative stress is established when there is a disruption/dysregulation of signaling and redox control caused by the increase of Reactive Oxygen Species (ROS) and/or a reduction in the antioxidant defence system (Jones, 2006). We have previously shown that MAYV-infected HepG2 cells generate ROS and induces significant oxidative stress, as indicated by the increase of malondialdehyde (MDA) and carbonyl protein levels, and by a significant decrease of the reduced versus oxidized glutathione (GSH/GSSG) ratio (Camini et al., 2017b). Thus, modulation of oxidative stress may pave the way toward important advances in the therapeutic approach of MAYV-induced acute disease.

The search for medicines derived from plants is an ancient practice in scientific research. There are many molecules able to provide health benefits being used in a large range of diseases and, among these molecules, silymarin has had a very important role (Federico et al., 2017). Silymarin is a complex extracted of *Silybum marianum* (milk thistle) and consists of seven flavonoglignans (silibinin A and B, isosilibinin A and B, silychristin, isosilychristin and silydianin) and a flavonoid (taxifolin) (Kim et al., 2003). It is used in different liver disorders, particularly chronic liver diseases, cirrhosis and hepatocellular carcinoma, due to its antioxidant, anti-inflammatory and antifibrotic activity (Federico et al., 2017). It also proved to have an antiviral effect against Chikungunya virus (CHIKV) (Lani et al., 2015) and Hepatitis C viruses (HCV) (Wagoner et al., 2010). Silymarin exhibited antiviral activity against CHIKV, another member of the *Alphavirus* genus, reducing both CHIKV replication and down-regulating production of viral proteins involved in replication (Lani et al., 2015). Thus, due to antioxidant and antiviral effects of silymarin, we hypothesized that this compound could present antiviral activity against MAYV and reduce cellular oxidative stress

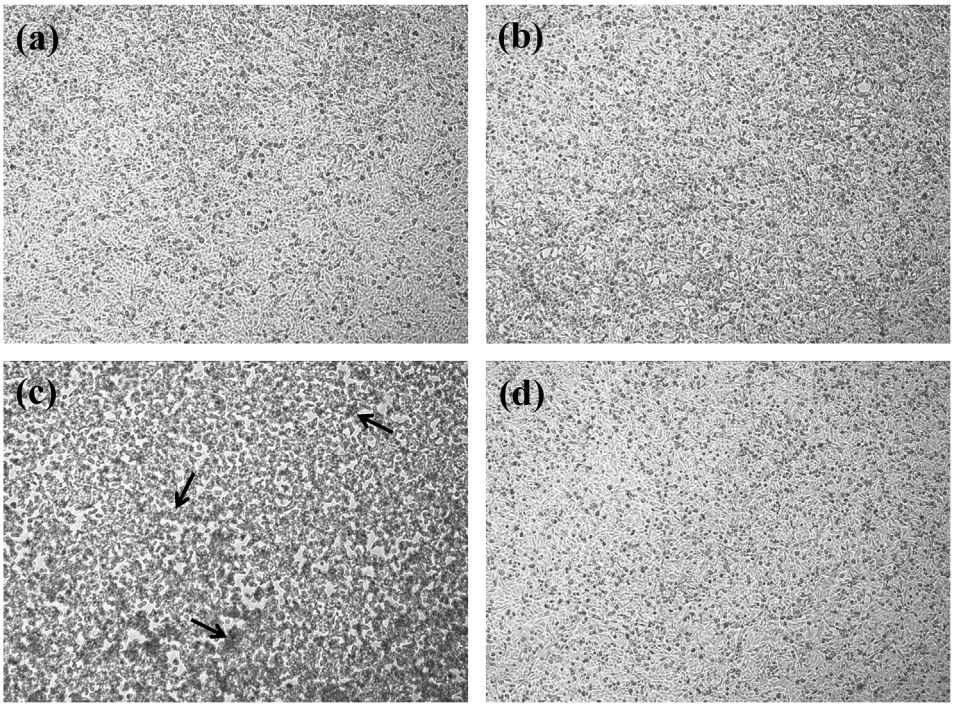

Fig. 2. Antiviral activity of silymarin in HepG2 cells infected with MAYV. HepG2 cells were infected with MAYV (MOI of 5), treated with silymarin (25 µg/ml) and photographed after 48 h of infection. (a) Uninfected and untreated cells, (b) Cells uninfected and treated with silymarin, (c) Infected cells, (d) Cells infected and treated with silymarin. Black arrows in (c) indicate some clusters of cells from cytopathic effect of the MAYV. Magnification, 100x.