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Inhibition of dengue virus production and cytokine/chemokine expression by ribavirin and compound A

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ABSTRACT

Dengue virus (DENV) infection is a worldwide public health problem with an increasing magnitude. The severity of disease in the patients with DENV infection correlates with high viral load and massive cytokine production – the condition referred to as "cytokine storm". Thus, concurrent inhibition of DENV and cytokine production should be more effective for treatment of DENV infection. In this study, we investigated the effects of the antiviral agent – ribavirin (RV), and the anti-inflammatory compound – compound A (CpdA), individually or in combination, on DENV production and cytokine/chemokine transcription in human lung epithelial carcinoma (A549) cells infected with DENV. Initially, the cells infected with DENV serotype 2 (DENV2) was studied. The results showed that treatment of DENV-infected cells with RV could significantly reduce both DENV production and cytokine (IL-6 and TNF-α) and chemokine (IP-10 and RANTES) transcription while treatment of DENV-infected cells with CpdA could significantly reduce cytokine (IL-6 and TNF-α) and chemokine (RANTES) transcription. Combined RV and CpdA treatment of the infected cells showed greater reduction of DENV production and cytokine/chemokine transcription. Similar results of this combined treatment were observed for infection with any one of the four DENV (DENV1, 2, 3, and 4) serotypes. These results indicate that combination of the antiviral agent and the anti-inflammatory compound offers a greater efficiency in reduction of DENV and cytokine/chemokine production, providing a new therapeutic approach for DENV infection.

1. Introduction

Dengue virus (DENV) infection, a mosquito-borne viral disease, is a major public health problem worldwide (Gubler, 2011; Halstead, 2007). DENV is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes, which are widespread in the tropical and subtropical regions (Bhatt et al., 2013; Kyle and Harris, 2008). Approximately 390 million people worldwide are at risk for DENV infection (Bhatt et al., 2013) with 500,000 dengue hemorrhagic fever (DHF) cases and more than 22,000 deaths each year (Gubler, 2002; Murray et al., 2013; Shepard et al., 2011). DENV is a member of *Flaviviridae* family and *Flavivirus* genus; its genome is a single positive-strand RNA with approximately 10.6 kilobases (Qi et al., 2008). It consists of four antigenically related serotypes, DENV 1, DENV 2, DENV 3, and DENV 4 (Blok, 1985).

The clinical manifestations of DENV infection range from asymptomatic or undifferentiated febrile illness, dengue fever (DF), dengue haemorrhagic fever (DHF), to dengue shock syndrome (DSS) (Simmons et al., 2012). Currently, there is neither licensed vaccine for prevention nor specific antiviral drug for treatment of DENV infection. Several studies have shown that severity of disease in the patients with DENV infection correlates with high viral load and host immune response, especially elevation of cytokines (Green and Rothman, 2006; Guilarde et al., 2008; Tricou et al., 2011; Vaughn et al., 2000, 1997). In the severe forms of DENV infection,

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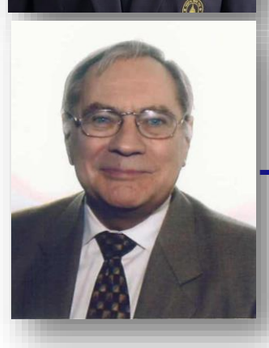

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Table 1 Sequences of primers for cytokine transcription study.

Primer	Orientation	Sequence (5′-3′)
IP-10_F	Forward	GAATCGAAGGCCATCAAGAA
IP-10_R	Reverse	AAGCAGGGTCAGAACATCCA
RANTES_F	Forward	TCCTGCAGAGGATCAAGACA
RANTES_R	Reverse	TCCTGCAGAGGATCAAGACA
IL-6_F	Forward	GTACATCCTCGACGGCATC
IL-6_R	Reverse	AGCCACTGGTTCTGTGCCT
TNF-α_F	Forward	TGCTTGTTCCTCAGCCTCTT
TNF-α_R	Reverse	ATGGGCTACAGGCTTGTCACT
GAPDH_F	Forward	CGACCACTTTGTCAAGCTCA
GAPDH_R	Reverse	AGGGGTCTACATGGCAACTG

Inhibition of dengue virus production and cytokine/chemokine expression by ribavirin and compound A

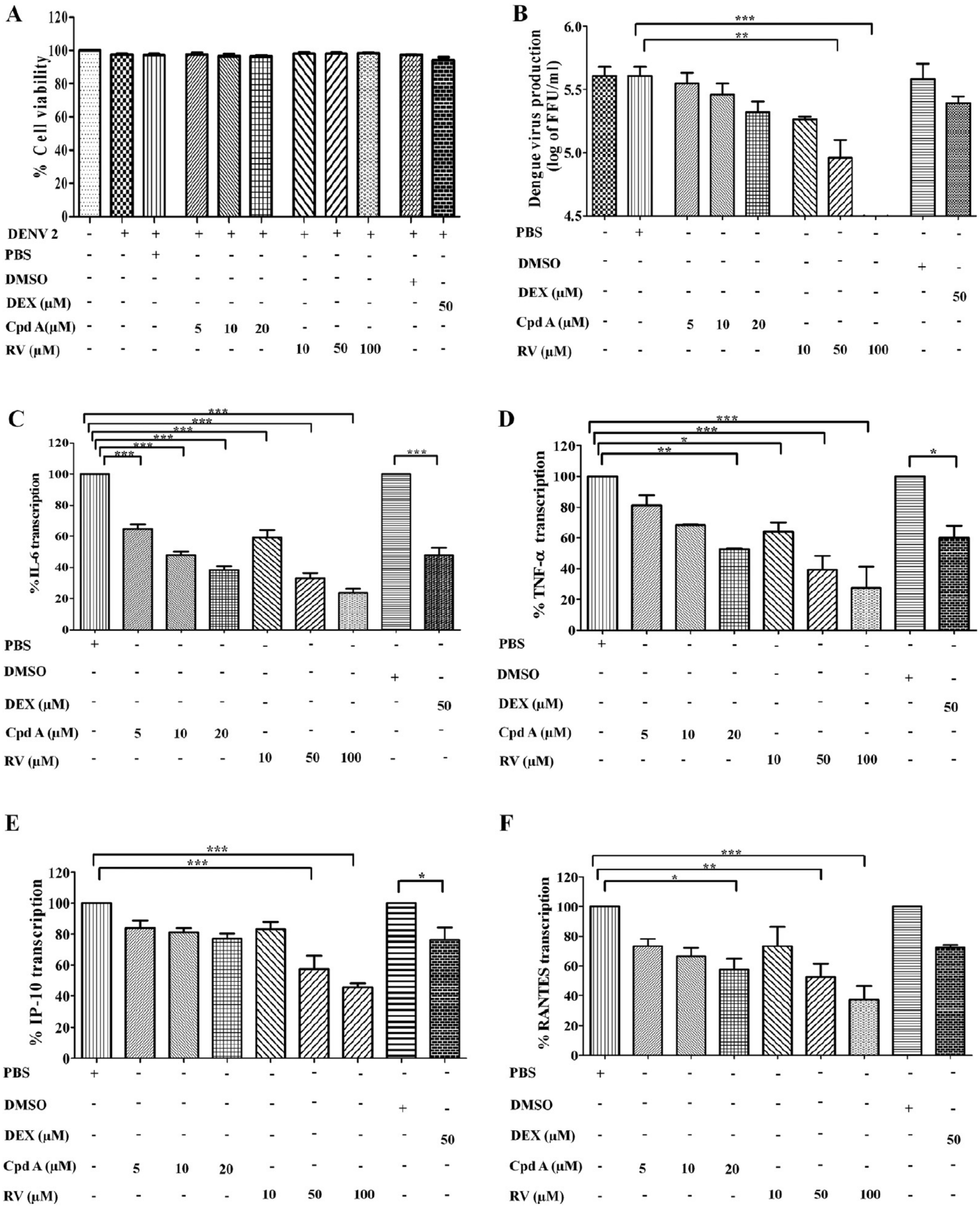

Fig. 1. Effects of ribavirin (RV) and compound A (CpdA) on DENV production and cytokine transcription.

Inhibition of dengue virus production and cytokine/chemokine expression by ribavirin and compound A

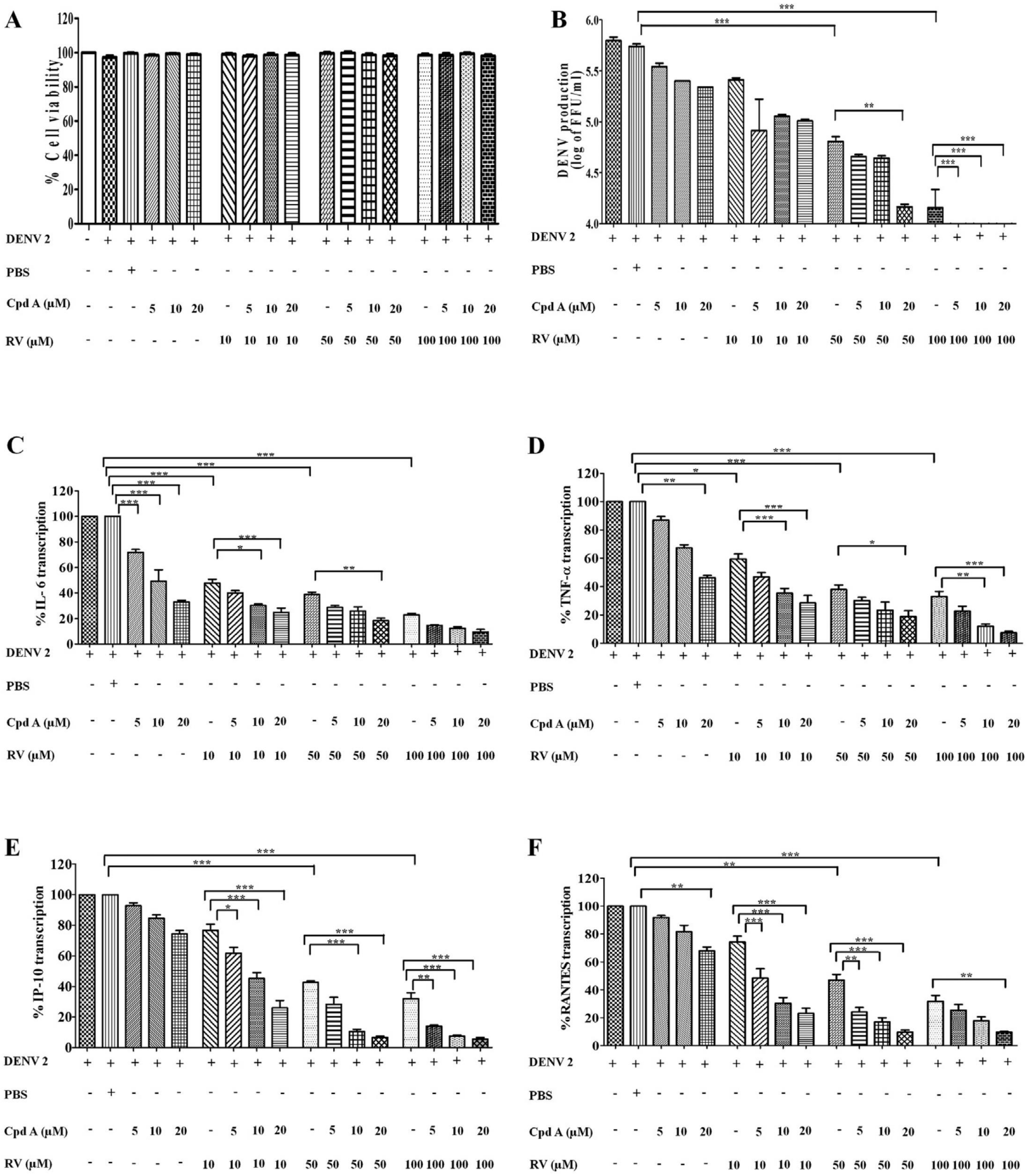

Fig. 2. Combined effects of ribavirin (RV) and compound A (CpdA) on DENV2 production and cytokine transcription.

Inhibition of dengue virus production and cytokine/chemokine expression by ribavirin and compound A

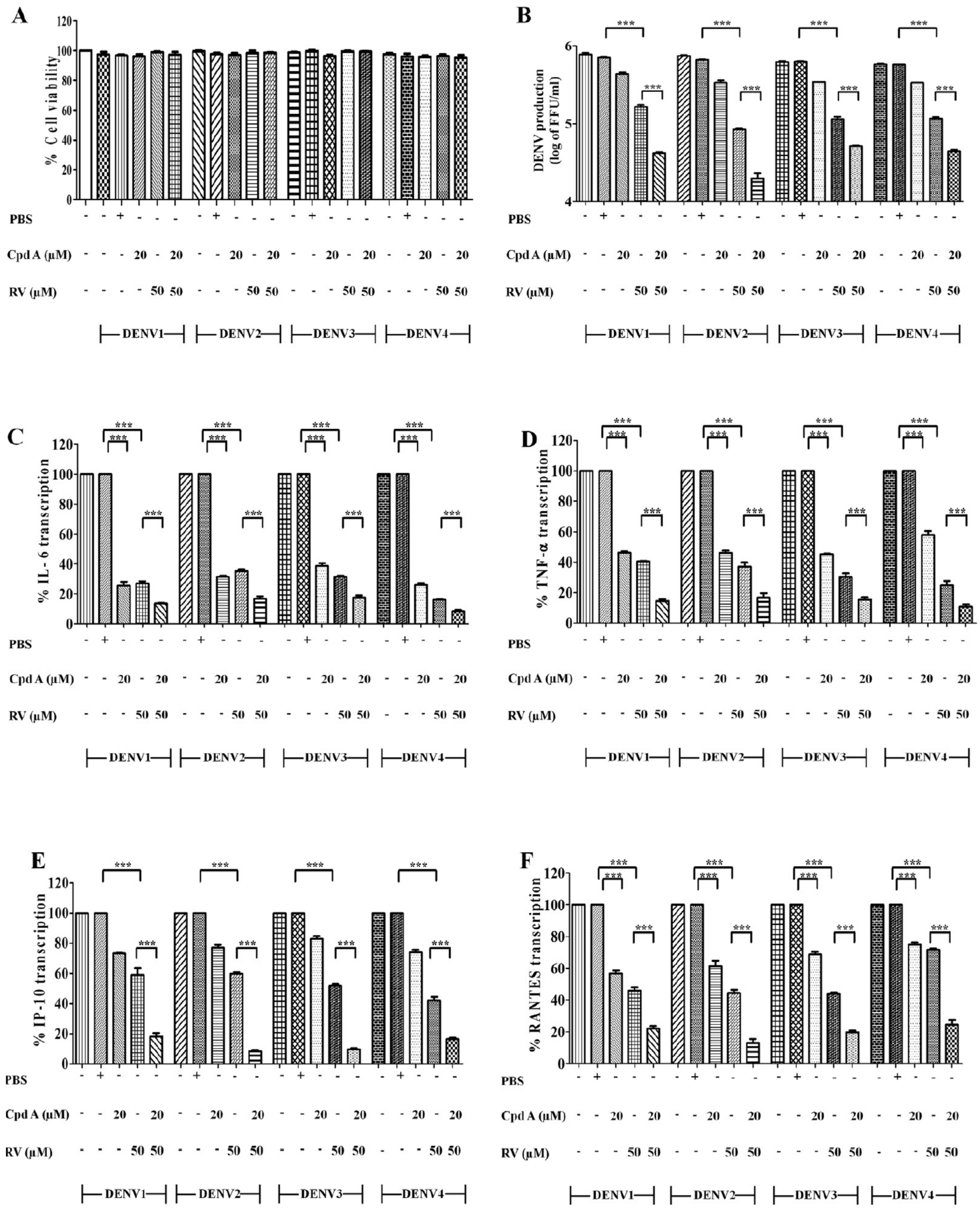

Fig. 3. Combined effects of ribavirin (RV) and compound A (CpdA) on DENV production and cytokine transcription in A549 cells infected with DENV serotypes 1, 2, 3, and 4.

Inhibition of dengue virus production and cytokine/chemokine expression by ribavirin and compound A

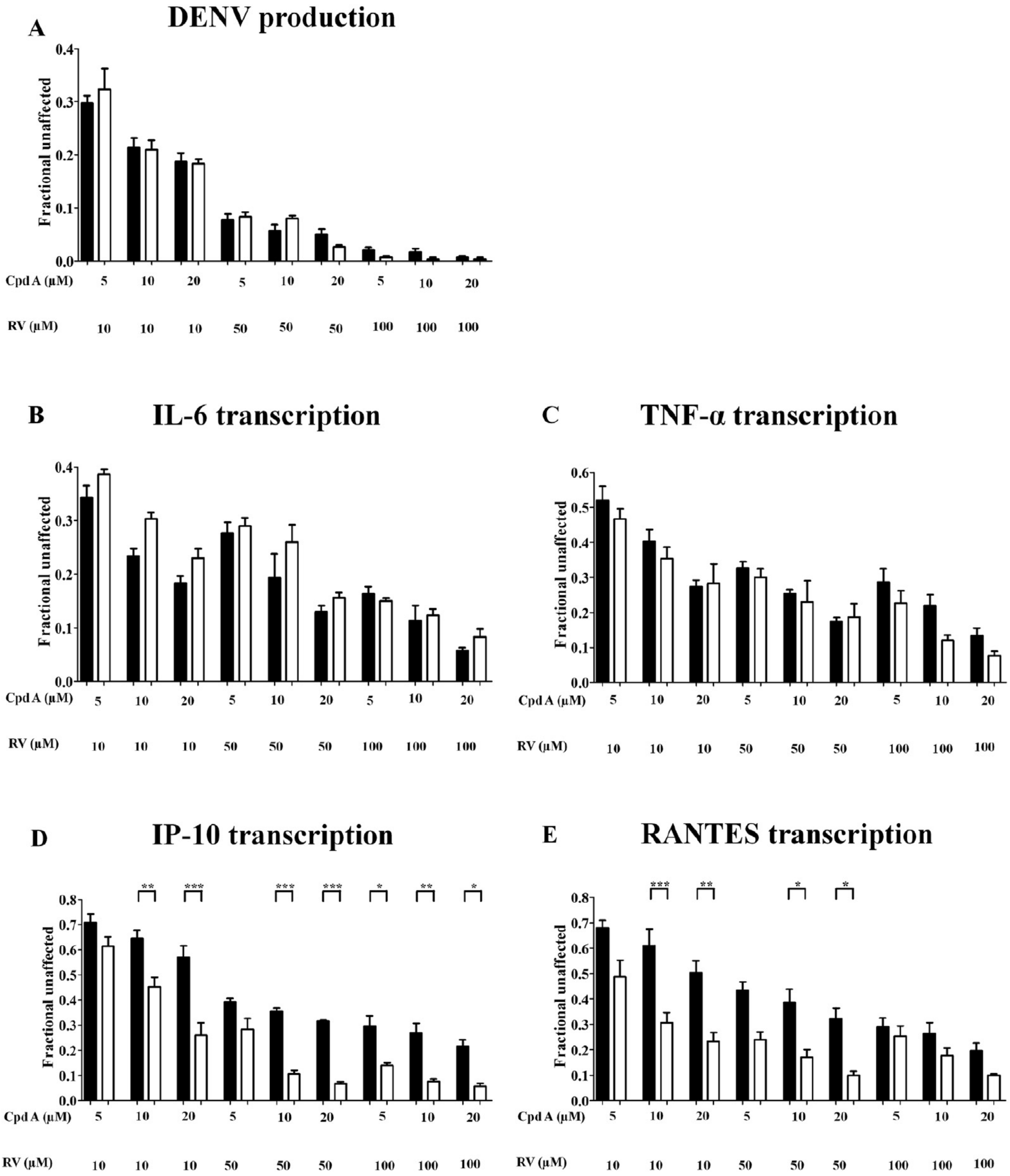

Fig. 4. Calculation of combinatorial effects of ribavirin (RV) and compound A (CpdA) together on DENV production and cytokine transcription.

Inhibition of dengue virus production and cytokine/chemokine expression by ribavirin and compound A

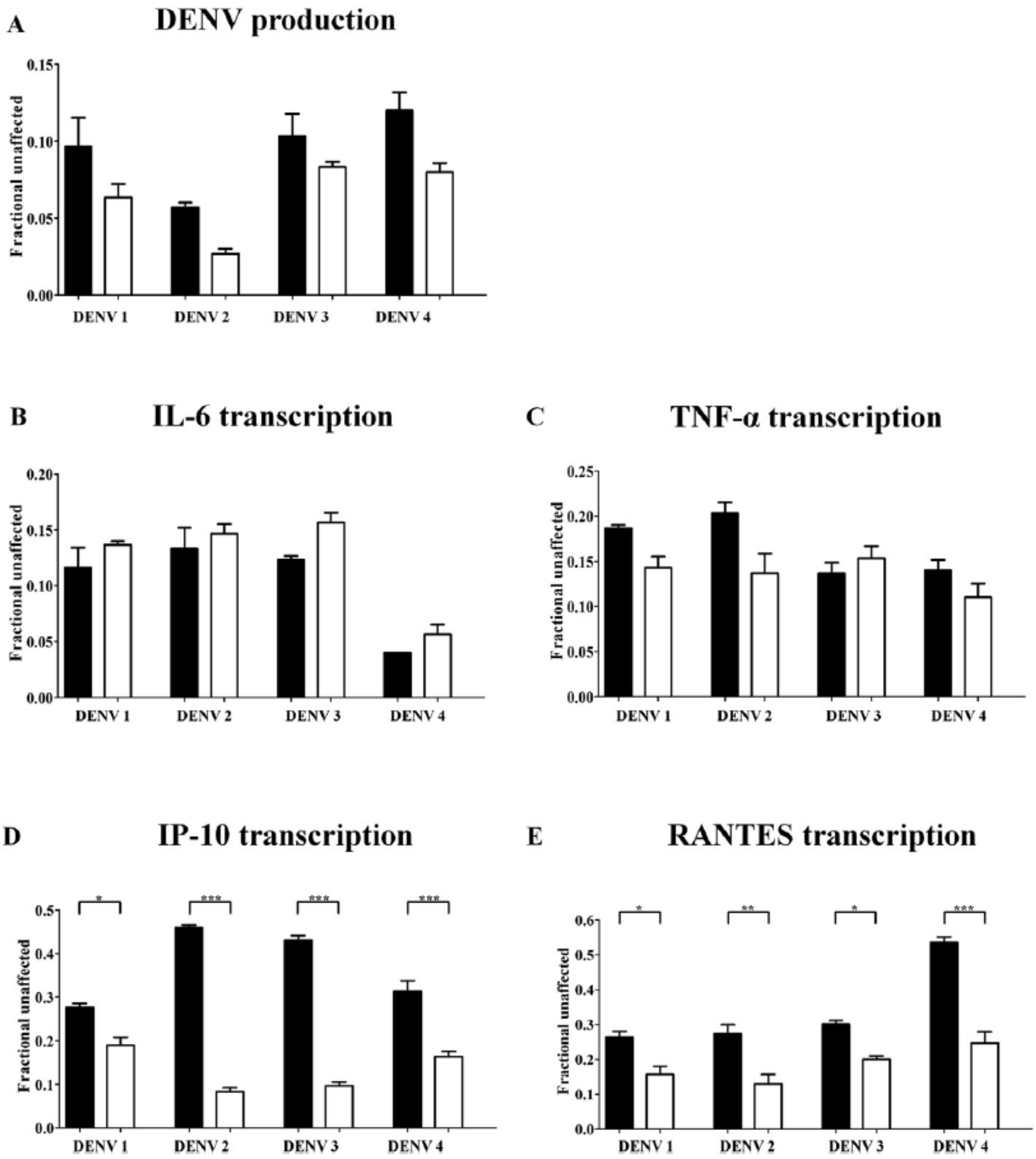

Fig. 5. Calculation of combinatorial effects of ribavirin (RV) and compound A (CpdA) together on DENV production and cytokine transcription in A549 cells infected by DENV serotype 1, 2, 3, and 4.

Inhibition of dengue virus production and cytokine/chemokine expression by ribavirin and compound A

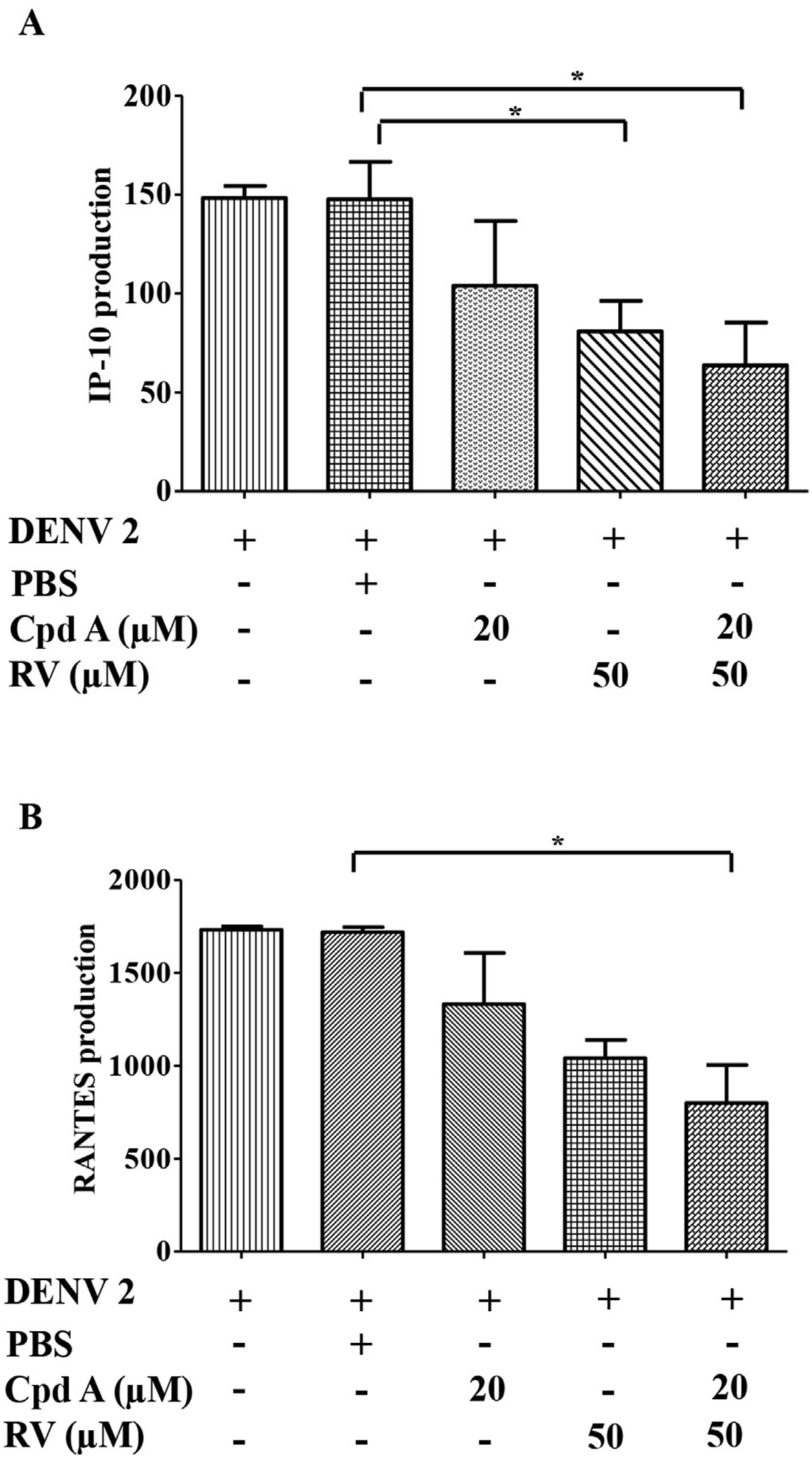

Fig. 6. Combined effects of RV and CpdA on IP-10 and RANTES protein production on DENV2 infected cells.