A novel arctigenin-containing latex glove prevents latex allergy by inhibiting type I/IV allergic reactions

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[ABSTRACT] The present study aimed at developing a natural compound with anti-allergic effect and stability under latex glove manufacturing conditions and investigating whether its anti-allergic effect is maintained after its addition into the latex. The effects of nine natural compounds on growth of the RBL-2H3 cells and mouse primary spleen lymphocytes were determined using MTT assay. The compounds included glycyrrhizin, osthole, tetrandrine, tea polyphenol, catechin, arctigenin, oleanolic acid, baicalin and oxymatrine. An ELISA assay was used for the in vitro anti-type I/IV allergy screening; in this process β-hexosaminidase, histamine, and IL-4 released from RBL-2H3 cell lines and IFN-γ and IL-2 released from mouse primary spleen lymphocytes were taken as screening indices. The physical stability of eight natural compounds and the dissolubility of arctigenin, selected based on the in vitro pharmacodynamic screening and the stability evaluation, were detected by HPLC. The in vivo pharmacodynamic confirmation of arctigenin and final latex product was evaluated with a passive cutaneous anaphylaxis (PCA) model and an allergen-specific skin response model. Nine natural compounds showed minor growth inhibition on RBL-2H3 cells and mouse primary spleen lymphocytes. Baicalin and arctigenin had the best anti-type I and IV allergic effects among the natural compounds based on the in vitro pharmacodynamic screening. Arctigenin and catechin had the best physical stability under different manufacturing conditions. Arctigenin was the selected for further evaluation and proven to have anti-type I and IV allergic effects in vivo in a dose-dependent manner. The final product of the arctigenin-containing latex glove had anti-type I and IV allergic effects in vivo which were mainly attributed to arctigenin as proved from the dissolubility results. Arctigenin showed anti-type I and IV allergic effects in vitro and in vivo, with a good stability under latex glove manufacturing conditions, and a persistent anti-allergic effect after being added into the latex to prevent latex allergy.

[KEY WORDS] Latex allergy; Arctigenin; RBL-2H3 cell; Mouse primary spleen lymphocytes

[CLC Number] R965

Introduction

Natural rubber latex (NRL) products are an integral part of human daily life. Rubber is manufactured after drawing the raw material from the *Hevea brasiliensis* tree [1-2]. NRL medical gloves and condoms are two kinds of common NRL products. NRL medical gloves are widely used in the medical environment for their good durability and ductility and their effectiveness in isolation of blood-borne infections [3].

Although NRL products are mostly safe in humans, there are certain populations suffering from allergy symptoms after exposure to NRL products, including contact dermatitis and contact urticaria, so called latex allergy [4]. Latex allergy mainly has effects on the skin, but itching and erythema can have a great impact on the quality of life [5]. It is reported that latex allergy can also damage other tissues besides skin and cause a series of severe symptoms. Latex allergy has gained lots of attention due to the
prevalence becoming higher and higher [1]. However, it is still unknown why latex products, especially latex gloves, are such potent sensitizers for allergies. Researchers have found that there are three main allergens causing latex allergy, including cornstarch powder, protein of latex, and chemical additives [3]. Cornstarch powder is used as a donning agent for gloves [6]. Experimental and clinical studies have confirmed that cornstarch promotes disease by two different mechanisms [7]; firstly, it acts as a foreign body that causes severe inflammatory responses and interferes with the host’s defenses against infection [8-9]; secondly, cornstarch may be a carrier for latex allergens [10-11]. The protein content of latex averages about 1% and makes contribution to most properties of gloves, like ductility and barrier function [12]. It usually causes immediate hypersensitivity (type I) mediated by immunoglobulin E (IgE), including localized urticaria (stage 1), angioedema (stage 2), asthma (stage 3), and anaphylaxis (stage 4) [13]. Chemical additives are referred to the chemicals added as preservatives, antiocoagulants, accelerators for vulcanization and antioxidants, such as thium or carbatame. They may cause delayed hypersensitivity (type IV), which is mediated by T lymphocytes [14-15].

NRL medical gloves are necessary protective equipments due to their good barrier function, and therefore taking off gloves in the hospital environment that is full of infection sources is not a good solution to latex allergy. Recently, in order to avoid latex allergy, some new kinds of gloves have been invented [3]. Powder-free gloves in the absence of cornstarch are produced with either a hydrogel polymer or surfactant as mold-release agents for latex gloves [7]. Though powder-free gloves provide a solution to latex allergy caused by cornstarch, they cannot prevent the allergic reaction caused by the main allergen-latex protein. Recently, a variety of synthetic gloves (e.g., vinyl, synthetic polyisoprene, polyurethane, nitrile, neoprene, and block polymers) which are latex-free, are available as substitutes for NRL gloves [14]. However, synthetic gloves may contain carbanates – which may cross-react with thium or carbatame and thioeac – and hence they may place NRL allergic patients with concomitant type IV reactions at risk for dermatitis [15]. However, this does not necessarily indicate that it is completely allergen-free, as both type I and type IV reactions to vinyl gloves have been reported [17]. Despite recent advances in synthetic gloves, latex gloves are still regarded as the best barrier against blood-borne pathogens. Latex is flexible with an extraordinary ability to stretch and tear resistant. It conforms to the shape of the hands, giving freedom of movement and retaining tactile sensitivity and fine manual dexterity [18].

Apparently, it is preferred to have a type of gloves with good ductility along with other advantages of latex but without causing latex allergy. Our hypothesis was that a natural compound with anti-allergic effect can prevent latex allergy after its incorporation into the latex during the manufacture process. However, there are more than 10 allergens related to latex protein, besides cornstarch and chemical additives [19]. Therefore, it is really hard to have one drug to prevent many allergens. In order to bypass this problem, our research design was based on the final biological pathways of all allergens, i.e., type I and type IV allergic reactions. As for the drug selection, natural compounds are commonly used for screening because of their low toxicity and high efficacy. Hence, in the present study, we selected nine natural compounds that have been reported in scientific literatures to possess anti-allergic effects, including glycyrrhizin, osthole, tetrandrine, tea polyphenol, catechin, arctigenin, oleanolic acid, baicalin, and oxymatrine. The in vitro pharmacodynamic model and the manufacturing stability evaluation experiments were established to select one natural compound that was further subject to an in vivo pharmacodynamic study using passive cutaneous anaphylaxis model and allergen specific skin response model. The dissolubility of the natural compound in the final latex was investigated in order to determine its dose in the final latex product and its dissolution under hand sweat simulation solution and three distilled water condition and to confirm the anti-allergic effect of the final latex containing the natural compound using the same pharmacodynamic model in vivo.

Materials and Methods

Reagents

Nine natural compounds, including glycyrrhizin, osthole, tetrandrine, tea polyphenol, catechin, arctigenin, oleanolic acid, baicalin, and oxymatrine were purchased from Zelang Technology Co., Ltd. (Nanjing, China). Fetal bovine serum (FBS), Dulbecco’s modified Eagle’s medium (DMEM), Roswell Park Memorial Institute-1640 medium (RPMI-1640), and penicillin–streptomycin were purchased from Gibco (Grand Island, NY, USA). DMSO, 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphnyl-2H-tetrazoliumbromide (MTT), ConA, anti-DNP IgE, DNP-BSA, ovabumin, aluminum hydroxide adjuvant. Evans blue saline, disodium cromoglycate p-nitrophenyl-V-acetyl-beta-D-glucosaminide, and 2, 4-dinitrofluorobenzene (2, 4-DNFB) were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Final latex gloves containing arctigenin were manufactured by Jiangsu Guotai International Group Guomao Co., Ltd. (Zhangjiagang, China). ELISA kits were purchased from R&D Systems (Minneapolis, MN, USA).

Drug preparation

The chemical structures of the nine natural compounds are shown in Fig. 1. For in vitro pharmacodynamic screening, stock solutions of the nine monomers (1 mol·L-1) were prepared in DMSO and diluted with DMEM or RPMI-1640 medium to final concentrations of 10–6, 10–5, and 10–4 mol·L-1, respectively. To investigate the stability under the latex manufacturing conditions, the compounds’ stock solutions (1 mg·mL-1) were prepared with HPLC-grade methanol. For in vivo pharmacodynamic confirmation of arctigenin, the
powder of arctigenin was added into an ointment matrix [oil phase: stearic acid (14.4 g), glycerol monostearate (4.2 g), albolene (7.2 g), lanolin (1.2 g); water phase: triethanolamine (0.48 g), tween 80 (0.12 g), and H2O (100 mL)]; both phases were water bathed at temperature 80 °C, the water phase was slowly added to the oil phase, and finally the mixture was stirred until a white paste was formed. After cooling, the ointment matrix was made to final concentrations of 20, 60, and 200 mg·kg⁻¹. Disodium cromoglycate’s stock solution was prepared with DMSO and then diluted with DMEM or RPMI-1640 medium to final concentrations of 10⁻⁶, 10⁻⁵, and 10⁻⁴ mol·L⁻¹.

Cell culture

RBL-2H3 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were maintained in DMEM supplemented with 10% heat-inactivated FBS, penicillin (100 U·mL⁻¹), and streptomycin (100 μg·mL⁻¹) at 37 °C in a humidified atmosphere with 5% CO₂.

Mouse primary spleen lymphocytes were obtained from the SPF BALB/c mice aged about 4 weeks, following the procedures based on the reports published before with some improvement [20]. After the mice were sacrificed by cervical dislocation, spleens were aseptically removed with scissors and forceps, kept in RPMI 1640 medium, cut into pieces, and then passed through a sterilized mesh (200 meshes) to obtain single cell suspensions. Erythrocytes in the cell mixture were washed by the ammonium chloride repeatedly. Finally, the cells were suspended to a final density of 5 × 10⁶ cells/mL in RPMI-1740 medium supplemented with 10% FBS and cultured at 37 °C in a humidified atmosphere with 5% CO₂.

Cell viability assay

Cell viability was determined with the MTT assay. The RBL-2H3 cells were seeded into a 96-well plate at a density of 5 × 10³/well, while primary spleen lymphocytes (1 × 10⁵/mL) were cultured in the presence of ConA (5 μg·mL⁻¹). After different concentrations (10⁻⁶, 10⁻⁵, and 10⁻⁴ mol·L⁻¹) of the nine compounds were added, the cells were incubated for 24 h. Then, 10 μL of MTT solution (5 mg·mL⁻¹ in PBS) was added into each well and the cells were further incubated at 37 °C for another 4 h. Following medium removal, 100 μL of DMSO was added to each well and the plates were gently shaken for 5 min. Optical absorbance was determined at 570 nm with a microplate spectrophotometer (BD Bioscience, USA). The absorbance of cells without treatment was regarded as 100% of cell survival. Control group were treated with the same amount of DMSO (0.1%) as used in the corresponding experiment. Each treatment was performed in quintuplicate and each experiment was repeated three times.

β-Hexosaminidase release assay

The degree of degranulation of RBL-2H3 cell lines, simulated by IgE and antigen was determined using the β-hexosaminidase release assay, according to previous report [21]. Briefly, RBL-2H3 cells (1 × 10⁵/well, in 96-well plates) were sensitized with anti-DNP IgE (50 ng·mL⁻¹) overnight and were washed once with PBS. 20 μL of different concentrations (10⁻⁶, 10⁻⁵, and 10⁻⁴ mol·L⁻¹) of the nine compounds were added to each well and the plate was incubated for 10 min, followed by the addition of 20 μL of antigen (DNP-BSA, final concentration 100 ng·mL⁻¹) at 37 °C for 30 min to stimulate the cellular degranulation. The reaction was stopped by cooling in an ice bath for 10 min. The supernatant (50 μL) was transferred to a 96-well microplate and incubated with 50 μL of substrate (1 mmol·L⁻¹ p-nitrophenyl-N-acetyl-β-D-glucosaminide) in 0.1 mol·L⁻¹ citrate buffer at 37 °C for 1 h. The reaction was stopped by
adding 200 μL of stop solution (0.1 mol·L⁻¹ Na₂CO₃/ NaHCO₃). The absorbance was measured at 405 nm with a microplate spectrophotometer (BD Bioscience). The inhibition of the release of β-hexosaminidase by the tested compounds was measured [22].

ELISA assay

Histamine and IL-4 released from RBL-2H3 cells treated with the nine natural compounds were measured by ELISA assay. Briefly, the RBL-2H3 cells (2 × 10^5 cells/mL) were sensitized overnight with 50 ng·mL⁻¹ of anti-DNP IgE in 6-wells plates, which were then washed once with PBS. Three concentrations (10⁻⁶, 10⁻⁵ and 10⁻⁴ mol·L⁻¹) of the nine natural compounds were added to each well at 20 μL/well and the cells were incubated for 24 h. 20 μL of antigen (DNP-BSA, final concentration 100 ng·mL⁻¹) was then added and the cells were further incubated for simulation at 37 °C for 30 min. The supernatant was collected and centrifuged at 400 g for 5 min. The fluorescence intensity was measured at an excitation emission wavelength of 450 nm following the manufacturer’s procedures.

IFN-γ and IL-2 released from mouse primary spleen lymphocytes treated with the nine natural compounds were measured by ELISA assay. Briefly, the mouse primary spleen lymphocytes were treated with three concentrations (10⁻⁶, 10⁻⁵ and 10⁻⁴ mol·L⁻¹) of the nine natural compounds in the presence of ConA (5 μg·mL⁻¹). The supernatant was collected and centrifuged at 400 g for 5 min. The fluorescent intensity was measured at an excitation emission wavelength of 450 nm, following the manufacturer’s procedures.

HPLC method

HPLC-UV (Kyoto, Japan) chromatography was used to investigate the stability of eight compounds under different latex manufacturing conditions. A Diamonsil C₁₈ analytical column (250 mm × 4.6 mm, 5 μm, Habon, Jiangsu, China) was used. The flow rate was 1.0 mL·min⁻¹, the column temperature was maintained at 30 °C, and the injection volume was 20 μL. The concentration of DMSO in each control group was the same as its corresponding sample group. The mobile phase and the wavelength used for detection of the nine compounds are shown in Table 1.

<table>
<thead>
<tr>
<th>Natural compounds</th>
<th>Wavelength (nm)</th>
<th>Mobile Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baicalin</td>
<td>280</td>
<td>Methanol–H₂O 50 : 50 V/V</td>
</tr>
<tr>
<td>Arctigenin</td>
<td>280</td>
<td>Methanol–H₂O 50 : 50 V/V</td>
</tr>
<tr>
<td>Osthole</td>
<td>320</td>
<td>Methanol–H₂O 50 : 50 V/V</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>252</td>
<td>ACN–H₂O 50 : 50 V/V</td>
</tr>
<tr>
<td>Catechin</td>
<td>280</td>
<td>ACN–H₂O 10 : 90 V/V</td>
</tr>
<tr>
<td>Tea polyphenol</td>
<td>280</td>
<td>ACN–H₂O 10 : 90 V/V</td>
</tr>
<tr>
<td>Oxyrmatrine</td>
<td>220</td>
<td>ACN–H₂O 10 : 90 V/V</td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>210</td>
<td>Methanol–H₂O 50 : 50 V/V</td>
</tr>
</tbody>
</table>

The preparation of sample solutions under different latex manufacturing conditions was as follows:

Different temperature conditions

Each compound’s stock solution was diluted 100 times to 500 μL with HPLC-grade methanol. After being heated in a water bath at constant temperature of 20, 40, 60, 80, and 100 °C for 3 h, the solutions were further heated in an oil bath at 120 °C, and made up to 500 μL with HPLC-grade methanol to prepare the sample solution.

Different pH conditions

1 mL of compound stock solution was mixed with 9 mL of HCl (1 mol·L⁻¹) and NaOH (1 mol·L⁻¹) for 2 h, respectively. After 10-times dilution with HPLC-grade methanol, the sample solutions were prepared.

Co-existed with natural rubber condition

Natural rubber latex (1 g) was put into 1 mL of monomer’s stock solution. After stirring for 2 h, the supernatant was collected and diluted 10 times with HPLC-grade methanol in order to prepare the sample solutions.

Co-existed with silicone oil condition

1 mL of monomer’s stock solution was mixed with 9 mL of silicone oil for 2 h, and then the aqueous solution was taken and diluted 10 times with HPLC-grade methanol to make the sample solutions.

Co-existed with vulcanized latex condition

Vulcanized latex (1 g) was put into 1 mL of monomer’s stock solution. After stirring for 2 h, the supernatant was taken and diluted 10 times with HPLC-grade methanol to prepare the sample solutions.

Co-existed with hand sweat simulation solution condition

1 mL of monomer’s stock solution was mixed with 9 mL of hand sweat simulation solution (1 mol·L⁻¹ urea, 1 mol·L⁻¹ NaCl, and 9 g·L⁻¹ lactic acid) for 2 h at 37 °C. The aqueous solution was taken and diluted 10 times with HPLC-grade methanol to prepared the sample solutions.

Co-existed with latex product condition

Latex products were cut into pieces and put in 18 mL of HPLC-grade methanol. After being mixed with 2 mL of monomer’s stock solution for 2 h, the aqueous solution was taken and diluted 10 times with HPLC-grade methanol to prepare the sample solutions.

Dissolubility test of arctigenin from the final latex product

We established a stable HPLC-UV chromatograph condition to investigate the stability of nine monomers under the different latex manufacturing conditions. A Diamonsil C₁₈ analytical column (250 mm × 4.6 mm, 5 μm) was used with a mobile phase consisting of 50% methanol and 50% water. The detection wave-length was set at 280 nm. The flow rate was 1.0 mL·min⁻¹, the column temperature was maintained at 30 °C, and the injection volume was 20 μL. The preparation of sample solutions was as follows: 3 g of final latex products with different concentrations of arctigenin (1 g/100 g, 2 g/100 g, and 6 g/100 g) were cut into pieces and mixed with 50
mL of three distilled water or hand-sweat simulation solution, then stood still for 2 h and stirred for 1 h in the water bath at 37 °C. Then, 2 mL of the above solution was centrifuged at a speed of 4 500 r·min⁻¹ for 20 min at 4 °C, and 150 μL of the supernatant was dried at 45 °C and the residual was made to the sample solution with HPLC-grade methanol.

The dissolubility from the final latex product of arctigenin was calculated using the following equation:

\[
\text{Dissolubility (\%) = \frac{A}{A_t} \times C_t \times V \times S \times 100}
\]

\(A\): Peak area of the test solution; \(A_t\): Peak area of self-control solution; \(C_t\): Concentration of self-control solution; \(V\): Volume of the test solution; \(S\): Diluted ratio of the test solution

Passive cutaneous anaphylaxis

Sixteen ICR mice (weighing 18–20 g, half male and half female) were immunized using an intraperitoneal injection of ovalbumin (OVA, 1 mg), with aluminum hydroxide adjuvant, on each of 7 alternate days. Fourteen days after the beginning of the sensitization, blood samples were drawn from the animals’ eyeballs and then centrifuged at 3 500 r·min⁻¹ to obtain the serum. Another 70 ICR mice (weighing 18–20 g, 10/group, half male and half female) were administrated percutaneously on their freshly shaved backs with the ointment matrix consisting of different concentrations of arctigenin for 3 days, while the final latex product tied to the dorsal skin of the mice. Then 3 spots (0.1 mL/spot) from each mouse were intradermally injected with the serum made above diluted by a saline solution (ratio = 1 : 15) and the mice were continuously administrated for 3 days. At 1 h after the last administration, each mouse received an injection of 200 μL of 0.5% Evans blue saline solution containing 1% OVA via the tail vein. 20 min later, the mice were sacrificed by cervical dislocation and their left and right ears were then removed, cut into 7-mm², and weighed. The thymus and spleen of each mouse were also removed and weighed, and the thymus/spleen index was calculated using the following equation:

\[
\text{Thymus/spleen Index = thymus / spleen’s weight (mg) / mouse’s weight (g) × 10}
\]

All数据 were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA with Turkey’s correction for multiple comparisons; \(P < 0.05\) was considered statistically significant.

Results

Effects of the nine natural compounds on cell viability

To investigate the anti-allergic effect of the nine natural compounds, MTT assay was performed to examine their toxicity in RBL-2H3 cells and primary spleen lymphocytes. As shown in Fig. 2A, most of the compounds had little cytotoxic effects on the RBL-2H3 cells, and the inhibitory rate was below 50% after 24 h treatment. As shown in Fig. 2B, most of the monomers were not cytotoxic, and rather the opposite, some monomers had proliferative effect on primary spleen lymphocytes, like arctigenin. We concluded that the nine compounds did not show a significant cytotoxic effect on both RBL-2H3 cells and primary spleen lymphocytes and that the three concentrations (10⁻⁶, 10⁻⁵, and 10⁻⁴ mol·L⁻¹) could be used in further in vitro experiments.

Fig. 2  Effects of nine natural compounds on RBL-2H3 cells (A) and primary mouse spleen lymphocytes (B) viability. RBL-2H3 cells (A) and primary mouse spleen lymphocytes (B) were treated for 24 h with various concentrations of nine natural compounds (10⁻⁶, 10⁻⁵, and 10⁻⁴ mol·L⁻¹) and cell viability were determined with MTT assay. Results shown are means ± SEM and are representative of three independent experiments. Data were analyzed by One-Way Analysis of Variance followed by post hoc Turkey test.
Anti-allergic effects of the nine natural compounds in vitro

After treatment with the nine natural compounds, β-hexosaminidase, histamine, and IL-4 released from RBL-2H3 cells for the anti-type I allergic effect, and IL-2 and IFN-γ released from primary spleen lymphocytes for the anti-type IV, were tested. Disodium cromoglycate, a well-known anti-type I allergic drug, was used as a positive control. As shown in Fig. 3A, all the nine compounds showed an inhibition on the release of β-hexosaminidase from RBL-2H3 cells, with higher potency from glycyrrhizin and osthole, compared to the control group. As shown in Fig. 3B, the nine monomers had inhibitory effects on histamine release, with baicalin and arctigenin having greater effects. Similar results were also obtained regarding the release of IL-4 from RBL-2H3 cells (Fig. 3C), and IL-2 and IFN-γ released from primary spleen cells (Figs. 4A and 4B).

Stability of eight natural compounds under the latex manufacturing conditions

Based on the anti-type I and type IV allergic screening, tetrandrine had the least anti-allergic efficacy, therefore it was excluded from further physical stability evaluation. The stability of the eight remaining compounds was detected by HPLC method under different latex manufacturing conditions. As shown in Table 2, all the natural compounds were stable at 100 °C, except for oleanolic acid and oxymatrine. All the HPLC peaks of all the eight natural compounds were abnormal at 120 °C. As shown in Table 2, the physicochemical stability of arctigenin, tea polyphenol, and catechin were stable under acid conditions, while other natural compounds was unstable under alkaline conditions. These results suggested that natural compounds should not be added into the latex under the condition of 120 °C or the alkaline environment. Arctigenin, osthole, and catechin were stable under conditions of coexistence with natural latex, vulcanized latex, silicone oil, hand sweat simulation, and final latex product. As shown in Table 2, arctigenin, catechin and osthole had better stability than other natural compounds, except under alkaline and high temperature conditions.

Fig. 3  Effects of nine natural compounds on RBL-2H3 cells’ β-hexosaminidase release (A), histamine (B) and IL-4 (C). RBL-2H3 cells were treated with different concentration of nine natural compounds and disodium cromoglycate as positive control (10^{-6}, 10^{-5} and 10^{-4} mol·L^{-1}) for 24 h and their effect on cytokine release of RBL-2H3 cells were determined as described in “Material and Methods”. Results shown are means ± SEM and are representative of three independent experiments. Data were analyzed by One-Way Analysis of Variance followed by post hoc Turkey test. *P < 0.05, **P < 0.01 vs model
Fig. 4 Effects of nine natural compounds on primary mouse spleen lymphocytes' IL-2 (A) and IFN-γ (B). Primary mouse spleen lymphocytes were treated with different concentration of nine natural compounds (10⁻⁶, 10⁻⁵ and 10⁻⁴ mol·L⁻¹) for 24 h and their effect on cytokine release of primary mouse spleen lymphocytes were determined as described in “Material and Methods”. Results shown are means ± SEM and are representative of three independent experiments. Data were analyzed by One-Way Analysis of Variance followed by post hoc Turkey test. *P < 0.05, **P < 0.01 vs model

Table 2 Physical stability of eight natural compounds under manufacturing conditions. The sample solutions under different manufacturing conditions (different temperature condition, different pH condition and co-existed with natural rubber (A), silicone (B), vulcanized latex (C), hand sweat simulation solution (D) and latex product (E) ) were prepared as described in “Material and Methods”. HPLC was used to determine physical stability of nine natural compounds under manufacturing conditions

<table>
<thead>
<tr>
<th>Glycyrrhizin</th>
<th>Osthole</th>
<th>Tea polyphenol</th>
<th>Catechin</th>
<th>Arctigenin</th>
<th>Oleanolic acid</th>
<th>Baicalin</th>
<th>Oxymatrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>H⁺</td>
<td>OH⁻</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>Condition</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Osthole</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Tea polyphenol</td>
<td>+</td>
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<td>+</td>
<td>±</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Catechin</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Arctigenin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Oleanolic acid</td>
<td>+</td>
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<td>±</td>
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<td>±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Baicalin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>–</td>
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<td>Oxymatrine</td>
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<td>–</td>
<td>–</td>
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</tbody>
</table>

“+” represents a stable state from HPLC results, “–” represents the opposite, and “±” as uncertain stability

Pharmacodynamic confirmation of arctigenin in vivo

Though the results above showed that arctigenin had an anti-allergic effect in vitro, the in vivo anti-allergic effect required further investigation. The PCA model was used to detect the anti-immediate hypersensitivity effect of arctigenin. As shown in Fig. 5A, the OD value of the blue spots on mouse’s

Fig. 5 In vivo confirmation for anti-type I/IV allergic effect of arctigenin. In vivo anti-type I allergic effect of different concentration of arctigenin (20, 60, 200 mg·kg⁻¹) and isodium cromoglycate as positive control (30 mg·kg⁻¹) was determined by the mice passive cutaneous anaphylaxis model (A); while anti-type IV allergic effect of arctigenin with the mice contact dermatitis model, whose details were in “Material and Methods”. Results shown are means ± SEM and are representative of three independent experiments. Data were analyzed by One-Way Analysis of Variance followed by post hoc Turkey test. *P < 0.05, **P < 0.01 vs control
back skins in arctigenin group decreased significantly in a dose-dependent manner, compared with the model group, which confirmed that arctigenin had an anti-immediate hypersensitivity (type I) effect in vivo. Fig. 5B shows that the difference in weight between left and right ears of each mouse from the arctigenin group was significantly lower, compared with the model group, demonstrating that arctigenin had also an anti-delayed hypersensitivity effect in vivo. Therefore, arctigenin exhibited an anti-immediate and an anti-delayed hypersensitivity effects in vivo.

**Dissolubility and in vivo pharmacodynamic confirmation of the final latex product with arctigenin**

The dissolubility of the final latex products with arctigenin under triple-distilled water and hand sweat simulation conditions was detected by HPLC. As shown in Fig. 6A, the dissolubility of the final latex product (2 g/100 g) with arctigenin under triple-distilled water condition was 3.8%, while it was 2.9% under hand sweat simulation condition. Based on the dissolubility and the in vivo anti-allergic effect of arctigenin, 6 g/100g was used as the dose of arctigenin in the final latex product. The PCA model and allergen-specific skin model were used to confirm the anti-allergic effects of the final latex product. As shown in Fig. 6B1, the OD value of blue spots on mice’s back skin from final latex product’s group decreased significantly, compared with the model group, which implied that the final product had an anti-immediate hypersensitivity effect in vivo. As shown in Fig. 6B2, the weight difference between left and right ears of each mouse from the final latex product’s group was significantly lower, compared with the model, demonstrating that arctigenin had an anti-delayed hypersensitivity effect in vivo. In summary, the final latex product with arctigenin had an anti-immediate and an anti-delayed hypersensitivity effects in vivo.

Fig. 6 Anti-type I/IV allergic evaluation of final latex product containing arctigenin in vivo. The dissolubility of final latex product containing 2 g/100 g (1) and 6 g/100 g (2) of arctigenin was determined by HPLC method under triple-distilled water (A1) and hand sweat stimulation(A2). Based on the dissolubility calculated and the results of arctigenin’s pharmacological experiment in vivo, that the dose arctigenin in final latex is 6 g/100 g was decided to be used for further anti-type I/IV allergic evaluation in vivo with the mice passive cutaneous anaphylaxis model (B1) and the mice contact dermatitis model (B2). Results shown are means ± SEM and are representative of three independent experiments. Data were analyzed by One-Way Analysis of Variance followed by post hoc Turkey test. *P < 0.05, **P < 0.01 vs model.
Discussion

Latex allergy gains more and more attention for causing serious immediate and delayed hypersensitivity. In the view of reducing latex allergens, several kinds of latex gloves have been invented. However, they could not satisfy human needs such as the flexibility and tear resistance. The present study was designed to target both types I and IV allergic reactions that are considered as the final biological pathways of all latex allergens. We confirmed that a natural compound with anti-type I and IV allergic effects prevents latex allergy after its incorporation into latex. Based on previous scientific reports and patents, we selected nine natural compounds for this present testing, including glycyrrhizin, osthole, tetrandrine, tea polyphenol, catechin, arctigenin, oleanolic acid, baicalin, and oxymatrine (Fig. 1). IL-4 and histamine released from RBL-2H3 cells and IL-2 and IFN-γ from primary spleen cells were taken as indices for the in vitro screening. HPLC method was used for the detection of the manufacturing stability under different conditions, such as gradient temperature and the incorporation with natural latex, silicone oil, and final latex product. Based on these results, arctigenin was selected to investigate anti-type I and type IV allergic reactions in vivo in which mice passive cutaneous anaphylaxis (PCA) model and mice allergen-specific skin response model were used. The dose of the final latex product containing arctigenin was selected at 6 g/100 g, based on the results from arctigenin’s in vitro anti-allergic evaluation and its dissolubility in the final latex product. The PCA model and mice allergen specific skin response model were used to detect the in vivo anti-allergic effect of the final latex product containing arctigenin, which was proven to be able to prevent latex allergy.

In the present study, appropriate cell models of type I and IV allergy reaction were established to screen the compounds with the best anti-allergic type I/IV effects. RBL-2H3 cells originated from rat basophilic leukemia are widely used for IgE-FceRI interactions and the screening for anti-allergic agents [23-24]. Hence, RBL-2H3 cells have been regarded as a useful cell-based model for the in vitro screening of these agents. Here, as demonstrated from MTT assay (Fig. 2), after treatment of RBL-2H3 cells and primary spleen lymphocytes with the nine natural compounds for 24 h, the inhibition rate was below 50%, indicating that the nine natural compounds had little cytotoxicity in vitro and that the doses of 10^{-9}, 10^{-5} and 10^{-4} mol·L^{-1} could be used for further in vitro study.

β-hexosaminidase is a common marker of mast cell degranulation [25–26], it is stored in mast cell granules and released with histamine induced by antigen. Histamine is one kind of pre-stored medium in mast cell granules that can induce type I allergic reaction by smooth muscle contraction, vasodilation, increasing capillary permeability and mucosal glands secretion [27]. Histamine is released after basophils stimulation by antigen and mast cell degranulation [28]. This substance is always regarded as a key index of type I allergic reaction. IL-4 does not only enhance TH2 cell growth but also induce the expression of IgE, which plays an important role in type I allergic reaction [29]. In the present study, β-hexosaminidase, histamine, and IL-4 were used as indices, while disodium cromoglycate (anti-allergic drug) was considered as a positive control, to screen compounds with best anti-type I allergic effects. We found that baiclin and arctigenin had the highest efficacy. As for the in vitro screening against type IV hypersensitivity, IL-2 and IFN-γ were used as key indices. IL-2 is secreted by T cell and is responsible for its activation, proliferation and secretion, while IFN-γ can activate and gather macrophages nearby to develop a granulomatous inflammation. In the present investigation, osthole and baicalin showed the best efficacy against anti-type IV allergy (Fig. 4).

In order to evaluate the physical stability of the nine natural compounds under the manufacturing conditions, HPLC method was used. The steps of NRL glove manufacturing include collection, centrifugation, compounding, coagulation oven curing, vulcanization and powder application [29-30]. Several conditions were selected to mimic the latex glove manufacturing processes and to evaluate the stability of the natural compounds; this set of conditions included variations in temperature and pH, co-existed with natural rubber, silicone oil and vulcanized latex, hand sweat simulation, and latex product conditions. All compounds were unstable at 120 °C or under the alkaline conditions, suggesting that these natural compounds should not be placed under these manufacturing conditions. In addition, arctigenin and catechin showed a better stability under most conditions of the process. Based on this result and observations from earlier experiments, we concluded that arctigenin had the best anti-allergic effect and highest stability, compared with the rest of the compounds. Arctigenin is one of the bioactive components extracted from Arctium lappa L, which has various pharmacological effects such as anti-tumor, anti-diabetic and anti-inflammatory activities [31-33]. Recent research has reported that arctigenin could decrease the production of IL-2 and IFN-γ in human T lymphocytes induced by anti-CD3/CD28 Ab, another cell-based model used for the study of immune responses [34]. Further, a research finding from a Korean group have suggested that arctigenin can inhibit TNF-α production via AP-1 activation [35]. These data all support the notion that arctigenin has anti-type I and type IV allergic effects by different mechanisms.

Though arctigenin has a good anti-allergic effect in vitro, the in vivo efficacy is still unknown. Acute toxicity evaluation of arctigenin showed no toxicity to animals (data not shown). The mouse passive cutaneous anaphylaxis (PCA) model [36] and allergen specific skin response model [37] were used to evaluate the anti-type I and anti-type IV allergic reactions, which are classical in vivo anti-allergic pharmacodynamic
models. Our results demonstrated that arctigenin can prevent both type I (Fig. 5A) and type IV (Fig. 5B) allergic reactions in a dose-dependent manner. However, there was no significant difference between the thymus and spleen indices in arctigenin group compared to that of the control, indicating the absence of any harmful effect of arctigenin on the animal immune system (data not shown).

For further study of the in vivo anti-allergic effect of the final latex product containing arctigenin, the dose of the latter in the final latex was selected. HPLC method was used to test the dissolubility of arctigenin in the final latex under both triple-distilled and hand sweat stimulation conditions (Fig. 6A); these results proved that arctigenin can dissolve from the final latex product containing this natural compound. Therefore, based on the dissolubility calculation and the results from arctigenin’s in vivo pharmacological experiment, the dose of 6 g of arctigenin per 100 g of final latex (6 g/100 g) was used. The results of PCA and allergen-specific skin response tests (Fig. 6B) indicated that the final latex product containing arctigenin had anti-type I and anti-type IV allergic effects.

In summary, we selected arctigenin from a set of nine natural compounds based on the in vitro pharmacological screening model and the evaluation under various latex manufacturing conditions. This compound was found to possess anti-type I and IV allergic effects, which were maintained after its incorporation into the final latex products.

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