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Antiviral Effects of Chestnut Honey Against Influenza A Virus Through Innate Immune Modulation

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Abstract

The continuous emergence and spread of viral infectious diseases, such as influenza, pose significant public health and economic challenges. Current antiviral treatments and vaccines have limitations due to viral mutations and drug resistance, necessitating the development of alternative therapeutic agents. Natural products have gained increasing attention for their potential antiviral properties. In this study, we investigated the antiviral effects of chestnut (Castanea crenata) honey against influenza A virus infection. Using an in vitro model with Raw 264.7 macrophage cells, chestnut honey treatment activated key innate immune signaling pathways, including TBK/IKK, IRF, and RIG-MAVS, leading to enhanced immune responses. In an in vivo mouse model, oral administration of chestnut honey prior to viral infection increased survival rates by 60% and reduced virus-induced weight loss by 20.9%, while also mitigating lung inflammation. These findings suggest that chestnut honey exhibits strong antiviral activity by modulating innate immunity and inhibiting viral replication. Therefore, chestnut honey has the potential to be developed as a functional natural remedy for the prevention and treatment of influenza and other viral infections.

Introduction

Influenza & Immunity

Influenza is one of the most significant respiratory diseases, affecting nearly 1 billion people each year and causing up to 650,000 deaths globally. Current prevention and treatment strategies, including vaccines and antiviral drugs, face limitations due to rapid viral mutation and drug resistance. As a result, there is a strong need for novel approaches that can enhance host defense. The innate immune system, the first line of defense, plays a central role by producing interferons and proinflammatory cytokines that restrict viral replication.

Materials & Methods

Sample: Castanea crenata honey (2020).

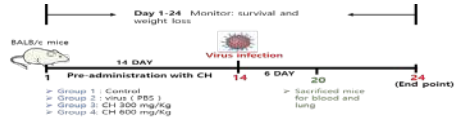
➢ In vitro assays:

- Murine macrophages (Raw264.7 cells) pretreated with CH.
- Infection with influenza A/PR8/34-GFP strain.
- Viral protein expression (M2, PB1, PA) measured by
- immunofluorescence, Western blotting, and plaque assays.

Cytokine secretion and signaling pathway activation analyzed by ELISA, RT-PCR, and EMSA.

➢ In vivo assays:

- BALB/c mice orally administered CH (300 or 600 mg/kg/day) for 14 days before IAV infection.
- Body weight, survival rate, lung pathology, cytokine levels, and NK cell activity assessed.



Results

Antiviral effect of CH on IAV-infected Raw264.7 cells

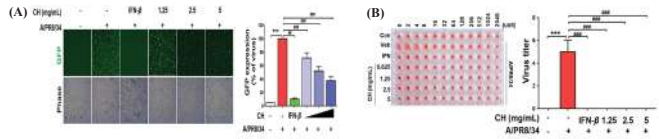


Fig. 1. Antiviral effect of CH on IAV-infected Raw264.7 cells. The cells were pretreated with CH (1.25, 2.5, and 5 mg/mL) or 1,000 units of IFN-b as a positive control for 24 h before infection with IAV-GFP. Detection of GFP using (A) fluorescence microscopy (10×) and flow cytometry. (B) Infected supernatant was added to round plates and then diluted with PBS. Finally, 0.5% cRBC in PBS was added to each well and incubated for 1 h at 25℃ before imaging.

CH activates the RIG-1-mediated MAVS pathway

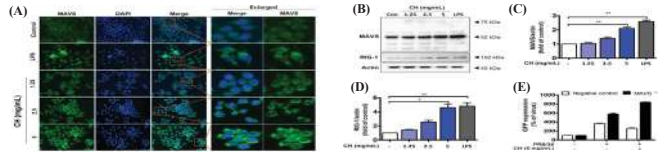


Fig. 2. CH activates the RIG-1-mediated MAVS pathway. The cells were pretreated with CH (1.25, 2.5, and 5 mg/mL) or 100 ng/mL LPS as a positive control for 24 h. (A) Expression of MAVS was detected via immunofluorescence. (B) Western blot analysis of RIG-1 and MAVS using whole-cell lysates and (C, D) quantitative analysis of protein bands (MAVS and RIG-1) was performed via ImageJ software. (E) Cells were transfected with siRNA against MAVS and then infected with GFP-IAV, followed by detection of viral GFP via flow cytometry. Bar graph (mean ± standard error of mean) statistical data were determined using one-way analysis of variance with Tukey's post hoc test. **P < 0.01 and *P < 0.05 compared with the untreated group (Con).

Antiviral effect of CH on IAV-infected BALB/c mice

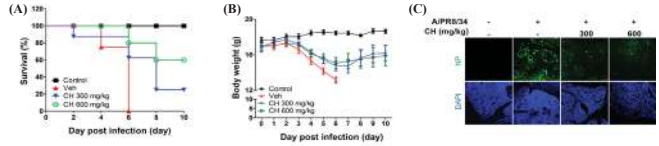


Fig. 3. Antiviral effect of CH on IAV-infected BALB/c mice. (A) Survival rate and (B) body weight was monitored daily until 10 dpi. (C) Immunofluorescence analysis revealed the levels of IAV NP in virus-infected mouse lungs. Scale bar = 100 mm.

Conclusion

Castanea crenata honey (CH) activated innate antiviral signaling by enhancing RIG-I/MAVS-mediated TBK/IKK phosphorylation and promoting IRF3/7 nuclear translocation, which stimulated type-I interferon production and IFN-stimulated genes (e.g., IFITM3). In vivo, CH pretreatment in mice significantly increased survival (up to 60%), reduced body weight loss (~20.9%), and alleviated lung inflammation and histopathological damage. Moreover, CH elevated serum cytokine levels and enhanced natural killer cell activity, suggesting a strengthened systemic immune response. Collectively, these findings demonstrate that CH enhances host immunity through the RIG-I/MAVS–TBK/IKK–IRF axis and provides effective protection against influenza infection, supporting its potential as a natural immunomodulatory agent.

Reference

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