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In Vitro Antiviral Activity of Morin Compound against Dengue Virus Type 1 in Vero Cells

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ABSTRACT

Introduction: Dengue Haemorrhagic Fever (DHF) is an irresistible ailment brought about by dengue infection. Recently, the medicine for dengue fever cannot be disseminated in various countries because of the price was high. One characteristic of a person infected with the virus was thrombocyte decreasing. In previous research it was known that guava can increase the number of thrombocyte patients of dengue fever. Guava was trusted can increase the number of thrombocyte because it contains a group of tannin and flavonoids as anti-bacterial. Some flavonoids in guava include morin, quercetin, and guajavarin.
Objective: Purpose in this study, was to decide the impact of morin compound to the replication of dengue infection.
Methods: This study used to test the inhibition of the virus is by Viral ToxGlo assay to know the value of 50% inhibition of morin compound on dengue virus replication (IC_{50}) and cytotoxicity to determine the toxicity level of the morin compound against body cell (CC_{50}).
Results: This study of IC_{50} morin for dengue compounds is 9.42 µg/ml and the result of CC_{50} in Vero cell is 12.46 µg/ml.
Conclusion: In summary, there was an investigation show that morin compound have harmful properties in the body.

Keywords: DENV-1, morin, inhibitor activity, cytotoxicity

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INTRODUCTION

Dengue shows up in two forms, classic dengue fever and acute dengue fever. In acute dengue fever, Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) which may cause stomach bleeding, haemorrhage and circulatory collapse. On the off chance that this extreme structure isn't treated with brief and legitimate administration it will prompt the deadly cases.[1] Dengue is a common arbovirus infection transmitted to humans by *Aedes aegypti*.[2] Dengue Haemorrhagic Fever (DHF) brought about by dengue infections is as yet a noteworthy issue in tropical nations. More than 2.5 billion individuals are at present in danger of DENV disease, with 100 million individuals assessed to be contaminated with DENV every year.[3] The main symptoms of Dengue Haemorrhagic Fever are fever, muscle and joint pain, which usually worsens after the first two days and then followed by a decreasing of the amount of thrombocytes. In the most recent decades, dengue has developed as a prime wellbeing worry in tropical and subtropical and until now there has been no effective and affordable drugs circulating.

Morin (2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one) is a derivative of flavones found in few Moracear families. Morin has various biological activities, such as antioxidants, antiinflammation, and antivirals for equid herpesvirus 1.[4] Physical properties of morin include the colour of yellow, water soluble, acid, pyridine and triclor acetic, alcohol, and acetone solutions, so they can be categorized as water soluble dyes.[5] Morin is a polyphenol compound extensively studied in its pharmacological activity to treat human health problems, with few side effects.[6] Morin was reported anti-CDV (canine distemper virus) activity at the time 0 hour, IC_{50} 34.02 µg/ml and CC_{50} 195.90 µg/ml. In the previous research derived of flavonoid, 5-hydroxy-7-methoxy-6-methylflavanone was inhibited DENV-2 infectivity with EC_{50} 12.31 ± 1.64 µM. Baicalein, fisetin and quercetagetin was reported for powerful inhibition of Chikungunya virus infection, with IC_{50} of 1.891 µg/ml, 8.444 µg/ml, and 13.85 µg/ml, and with CC_{50} of 356.3 µg/ml, 194.4 µg/ml, and 226.7 µg/ml, respectively.[7] Thus, rutin and quercetin on DENV-2 in $C_{6/36}$ cells inhibited virus replication with IC_{50} of 362.68 µg/ml and 500 µg/ml, separately. No cytotoxicity was seen with any of these compounds at up to 1000 µg/ml.[8]

In this research, we have several characterizations that were by doing an activity test in *in vitro* and also the cytotoxicity against body cells. All of the characterizations were performed to determine the inhibitory activity of dengue virus using morin compounds.

MATERIALS AND METHODS

Materials

The chemical reagents used in this research were Morin compound, Ethanol 96% (Merck, Germany), aquades, Dimethyl Sulfoxide (DMSO) (Merck, Germany), Dengue virus serotype 1 (DENV-1) Isolate from Surabaya with Genbank Accession AB915377, Minimum Essential Eagle

Medium (Sigma-Aldrich, Germany), Vero cell (African green monkey kidney), Viral Toxglo Assay (Promega, USA), and Cell Proliferation Reagent WST-1 by Roche Applied Science.

METHODS

Antiviral Activity Assay

Vero Cells with concentration 1.10^6 cells/10 ml were seeded into a 96 well plate and incubated plates containing cells at 37°C in a humidified CO_2 incubator for at least 4 hours (and up to 24 hours) to facilitate attachment and allow cells to recover from seeding stresses. One hundred microliters of dengue virus with concentration $4x10^5$ FFU/ml stock was combined with various concentrations of morin (50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.13 µg/ml, 0.78 µg/ml). After one hour of exposure to the dengue virus at room temperature, 100 µl of the morin treated virus was added to Vero cells in individual wells. Cells were infected for 1 hour. Unabsorbed virus was removed and replaced with MEM. After 48 hours post-infection added 100µl of ToxGlo (Promega, Madison, WI). 100 µl of ATP Detection Reagent was added to each well of a 96-well plate (25 µl to each well of a 384-well plate) and wait at least 10 minutes prior to measuring luminescence. Calculate IC_{50} values by plotting net RLU (relative luminescence units) values (subtracting the average of blank wells) versus compound concentration. The IC_{50} value is the compound concentration that caused 50% inhibitory effect on the viral replication or viral cytopathic effect (CPE).

Cytotoxicity Assay

Cytotoxicity used WST-1 cell proliferation reagent by Roche Applied Science, Mannheim, Germany. The assay is very sensitive: it can detect 1,000 cells/well of a 96-well plate reader. Vero cells (1×10^5 cells/ml), 50 µl of serial dilution compound, and a total of 10 µl of Cell Proliferation Reagent was added to each well of a 96-well plate and incubated under 5% CO_2 at 37°C for 1 hour. The plate was read at 450 nm (main filter) and 655 nm (reference filter) using an iMarkTM Microplate Absorbance Reader.

RESULTS AND DISCUSSION

To decide the antiviral impact of morin, DENV was treated with morin. The IC_{50} was analysed from the linear regression equation of the percent infectivity. We found that IC_{50} of morin was 9.42 µg/ml (Figure 1).

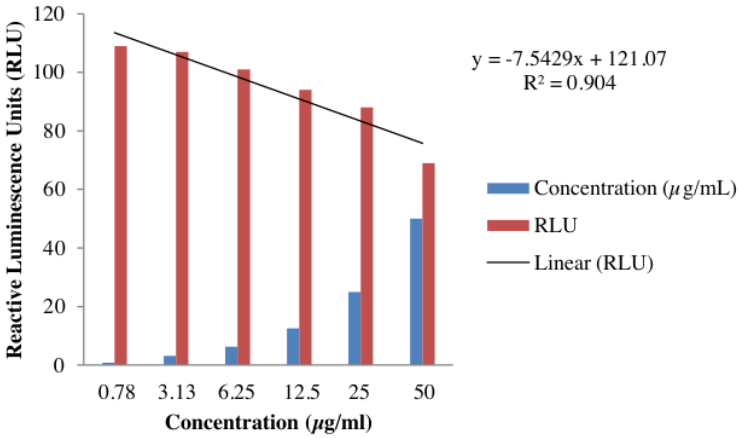

Figure 1. Antiviral activity of Morin for Vero Cells at Varying Concentrations. IC_{50} (maximal inhibitory concentration) is measure of the effectiveness of a substance in inhibiting a specific DENV-1. In this curve was used 6 concentration; 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.13 µg/ml, and 0.78 µg/ml. IC_{50} was calculated from regression linier curve; y = -7.5429x + 121.07

Morin has been used in several studies, one of them on the development of sensitized coloured solar cells (*dye sensitized solar cell*). The expansion of morin in previous study as a sensitiser in DSSC material did not cause changes in crystal composition on TiO_2 anatase or TiO_2 synthesis results.[5] As of late, there have been numerous examinations on dengue antivirals from various organic and crystalline chemicals in TiO_2 anatase and TiO_2 from synthesis. Based on previous research, omega 3/6 fatty acids can be used as potential inhibitors of DENV envelope protein fusion process.[9] Other studies of antivirals for dengue have also been performed using curcumin and Pentagamavunon-0.[10] From the results of this study known the concentration of curcumin and Pentagamavunon-0 are good against body cells. An anti-dengue study of organic matter has also been carried out, the results showed that the extract from *Psidium guajava (Guava), Euphorbia hirta (Patikan kerbau), Piper betel L. (Sirih), Carica papaya (Papaya), Curcuma longa L. (Turmeric), Phyllanthus niruri L. (Meniran), Andrographis paniculata (Sambiloto), and Cymbopogon citratus (*lemongrass*)* were considered to have *in vitro* activity dependent on three criteria, for example cell viability >50%, IC_{50} ≤25 µg/ml and the selectivity index >3.15-17.[11] To ensure that morin was not dangerous to the cell, the half cytotoxic concentration (CC_{50}) was measured with the WST-1 test. The CC_{50} was analysed from the linear regression equation of the percent viability. We found that the CC_{50} of morin was 12.46 µg/ml (Figure 2).

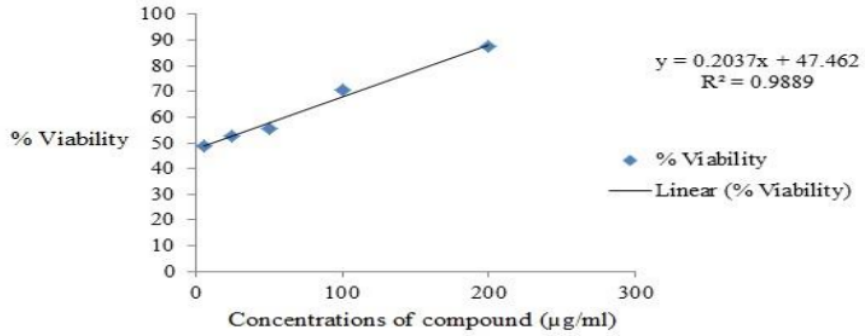

Figure 2. Cytotoxicity of Morin for Vero Cells at Varying Concentrations. CC_{50} is cytotoxicity level of morin compound to cause death to 50% of Vero cells. CC_{50} was calculated from regression linier curve; y = 0.2037x + 47.462 with the axis (x) is concentration of compound and ordinate (y) is %viability.

The results show that from the value IC_{50} and CC_{50}, the CC_{50} value was found to increase with an increasing concentration of the test compound. Morin for Vero cells was 12.46 µg/ml, with an R^2 value of 0.9889. When compared with a previous study, morin is more toxic than plant extracts with an IC_{50} value of 35.7 µg/ml. and CC_{50} value 250 µg/ml.[11] The good IC_{50} value has a concentration close to 0, whereas for a good CC_{50} should be above 100 µg/ml so that the results of this study can be seen from the values of IC_{50} and CC_{50} indicating that morin is toxic to Vero cells.

In the previous research, bioflavonoids derived are of intrigue as a result of their natural and health advantages. Balcalein inhibited DENV-2 replication in Vero cells with IC_{50} was 1.55 µg/ml and exhibited cytotoxic effects with CC_{50} was 109 µg/ml[12] and showed potent anti-JEV activity with IC_{50} = 14.28 µg/ml when it was acquainted to the Vero cells.[13] The IC_{50} values for the aqueous extract of *Sculellaria baicalensis* on Vero cells following DENV adsorption ranged from 86.59 to 95.19 µg/ml with CC_{50} of the extract was 912.6 µg/ml. The cytotoxicity suggests that the extract is in general non-cytotoxic and could be well-endured by Vero cells.[14]

The mechanisms of how morin exerts it is anti-DENV effects are not known. A possible mechanism of action of intracellular anti-DENV activity is binding of morin to the viral RNA, interaction with DENV structural and non-structural protein, and also prophylactic activity of morin against DENV replication, is about the aggregation of the compound in the cells during the treatment. However, avoidance of infection adsorption to the cells is another plausibility that may prompt hindrance of infection passage to the cells.[13]

CONCLUSION

In conclusion, from screening antiviral activity of morin did not potential against DENV-1 because it was shown to be medium toxic to Vero cells. Further study is expected to affirm antiviral activity through a larvicidal test.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflicts of interest.

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