

Evaluation of antiviral activity of Manuka honey against SARS-CoV-2

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INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) has been documented in Wuhan city in China during the end of the year 2019. On 11 March 2020 the World Health Organization (WHO) announced COVID 19 as a pandemic. COVID-19 has a variety of indications from asymptomatic infection to normal cold and flu or severe pneumonia or death. Hence, boosting of the immune system is crucial to ease the complication coupled with it. Natural immunity boosters, such as honey, have been utilized by humans for centuries. An *in silico* study showed the latent possibility of Manuka honey capability in inhibiting SARS-CoV-2 proteases (Hashem, 2020). Manuka honey contains high concentrations of methylglyoxal (MG) that can inhibit enveloped virus growth *in vitro*. MG is dicarbonyl compound that is endogenously formed under physiological conditions, contributing to modifying proteins and DNA that cause misfolding and deactivation. However, in a healthy human MG exogenously consumed has limited bioavailability (Degen et al., 2013), likely due to metabolism by intestinal bacteria. MG content in fresh Manuka honey ranges between 50 - 250 mg/kg, while in commercial Manuka honey it varies between 70 - 700 mg/kg (Atrott et al., 2012). Degan *et al.* calculated the daily quantity of MG intake between 5 - 20 mg/day (0.1 - 0.3 mmol/day) where Rabbani *et al.* (2016) estimated this value was lower, <0.03 mmol MG/day. Endogenous formation of MG in healthy human adults subjects is ca. 3 mmol per day (Rabbani *et al.* 2016). The MG content of Manuka honey is unlikely to be hazardous to health and be readily metabolized. Manuka honey may have antiviral activity against SARS-CoV-2 linked to its MG content. The study aims to characterize this by incubating SARS-CoV-2 with Manuka honey in cell-free medium before exposing it to Vero cells and assessing viral infectivity and growth at multiplicity of infection (MOI) of 0.1.

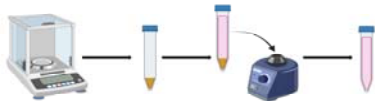
METHODOLOGY

Day 1:

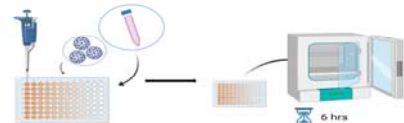


I. Cells culture: In a 96-well plate 15,000 vero cells were seeded and incubated for 24 hours.

Day 2:



I. Preparation of honey solution: Manuka honey (250+ MG) was diluted with basal medium 10-folds to a final volume of 10 mL attaining a final concentration of 500 μ M MG.



II. Honey dilution plate: The honey solution was further diluted to 250 μ M, 225 μ M, 200 μ M, 175 μ M, 150 μ M, 125 μ M. The virus titre was 0.1 MOI of SARS-CoV-2 virus. Controls used were : manuka honey (500 μ M) with aminoguanidine (1 mM), Manuka honey only and the virus only. To assess if MG is the active ingredient in manuka honey. Manuka honey was incubated (3 hours) with aminoguanidine –it is a potent scavenger of MG.



III. Viral infection: The cells were incubated with the virus until a significant CPE was seen. TCID₅₀ was then performed to evaluate the virus inhibition.

RESULTS

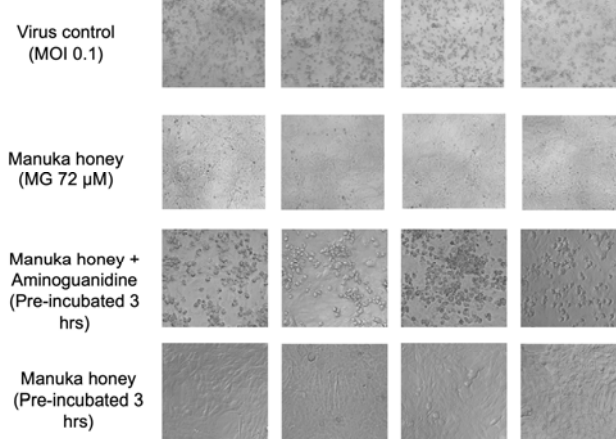


Figure 1: Effects of manuka honey on inhibiting SARS-CoV-2 at different MG concentrations with and without Aminoguanidine incubation. Vero cells treated with wild-type SARS-CoV-2 with titers of multiplicities of infection (MOI) 0.1 incubated with different dilutions of manuk

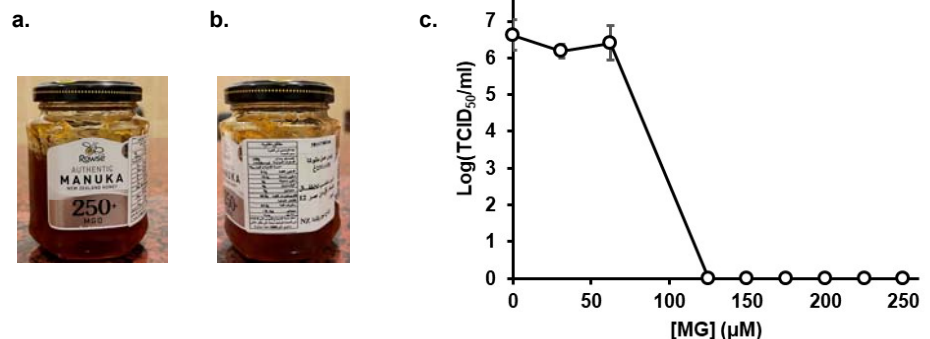


Figure 2: a. and b. Manuka honey used in the experiments. c. Inhibition of SARS-CoV-2 by manuka honey dilutions. Diluted Manuka honey – shown at equivalent MG concentration - inhibited SARS-CoV-2 replication in Vero cells (MOI = 0.1) . SARS-CoV-2 was incubated in diluted Manuka honey in medium at 37°C for 3 h before adding to Vero cells. Cell supernatants were collected for virus titer (TCID₅₀) determination. Data are mean \pm SD, n = 4 - 8 biological replicates. Data analysis was by logistic regression: $\ln(E/E_{\max} - E)$ against $\ln([MG]/\mu\text{M})$ where E is cytopathic effect. TCID₅₀ (mean \pm SD) was deduced by interpolation. EC₅₀ = 72 \pm 2 μ M MG equivalents.

DISCUSSION

Antiviral activity of Manuka honey against SARS-CoV-2 was detected for the first time *in vitro*. The virus titer was inhibited by 100% with MOI 0.1 when treated with ≥ 125 μ M MG equivalents of commercial 250+ grade Manuka honey. The inhibition of the virus was microscopically viewed for the presence or absence of cytopathicity in quadruplicate wells. Virus titer (TCID₅₀) determination further confirmed the efficacy of inhibiting SARS-CoV-2 MOI 0.1 replicated in Vero cells (Figure 2). Prior incubation of diluted Manuka honey with the MG scavenger, aminoguanidine (AG), abolished the antiviral effect. This suggests that MG content of Manuka honey may be the active component producing the antiviral activity.

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