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IMMUNOMODULATORY POTENTIAL OF HESPERIDIN FROM GARUT ORANGE PEEL (CITRUS NOBILIS VAR. CHRYSOCARPHA) AGAINST HUMAN METAPNEUMOVIRUS (HMPV) INFECTION

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Abstract

Acute respiratory infections (ARIs), including those caused by Human Metapneumovirus (HMPV), remain a global health concern, and no specific therapy or vaccine is currently available. Hesperidin, the main flavonoid in Garut orange peel (Citrus nobilis var. chrysocarpha), is known for its anti-inflammatory, antiviral, and immunomodulatory activities. This study was an in vivo pilot study using 35 male Balb/c mice divided into seven groups: normal control, negative control (5% imiquimod), positive control (levamisole), and hesperidin-treated groups at 50, 100, and 150 mg/kg body weight, either alone or in combination with imiquimod. Observed parameters included total leukocytes, lymphocytes, neutrophil-to-lymphocyte ratio (NLR), body weight, organ weight, and lung histology. Hesperidin was extracted using 70% ethanol and identified by thin-layer chromatography (TLC).Results showed that body weight data were normally distributed, with no significant differences between groups ($p > 0.05$), indicating that hesperidin was safe at the tested doses. Hematological analysis revealed significant differences in leukocyte counts ($p = 0.001$), lymphocytes ($p = 0.002$), and NLR ($p = 0.001$). Imiquimod induced systemic inflammation, as evidenced by increased leukocytes, lymphocytes, NLR, and lung inflammation scores. Hesperidin exhibited dose-dependent immunomodulatory effects; the 100 mg/kgBW dose combined with imiquimod produced the most optimal results, characterized by low NLR and a histology score of 0, reflecting controlled inflammation and effective adaptive immune activation. In contrast, the 150 mg/kgBW dose triggered excessive inflammatory responses.

Keyword: Acute Respiratory Infection, Hespiridin, Immunodulation, Human Metapneumovirus (HMPV), Imiquimod 5%

INTRODUCTION

Acute respiratory infections (ARIs) remain one of the leading causes of morbidity and mortality worldwide, particularly among children, the elderly, and individuals with weakened immune systems(Billard et al., 2025). One of the causative viruses of ARIs that is frequently found but still not well known is Hman Metapneumovirus (HMPV) (Porwal et al., 2025). This virus belongs to the Paramyxoviridae family and is similar to Respiratory Syncytial Virus (RSV), causing respiratory symptoms ranging from mild to severe, including pneumonia and bronchiolitis (Kaseena et al., 2025).

To date, no specific antiviral therapy or effective vaccine is available for HMPV, so management remains supportive. One promising strategy to combat viral infections is by enhancing the body's immune system using natural immunomodulators (Goldstein et al., 2025). One natural compound with potential immunomodulatory and antiviral activity is hesperidin, the main flavonoid commonly found in citrus peels, including Garut orange peel (Akingbola et al., 2025).

In this study, Garut orange peel represents an underutilized agricultural by-product, yet it contains a high amount of hesperidin (Rehman et al., 2021). The Garut orange comes from the local variety *Citrus nobilis* var. *chrysocarpha*, native to the Garut region in West Java. This orange is known for its sweet taste, distinctive aroma, and yellow-orange peel color when ripe (Sundaram et al., 2019). Citrus peels have been widely explored due to their high content of methoxy-flavonoids, their practical non-toxicity, and their immunomodulatory activity, making them promising candidates for applications in the nutraceutical and pharmaceutical fields (Sulhan, 2019).

Previous studies have shown that hesperidin from citrus peel exhibits antiviral activity against Influenza A virus (WSN/33) in mice (Sundaram et al., 2019). Pre-treatment with hesperidin at 100 mg/kg/day protected mice from influenza-induced mortality, reduced lung histological damage and neutrophil / monocyte / lymphocyte infiltration, and limited viral spread in the lungs (IHC NP/NS1) (Loubet et al., 2025). Another study using mice with respiratory syncytial challenge (bronchiolitis) at hesperidin doses of 18 and 366 mg/kg demonstrated reduced lung inflammation scores and mucus hypersecretion, decreased IL-4, IL-

6, and TNF-α levels in bronchoalveolar lavage fluid (BALF), shifted macrophage polarity from M1 to M2, and improved histological appearance. Hesperidin also increased interferon-gamma (IFN-γ) production and modulated lymphocyte composition, including increased TCRαβ+ cells and decreased B cells. These findings indicate that hesperidin has potential as an immunomodulatory agent capable of influencing immune response balance (L. Chen et al., 2021). The compound may be further developed as a basis for natural immunomodulatory supplements or phytopharmaceuticals (TYAS, 2019). Additionally, hesperidin possesses anti-inflammatory and antioxidant activities that can enhance the body's immune response against viral infections (Handayani, 2022).

Studies on the effects of hesperidin in enhancing immune responses against Human Metapneumovirus (HMPV) infection are still limited (Iyer et al., 2025). Therefore, this research was designed as a pilot study focusing on basic but informative parameters, including total and differential leukocyte counts (including NLR), body weight, major organ weights (lung, spleen, liver, kidney), and lung histology. This approach was chosen to provide an initial overview of the immunomodulatory effects of hesperidin with more efficient use of resources. The results of this pilot study will serve as a foundation to determine the feasibility, direction, and design of subsequent, more comprehensive studies (Ramocha et al., 2021).

If positive results are obtained, follow-up studies will measure molecular parameters such as proinflammatory cytokine levels (IL-6, TNF-α, IFN-γ) using ELISA. Due to limited access to HMPV for in vivo experiments, this study employed 5% imiquimod cream as an alternative model to induce systemic inflammation that mimics the immune response to viral infection via TLR7 activation. Imiquimod is a TLR7 agonist (and partially TLR8 in humans) capable of inducing proinflammatory cytokine production in vivo, including IFN-α, IL-6, TNF-α, and other chemokines (Guo et al., 2023).

Activation of TLR7 by imiquimod mimics the innate immune response to RNA viruses such as HMPV or influenza. These cytokines trigger chemokine release, recruiting inflammatory cells—including neutrophils, macrophages, and lymphocytes—to the site of activation

(Heim et al., 2025). Due to this effect, imiquimod is often used as a non-specific model to stimulate the immune system in immunomodulator studies, including research on natural compounds such as flavonoids (Thakur et al., 2025). Therefore, the use of imiquimod in this study is considered relevant for evaluating the immunomodulatory potential of hesperidin from Garut orange peel as a preventive or supportive therapeutic agent against inflammation resembling HMPV infection (Ansori, 2025).

RESEARCH METHOD

This study was designed as a quantitative pilot study using an in vivo approach with male Balb/c mice (Dhamodharan et al., 2025). Measured parameters included total leukocyte and lymphocyte counts, neutrophil-to-lymphocyte ratio (NLR), body weight, major organ weights (lung, spleen, liver, kidney), and lung histology scores**.** The mice were randomly assigned to seven treatment groups: normal control, negative control, positive control, and hesperidin-treated groups at doses of 50, 100, and 150 mg/kg body weight. Hesperidin was extracted from Garut orange peel (*Citrus nobilis* var. *chrysocarpha*) using 70% ethanol and identified via Thin-Layer Chromatography (TLC) (Nidhi et al., 2020)**.** Data were analyzed descriptively to provide an initial assessment of hesperidin's immunomodulatory potential, forming a foundation for future, more comprehensive studies.

RESULT AND DISCUSSION

This study began with the extraction and identification of hesperidin from Garut orange peel (*Citrus nobilis* var. *chrysocarpha*) (Victor et al., 2023). Extraction was performed using a maceration method with 70% ethanol, and the resulting solution was evaporated using a rotary evaporator to obtain a golden-brown extract (Pasdaran et al., 2023). Compound identification was conducted using Thin-Layer Chromatography (TLC) with a mobile phase of ethyl acetate:formic acid:water (41:6:6). The TLC plate was observed under UV light, sprayed with citroborate reagent, and heated. Hesperidin appeared as a yellow–orange spot with an Rf value of 0.45, matching the reference standard, and exhibited fluorescence under UV at 254 nm and 366 nm. This confirmed that the detected compound was a flavonoid, consistent with literature

reporting hesperidin Rf values ranging from 0.40 to 0.47 in polar–semi-polar eluent systems (Khalil et al., 2022).

The next step was the acclimatization of mice for the in vivo experiment. This study used 35 male Balb/c mice aged 6–8 weeks, weighing 20–30 g. All animal research procedures were approved by the Animal Research Ethics Committee of STIKes Karsa Husada Garut (No: 004884/KEP STIKes KHG/2025), ensuring that the study adhered to the 3R principles (Replacement, Reduction, Refinement) and considered animal welfare (Joshi et al., 2022). The mice were acclimatized for 7 days to adjust to laboratory conditions and were then assigned to treatment groups according to the study design (Carmello et al., 2019).

During acclimatization, the mice exhibited normal activity and showed no clinical signs of disease. Monitoring of body weight (table 1) indicated stable changes without significant decreases, suggesting that the administration of hesperidin extract at the experimental doses was safe and well-tolerated. These findings are consistent with previous toxicity studies of hesperidin, which demonstrated high safety in animal models, as well as animal welfare guidelines according to NRC and Morton et al..

Table 1. Average Final Body Weight of Experimental Animals During Hespiridin Treatment

Treatment Group	Average Final Body Weight (Day 0 to Day 6)
Normal Group (CMC 0.5% + Vaseline)	35 ± 4,38
Negative Control (CMC 0.5% + Imiquimod 5%)	28,4 ± 5,38
Positive Control (Levamisole + Imiquimod 5%)	26,8 ± 4,95
Hesperidin 100 mg/kg Body Weight (BW)	34,8 ± 2,13
Hesperidin Dose 50 mg/kg Body Weight (BW) + Imiquimod 5%	32,8 ± 1,72
Hesperidin Dose 100 mg/kg Body Weight (BW) + Imiquimod 5%	31,4 ± 5,95
Hesperidin Dose 150 mg/kg Body Weight (BW) + Imiquimod 5%	32 ± 5,17

Table 1 shows that there was variation in the final body weight of the experimental animals after 6 days of treatment. The normal control group had the highest body weight (35 ± 4.38 g), while the negative control group treated with imiquimod experienced a considerable decrease in body weight (28.4 ± 5.38 g). This is consistent with reports that imiquimod can trigger a systemic inflammatory response via TLR-7/8 activation, leading to reduced

food intake, metabolic stress, and body weight loss in mice (23,24). The positive control group (levamisole + imiquimod) showed lower body weight (26.8 ± 4.95 g), indicating that levamisole as an immunomodulator was not fully able to counterbalance the acute inflammatory effects induced by imiquimod (Z. Chen et al., 2019). Statistical analysis of body weight from day 0 to day 6 showed that all significance values were above 0.05 ($p > 0.05$), indicating no significant differences between groups during the treatment period.

Thus, administration of hesperidin at various doses—either alone or in combination with imiquimod—did not cause significant changes in body weight (Wahyudin & Perceka, 2019). These results indicate that hesperidin is well tolerated and does not induce systemic toxicity, in line with previous studies reporting that hesperidin is safe and does not affect the body weight of mice at therapeutic doses. Stable body weight also confirms that the animals' basic physiological status remained intact throughout the study, consistent with animal welfare guidelines that use body weight changes as a non-invasive indicator to assess toxicity or physiological distress (Wahyudin & Perceka, 2021). These findings align with prior reports on hesperidin safety. Sub-chronic studies in Sprague-Dawley rats reported that administration of hesperidin up to 500 mg/kg did not cause significant changes in body weight, hematological parameters, or organ pathology (Geraldes et al., 2020). A 13-week toxicity study on glucosyl hesperidin also showed a high NOEL value with no adverse effects on body weight or genotoxicity. Furthermore, a combination formulation of naringenin and hesperidin at 2000 mg/kg reportedly did not cause mortality or significant changes in body weight (Li et al., 2019).

Although most of these toxicity studies were conducted in rats, the physiological similarities between rats and mice as rodents make the data still relevant. The doses used in this study (50–150 mg/kg) are far below the toxicity test doses reported in the literature, and the stable body weight of the experimental animals further reinforces that hesperidin is safe and does not induce systemic toxic effects within the tested dose range.

Administration of 5% imClick or tap here to enter text.iquimod as a mimic of HMPV infection in the negative control

group caused an increase in leukocyte and lymphocyte counts, as well as a rise in the neutrophil-to-lymphocyte ratio (NLR) compared to the normal group (Silva et al., 2025). This indicates that 5% imiquimod is capable of triggering an immune response through the activation of inflammatory cells. This mechanism aligns with imiquimod's properties as a Toll-like receptor 7 (TLR-7) agonist, which stimulates the production of proinflammatory cytokines (30). In contrast, the positive control group (levamisole + 5% imiquimod) showed a decrease in NLR values despite maintaining high lymphocyte counts. This finding indicates immune modulation toward a dominant adaptive immune response. Levamisole is known to act as an immunostimulant that can enhance lymphocyte proliferation and suppress neutrophil-dominated responses (Acharya & Byrareddy, 2025).

Hesperidin exhibited dose-dependent immunomodulatory effects. When administered alone without 5% imiquimod, hesperidin did not induce excessive inflammation, as indicated by consistently low NLR values, suggesting its immunoprotective properties (Oktaviani et al., 2024). The combination of hesperidin at 50 mg/kgBW with 5% imiquimod showed the most favorable outcome, with the lowest NLR value (0.08), indicating lymphocyte dominance and optimal inflammation control. At a dose of 100 mg/kgBW, the effect was moderate, while the higher dose of 150 mg/kgBW led to marked increases in leukocyte and lymphocyte counts, accompanied by signs of exaggerated immune activation, suggesting a potential proinflammatory response.

The response observed at 150 mg/kgBW indicates a possible biphasic dose-dependent effect of hesperidin. While moderate doses exert immunomodulatory and anti-inflammatory activity, higher doses may overstimulate immune pathways such as NF-κB and MAPK, leading to increased production of proinflammatory cytokines. Clinically, this finding highlights the importance of defining an optimal therapeutic window to prevent immune overactivation. Similar dose-dependent patterns have been reported in previous studies, where high-dose hesperidin triggered elevated inflammatory markers and oxidative stress responses, reinforcing the need for careful dose

optimization in future translational applications. These findings are consistent with previous reports indicating that hesperidin can increase lymphocyte counts and suppress inflammatory mediators such as TNF-α and IL-6 in animal models (and & ..., 2019). Moreover, recent meta-analyses have shown that hesperidin supplementation in humans can reduce systemic inflammatory markers such as CRP and IL-6 in(Lin & Micic, 2021). Therefore, the results of this study further support the potential of hesperidin from Garut orange peel as a natural immunomodulator against respiratory viral infections, including HMPV.

Normality testing using the Shapiro–Wilk method confirmed that body weight data in all experimental groups were normally distributed ($p > 0.05$). Consequently, one-way ANOVA analysis demonstrated no statistically significant differences in mean body weight among groups during the treatment period ($p > 0.05$). Although descriptively the normal group and the hesperidin 100 mg/kgBW group exhibited slightly higher body weight compared to the negative control, these variations were not statistically significant. Overall, the results indicate that hesperidin administration at the tested doses did not affect body weight and did not induce systemic toxicity, suggesting good tolerability throughout the experimental period .(Utomo et al., 2020).

Normality testing of body weight data in all experimental groups showed p-values > 0.05, indicating that the data were normally distributed. Based on these results, a one-way ANOVA was performed, which showed no significant differences between groups ($p > 0.05$). This indicates that administration of hesperidin at various doses did not significantly affect the body weight of the animals during the treatment period. However, descriptively, there was a tendency for increased body weight in the normal group and in the group receiving hesperidin at 100 mg/kgBW compared to the negative control group (CMC + 5% imiquimod). Normality testing of body weight data in all experimental groups showed p-values > 0.05, indicating that the data were normally distributed. Based on these results, a one-way ANOVA was performed, which showed no significant differences between groups ($p > 0.05$). This indicates that administration of hesperidin at various doses did not significantly affect the body weight of the animals during the treatment period. However, descriptively, there was a tendency for increased body weight in the normal group and in the group receiving hesperidin at 100 mg/kgBW compared to the negative control group (CMC + 5% imiquimod).

In addition to body weight measurements, evaluation of major organ

weights showed that administration of 5% imiquimod as a mimic of HMPV infection in the negative control group caused a relative change in spleen weight (0.94 ± 0.27%) compared to the normal group (0.82 ± 0.14%). This change is consistent with the mechanism of imiquimod as a Toll-like receptor 7 (TLR7) agonist, which can activate dendritic cells and macrophages, thereby inducing the release of proinflammatory cytokines and increasing lymphocyte proliferation in the spleen (Raharjo & Hadi, 2019).

In the hesperidin-treated groups at various doses, no significant differences were observed in the weights of the lungs, liver, or kidneys compared to the control groups, indicating that administration of hesperidin extract is relatively safe and does not cause organ toxicity. Interestingly, the spleen showed a tendency for increased relative weight in the group receiving 100 mg/kgBW hesperidin combined with 5% imiquimod (1.35 ± 0.65%) compared to the normal control group, suggesting an adaptive immune response modulated by hesperidin. These findings support previous studies reporting that hesperidin can enhance lymphocyte proliferation, balance the neutrophil-to-lymphocyte ratio, and regulate cytokine expression such as IL-4, IFN-γ, and TNF-α.

Evaluation of the absolute and relative weights of organs (lungs, kidneys, spleen, and liver) showed that most parameters did not differ significantly among the treatment groups. In the negative control group (CMC + 5% imiquimod), there was an increase in the relative spleen index (0.94 ± 0.27%) compared to the normal control group (0.82 ± 0.14%). This finding is consistent with the mechanism of imiquimod as a TLR-7 agonist, which activates dendritic cells and macrophages, thereby stimulating lymphocyte proliferation in the spleen (Raharjo & Hadi, 2019).

In the groups receiving hesperidin at various doses, no significant differences were observed in the absolute or relative weights of the lungs, kidneys, and liver, indicating that hesperidin did not induce organ toxicity. However, the spleen showed a tendency for an increased relative index at the 100 mg/kgBW dose combined with 5% imiquimod (1.35 ± 0.65%), which may reflect activation of the adaptive immune response through enhanced lymphocyte proliferation. This observation aligns with hematological parameters showing a decreased NLR and increased lymphocyte count in the hesperidin-treated groups table 2 (Sazhenova et al., 2021).

Table 2. Hematological Profiles of Experimental Groups Following Hesperidin and Imiquimod

Kelompok	Leukosit total ((10³/µL)	Limfosit (%)	NLR
Normal (CMC 0,5% + Vaseline)	1040 ± 378	960 ± 328,634	0,20
Negatif (CMC 0,5% + Imiquimod 5%)	6100 ± 3601	3920 ± 3399,56	0,31
Positif (Levamisole + Imiquimod 5%)	4100 ± 1434	3800 ± 1135,78	0,09
Hespiridin 100 mg/kgBB	6680 ± 5983	5500 ± 5024,94	0,15
Dosis Hespiridin50 mg/kgBB + Imiquimod 5%	8200 ± 4183	7120 ± 3688,77	0,08
Dosis Hespiridin 100 mg/kg BB + Imiquimod 5%	3260 ± 1996	2600 ± 1958,32	0,16
Dosis Hespiridin 150 mg/kg BB + Imiquimod 5%	16720 ± 3354	14400 ± 2979,93	0,12

Administration Statistical analysis showed that the data for leukocytes, lymphocytes, and NLR were normally distributed (Sig > 0.05), but the variances between groups were not homogeneous (Sig < 0.05). Significant differences were observed in leukocytes (p = 0.001), lymphocytes (p = 0.002), and NLR (p = 0.001), confirming that each treatment—imiquimod, levamisole, or hesperidin—induced distinct and meaningful immunomodulatory effects. In the negative control group (5% imiquimod), significant increases were observed in total leukocytes, lymphocytes, and NLR. Imiquimod is a TLR-7 agonist that recognizes viral ssRNA, activates the MyD88–NF-κB pathway, and enhances the production of IL-6, TNF-α, and IFN-α/β (39,40,41). This inflammatory activation is consistent with lung histopathology findings, where the inflammation score reached 2, characterized by alveolar septal thickening and moderate inflammatory cell infiltration (score 2). This pattern is typical of acute inflammation resembling RNA virus infections, including HMPV (39,42). The significant increase in NLR further supports that imiquimod induces a robust systemic inflammatory response, aligning with reports that NLR is sensitive to the severity of respiratory viral inflammation (Shrestha et al., 2023).

In the levamisole + imiquimod group, NLR significantly decreased despite lymphocyte counts remaining elevated. Histologically, this group exhibited an inflammation score of 1, characterized by mild inflammatory cell infiltration and minimal alveolar narrowing. Levamisole enhances T cell activation, strengthens dendritic cell function, and promotes a more controlled adaptive immune response (Ruiz-Pozo et al., 2025). This indicates a shift from acute neutrophilic inflammation toward a more directed and stable lymphocyte-mediated response.

Hesperidin exhibited dose-dependent immunomodulatory effects. In the groups without imiquimod, the NLR remained low,

reflecting hesperidin's anti-inflammatory action through inhibition of NF-κB, MAPK pathways, and reduction of ROS (Stanisic et al., 2018). Histopathologically, hesperidin alone showed an inflammation score of 2, characterized by alveolar narrowing and moderate inflammatory cell infiltration, although alveolar structure remained preserved. These findings are consistent with literature reporting that hesperidin can maintain tissue integrity despite ongoing inflammation (47).

The administration of hesperidin combined with imiquimod elicited a dose-dependent response. At a dose of 50 mg/kgBW + imiquimod, an inflammation score of 1 was observed, characterized by mild congestion and inflammation while the alveolar structure remained intact. This is typical of low-dose flavonoids, which generally act as mild antioxidants and cell membrane stabilizers. The 100 mg/kgBW + imiquimod dose produced the most optimal results. Histologically, this group had an inflammation score of 0, with normal alveoli and minimal inflammatory cell infiltration. These findings support literature reports that medium-dose hesperidin reduces proinflammatory cytokines and preserves lung structure under virus-induced inflammatory conditions (Bischoff et al., 2023). Hematologically, the combination of increased lymphocytes with low NLR indicates efficient adaptive immune activation without triggering hyperinflammation. At the high dose of 150 mg/kgBW + imiquimod, although the inflammation score was recorded as 0, histological examination revealed areas of extensive inflammatory cell infiltration and congestion, indicating an excessive inflammatory response. This phenomenon aligns with reports that flavonoids at high doses can act as pro-oxidants or induce immune hyperactivation. Hematologically, leukocytes and lymphocytes increased sharply, while NLR remained low, indicating strong immune activation that did not correlate with the severe tissue inflammation observed (Logroño et al., 2021).

The integration of hematological, histopathological, and inflammation score findings suggests that hesperidin exerts its optimal immunomodulatory effect at a dose of 100 mg/kgBW, as evidenced by an inflammation score of 0, increased adaptive lymphocyte response, and well-controlled systemic inflammation, indicated by a low

NLR value. These results support the potential of hesperidin derived from Garut orange peel as a natural immunomodulator against respiratory viral infections. However, its specific efficacy against HMPV should be interpreted cautiously and requires further confirmation using a live viral model.

This study has several limitations that should be explicitly acknowledged. First, direct measurement of pro- and anti-inflammatory cytokines was not performed; therefore, the immunological effects were inferred solely from hematological parameters and histopathological findings without molecular-level confirmation. Second, the experimental model utilized imiquimod-induced inflammation as a surrogate for RNA viral infection, which does not fully replicate the complex immunopathological mechanisms of live HMPV infection. Consequently, future studies employing direct HMPV challenge models along with comprehensive cytokine profiling are strongly recommended to enhance the mechanistic understanding and translational relevance of these findings.

CONCLUSION

This study demonstrates that hesperidin extracted from Garut orange peel (*Citrus nobilis* var. *chrysocarpha*) exhibits effective and safe immunomodulatory activity in Balb/c mice induced with imiquimod as a model of RNA viral infection resembling HMPV. TLC analysis confirmed the presence of hesperidin with an Rf value of 0.45, consistent with standard references. Administration of hesperidin at doses of 50–150 mg/kgBW did not cause significant changes in body weight or major organ weights, indicating the absence of systemic toxicity. Imiquimod induced a strong inflammatory response, evidenced by increased leukocytes, lymphocytes, NLR, and lung histopathological damage.Hesperidin showed dose-dependent immunomodulatory effects, with the 100 mg/kgBW dose producing the most optimal outcome, characterized by reduced NLR, controlled lymphocyte increase, and preserved normal alveolar structure without inflammation. In contrast, the 150 mg/kgBW dose triggered excessive immune activation and extensive inflammatory infiltration, suggesting potential pro-inflammatory effects at higher doses. Overall, hesperidin shows

strong potential as a natural immunomodulator for controlling inflammatory responses in respiratory infections resembling HMPV, with 100 mg/kgBW identified as the optimal dose.

RECOMMENDATION

It is recommended to conduct a more in-depth molecular mechanism analysis, including the measurement of pro- and anti-inflammatory cytokines (IL-6, TNF-α, IFN-γ, IL-10) as well as immune signaling pathways (NF-κB, MAPK), to clarify the mechanism of action of hesperidin as an immunomodulator. Long-term studies (subchronic and chronic) are also necessary to ensure the safety of repeated hesperidin use and to evaluate its potential long-term toxic effects.

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