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Gentiopicroside from Gentiana scabra Bunge: In Vitro Anti-Allergic Mechanism and Skincare Potential

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1. Introduction

Gentiana scabra Bunge is a highly valued herbal remedy commonly prescribed for inflammatory skin conditions attributed to wind-heat or dampness-heat[1]. Its rhizomes and roots, traditionally used in Chinese, Japanese, and Korean medicine, are known for their ability to clear heat, dry dampness, and purge fire from the liver and gallbladder. Consequently, *G. scabra* has been employed to treat various ailments stemming from lower energizer dampness heat, such as jaundice, pudendal swelling, vulvar pruritus, leukorrhea, and eczema. Despite its medicinal applications, its potential in skincare remains largely unexplored. One of the active components of *G. scabra* is gentiopicroside[2, 3]. Allergic reactions, a form of hypersensitivity, arise from abnormal immune responses, with Type I being the most prevalent, primarily mediated by IgE antibodies[4]. In a recent study, the anti-allergic mechanism of gentiopicroside was investigated using in vitro cultures of RBL-2H3 cells. The IgE/Ag complex-mediated allergic reaction experiments revealed that gentiopicroside could inhibit cell degranulation in a dose-dependent manner, as well as the expression of cytokines like tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-6. These findings suggest that gentiopicroside can effectively suppress allergic reactions, highlighting its potential as a therapeutic agent and its promising application in skincare products.

2. Materials and Methods

Cell culture

RBL-2H3 cells were adherent and cultured in MEM medium supplemented with 15% fetal bovine serum and 100 U/mL penicillin and 100 μg/mL streptin at 37℃ in a 5% CO_2 incubator.

Detection of degranulated β-Hex by cell activation

RBL-2H3 cells (6×10^4/mL) were seeded in a 24-well plate (1 mL/well) and divided into groups: control (sensitized, no stimulation, no drug), model (sensitized, stimulated, no drug), drug group (sensitized, stimulated, gentiopicroside 3.12/6.25/12.5 μM), and positive control (sensitized, stimulated, 25 μM ketotifen fumarate), each with three replicates. After 24 h, cells were

washed with PBS, sensitized with 500 μL anti-DNP-IgE (0.2 μg/mL) for 12 h. Drug group received corresponding drugs, normal group received MEM medium. After 2 h, cells were washed and stimulated with 400 μL DNP-HAS (0.2 μg/mL) or Tyrode's buffer for 1 h, then terminated by ice-bathing for 10 min. Supernatants were collected (50 μL/well to 96-well plate). Cells were lysed with 0.5% Triton X-100 (30 min on ice), lysates collected (50 μL/well to 96-well plate), and 50 μL colorimetric reagent added. After 1 h at 37℃, 200 μL stop solution was added, and absorbance was measured at 405 nm within 5 min using a microplate reader.

Expression of inflammatory factors

Total RNA was extracted from each group of cells, reverse transcription was performed, gene expression was performed according to the instructions, and gene expression was detected by quantitative real-time PCR (qRT-PCR).

Table 1. The primers of qRT-PCR.

Gene name	Primer sequence
GAPDH	F: TTCACCACCATGGAGAAGGC R: GGCATGGACTGTGGTCATGA
IL-4	F: GGATGTAACGACAGCCCTCT R: GTGTTCCTTGTTGCCGTAAG
TNF-α	F: TCAGCCTCTTCTCATTCCTGC R: TTGGTGGTTTGCTACGACGTG
IL-1β	F: CAGCTTTCGACAGTGAGGAGA R: TTGTCGAGATGCTGCTGTGA
IL-6	F: ACAAGTCCGGAGAGGAGACT R: TTGCCATTGCACAACTCTTTTC
IL-13	F: GCTCTCGCTTGCCTTGGTGGTC R: CATCCGAGGCCTTTTGGTTACAG
IFN-γ	F: GAGGTGAACAACCCACAGATCCA R: CGACTCCTTTTCCGCTTCCTTAG
FcεR Ⅰα	F: AGATGCCGTAGCTGGAAGAAA R: AATGATGGGAAAATGAGTTGTAGC

3. Results

Effect of gentiopicroside on the viability of RBL-2H3 cells

In order to obtain the appropriate range of gentiopicroside action dose, the viability of RBL-2H3 cells was first measured by CCK8 assay. The results showed that gentiopicroside had no significant toxic effect on the cells in the concentration range of 0-200 μM. The concentrations of gentiopicroside were 3.12, 6.25, and 12.5 μM for subsequent experiments (Figure.1a).

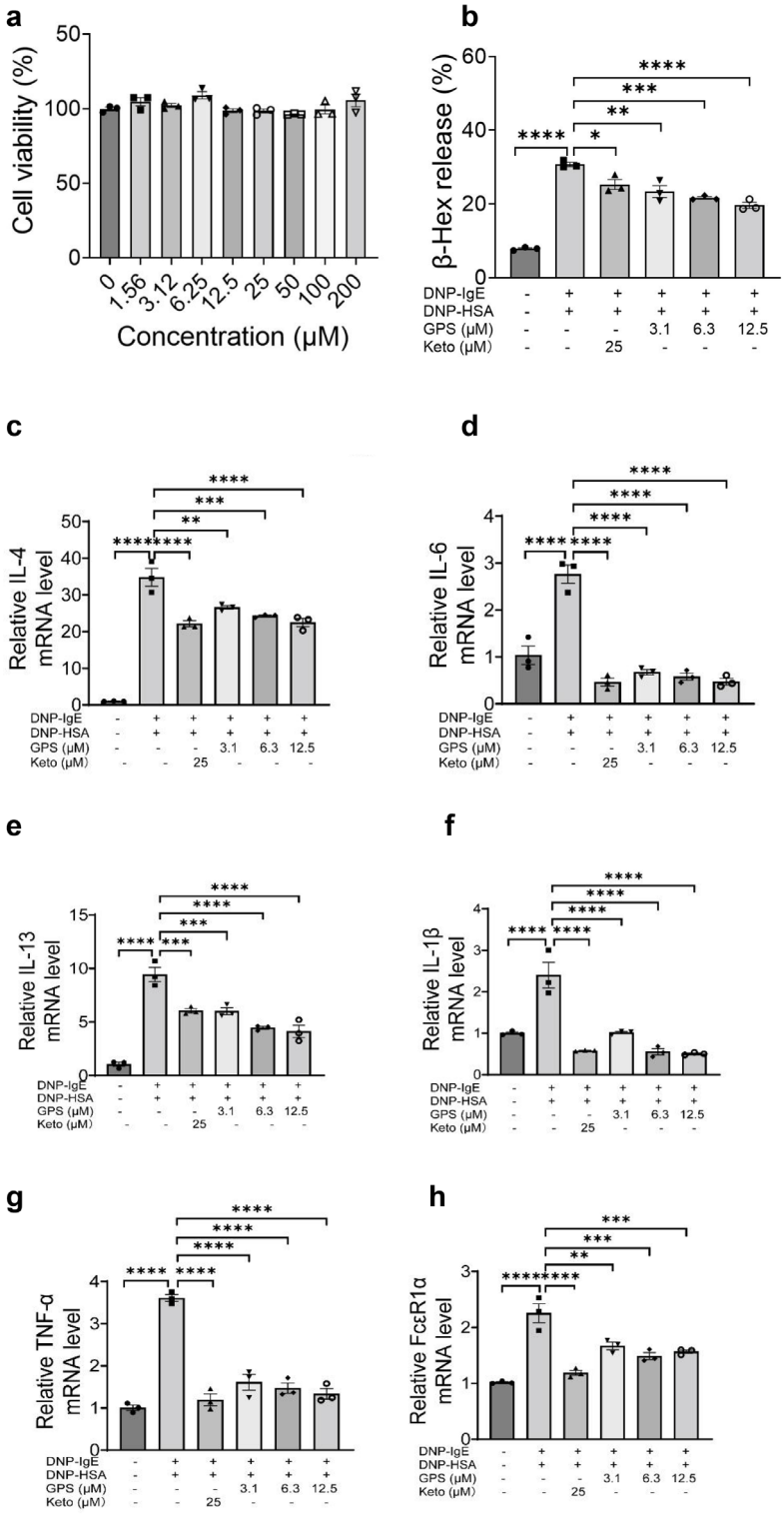

Figure 1. Effect of gentiopicroside on IgE-mediated type I anaphylaxis: (a) The effect of gentiain on the viability of RBL-2H3 cells; (b) The effect of gentian glycoside on the degranulation and release of β-Hex in RBL-2H3 cells; (c-h) Effect of gentiopicroside on mRNA expression in RBL-2H3 cells.

Effect of gentiopicroside on degranulation release of β-Hex from RBL-2H3 cells

The release rate of β-Hex in the control group was 7.35%. The release rate of β-Hex in the model group was 31.88%, which was significantly different from that in the control group. Compared with the model group, the release rate of gentiopicrin group decreased in a concentration-dependent manner, and the inhibitory effect of each group was better than that of ketotifen fumarate group (Figure.1b). These results indicated that gentiopicroside could inhibit the degranulation of RBL-2H3 cells.

Effect of gentiopicroside on RBL-2H3 cytokine mRNA expression

When allergic reaction occurs, inflammatory immune response occurs, mast cells are activated, and biological mediators such as inflammatory factors are released. In contrast, cross-linking of IgE with the high-affinity IgE receptor (FcεRI) on the surface of mast cells and basophils results in cell activation. The continuation of the allergic response requires the involvement of pro-inflammatory factors released by mast cells, and to further investigate the involvement of inflammatory factors in the inhibition of the allergic response by gentiopicroside, qRT-PCR experiments were performed. The results showed that compared with the control group, the mRNA expression of FcεR1α, IL-1β, IL-4, IL-6, IL-13 and TNF-α in the model group was increased ($p<0.01$), indicating that the cells were activated in the allergic reaction and participated in the inflammatory response (Figure.1c-h). Compared with the model group, gentiopicroside treatment group could reduce its expression in a dose-dependent manner ($p<0.01$). These results indicate that gentiopicroside inhibits inflammation and allergic reactions by inhibiting the expression of cytokines.

4. Discussion

The present study demonstrates that gentiopicroside exhibits significant anti-allergic and anti-inflammatory effects in IgE-mediated RBL-2H3 mast cells without inducing cytotoxicity at concentrations up to 200 μM. The CCK-8 assay confirmed the safety of gentiopicroside, allowing its use at 3.12–12.5 μM in subsequent experiments. Notably, gentiopicroside effectively suppressed β-hexosaminidase (β-Hex) release in a concentration-dependent manner, outperforming the reference drug ketotifen fumarate. This suggests that gentiopicroside may stabilize mast cell membranes or interfere with FcεRI-mediated degranulation, a critical step in allergic responses.

Furthermore, gentiopicroside significantly downregulated the mRNA expression of key pro-inflammatory cytokines (IL-1β, IL-4, IL-6, IL-13, TNF-α) and FcεR1α, which are pivotal in mast cell activation and allergic inflammation. The dose-dependent inhibition of these markers aligns with the observed reduction in degranulation, reinforcing gentiopicroside's dual role in blocking both immediate (degranulation) and delayed (cytokine-driven) phases of allergic reactions. These findings suggest that gentiopicroside modulates the FcεRI signaling pathway, potentially disrupting the IgE-mediated cascade.

In conclusion, gentiopicroside holds promise as a therapeutic agent for allergic diseases by targeting mast cell activation and inflammatory cytokine production. Future studies should explore its in vivo efficacy and precise molecular mechanisms, particularly its interaction with FcεRI and downstream signaling pathways such as Syk or NF-κB.

5. Conclusion

In this study, gentiopicrin was used as the research object, and RBL-2H3 cell activation degranulation model was used to explore the effect of gentiopicrin on cutaneous type I allergic reaction. Cell experiments confirmed that gentiopicrin played an anti-allergic role by reducing

the expression of β-Hex inflammatory factors. Taken together, our findings suggest that gentiopicrin has some therapeutic potential for type I allergic reactions and can be used as a potential therapeutic agent.

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