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Inhibitory effects of Quillaja saponin on IgE-mediated degranulation of rat basophilic leukemia RBL-2H3 Cells

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ABSTRACT

The anti-allergic immunomodulatory effects of Quillaja saponin in antigen-stimulated rat basophilic leukemia cell line RBL-2H3 were investigated. Quillaja saponin exhibited a significant inhibitory activity using β -hexosaminidase and histamine release assays on anti-DNP-IgE-sensitized rat basophilic leukemia (RBL)-2H3 cells in a dose-dependent manner when tested in the range of 0.5–8.0 μ g/mL. In addition, Quillaja saponin also suppressed mRNA expression of interleukins (IL)-4 and IL-6. In order to explore the antigen-allergy mechanisms of Quillaja saponin in mast cells, intracellular Ca^{2+} level and IgE receptor FcεRI mRNA expression were also determined. It was found that the intracellular Ca^{2+} level was also decreased with Quillaja saponin tested at various concentrations. Furthermore, real time-PCR results showed that mRNA expression of FcεRI α chain was significantly suppressed by Quillaja saponin, and not of the β and γ chains.

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1. Introduction

Allergies are caused by an inappropriate reaction against proteins (Denery-Papini et al., 2007). The prevalence and severity of allergic diseases has increased dramatically during the past decade around the world, especially affecting an estimated 22% of the population in the developed countries (Li, Lee, Le, Kim, & Kim, 2008). Allergic reactions are mediated by allergen-specific IgE antibodies. The chronic nature of allergy and lack of preventive and curative therapies are leading researchers to seek complementary and alternative natural medicines. Evidence-based research into such treatment modalities and their related mechanisms of efficacy is still in its infancy. Mast cells play a central role in not only causing immediate allergic reactions, but also in developing late phase reactions, and have been recognized as one of the most important cell type in the pathophysiology of allergic diseases (Kimata et al., 2000). Cross-linking of cell surface high-affinity

IgE receptors (FcεRI) by initiating the binding of IgE with polyvalent antigens causes degranulation of the mast cells and the release of various mediators such as histamine, prostaglandins, β -hexosaminidase, protease and other chemotactic factors that trigger the allergy response (Corry & Kheradmand, 1999). The enzyme β -hexosaminidase can be used in basophil release assays as a biomarker of basophil activation for antigen-induced degranulation in RBL-2H3 cell line (Ho, Choi, Yoo, Kim, & Ryu, 1998). Mast cells also play an important role in initiating and perpetuating the inflammatory response in allergic reactions by secreting abundant amounts of proinflammatory mediators such as IL-4, IL-5, IL-6, tumour necrosis factor (TNF)- α , and leukotrienes (Bradding et al., 1994; Stelekati, Orinska, & Bulfone-Paus, 2007). In particular, IL-4 has a prominent role in the initiation of an allergic reaction, and promotes the switch from naive T cells to the allergic type Th2 cells. Therefore, identifying novel compounds which can immunomodulate mast cell function may not only be

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useful for the prevention of allergic diseases, but could also be applicable to numerous other conditions where immunity may be compromised.

In recent studies, it has been reported that various natural products suppress the degranulation in cellular systems and it was found that these dietary natural compounds have less cytotoxicity and side effects (Fujimura, Tachibana, & Yamada, 2001). However, the underlying mechanism of suppression and/or immune modulation is not fully understood. Saponins are naturally occurring amphiphilic triterpene glycosides found in various plants. They consist of a lipophilic aglycone backbone with hydrophilic sugar side chains (Van Setten & Vande Werken, 1996). Quillaja saponin is obtained from the bark of a South American tree (*Quillaja saponaria* molina) and has two normonoterpene ester moieties, linked linearly to their fucosyl residue, which possess powerful adjuvant activities (Soltysik et al., 1995; Toru et al., 1996). Saponins have been reported to have a wide range of functional properties, including hypocholesterolemic, immunostimulatory, antitumorigenic, and antimutagenic activities, depending on the structure (Berhow, Wagner, Vaughn, & Plewa, 2000) and has also been reported to have immunomodulating properties (Soltysik et al., 1995). It was recently demonstrated that saponin may act as a promising herbal medicine with potent anti-inflammatory actions (Byeon et al., 2009). Some investigations have also demonstrated that saponins may have antiallergic activity (Park, Park, Koh, & Lim, 2002). Our previous research showed that Quillaja saponin can suppress the allergen-specific IgE-mediated allergic reaction in a murine model of food allergy, which resulted in shifting the Th2- to a Th1-dominated immune response (Katayama & Mine, 2006). This may suggest possible use of Quillaja saponin in preventing IgE-mediated allergy.

However, the effect of anti-allergic activity of Quillaja saponin remains unclear and in this context, it may be postulated that Quillaja saponin may directly affect the IgE receptors on the mast cells. In the present study, the mechanisms underlying the anti-allergic activity of Quillaja saponin, including mast cell functions were explored using the rat basophilic leukemia cell line RBL-2H3 both at the cellular and at the molecular levels.

2. Materials and methods

2.1. Materials and chemicals

Quillaja saponin (>95% purity) was obtained from Superfos Biosector (Vedbaed, Denmark). The rat basophilic leukemia cell line (RBL-2H3) was obtained from the American Type Culture Collection (ATCC- CRL-2256). Eagle's Minimal Essential Medium (EMEM), sodium pyruvate, penicillin–streptomycin and HEPES were all obtained from Invitrogen (Grand Island, NY). Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT). Rat anti-DNP monoclonal antibody (anti-DNP-IgE), dinitrophenol-human serum albumin (DNP-HAS), Tyrode's solution, water-soluble tetrazolium-1 (WST-1), and 4-nitrophenyl N-acetyl- β -D-glucosaminide were purchased from Sigma–Aldrich (St. Louis, MO). Bovine serum albumin (BSA) was obtained from Fisher Scientific (Fair Lawn, NJ,

USA). All other chemicals and solvents used in this study were of analytical grade.

2.2. Cell culture

The RBL-2H3 cells were grown in EMEM medium supplemented with 1 mM sodium pyruvate, 50 U/mL penicillin–streptomycin and 100 g/L of heat-inactivated FBS, and cultured at 37 °C in a humidified atmosphere with 5% CO₂. The medium was replaced every 2–3 days. The cells were subcultured every 4–5 days until the cells reached 70–80% confluency.

2.3. Water-soluble tetrazolium-1 (WST-1) cell viability assay

The WST-1 cell viability assay is based on the cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases. Expansion of viable cell numbers results in an increase in the overall activity of the mitochondrial dehydrogenases in the sample, corresponding to an increase in formazan dye metabolism which can be photometrically detected (Ishiyama, Miyazono, Sasamoto, Ohkura, & Ueno, 1997). Briefly, RBL-2H3 cells were seeded into 48-well plates (Corning Incorporated, Corning, NY) at a density of 1×10^5 cells/well and allowed to adhere for 3 h at 37 °C in 5% CO₂. The culture medium was then aspirated and replaced with 250 μ L of fresh medium containing 0.5 μ g/mL of anti-DNP IgE and incubated overnight 37 °C in 5% CO₂. The cells were then rinsed twice with Tyrode's buffer containing 10 mM HEPES and 1 g/L BSA, and pre-incubated with various concentrations (0–8 μ g/mL) of Quillaja saponin in Tyrode's buffer for 1 h. The buffer was then removed and 290 μ L of media and 10 μ L of WST-1 solution were added to each well. The plate was incubated for 30 min at 37 °C for color development. Supernatants were transferred onto a 96-well microtiter plate for measurement at 450 nm with a plate reader (BioRad model 550, Bio-Rad Laboratories, Hercules, CA).

2.4. IgE-mediated degranulation assay

The RBL-2H3 cells were seeded into 48-well plates (Corning Incorporated) at a density of 1×10^5 cells/well and were allowed to adhere for 3 h at 37 °C with 5% CO₂. The culture medium was then aspirated and replaced with 250 μ L of fresh medium containing 0.5 μ g/mL of anti-DNP IgE and incubated overnight at 37 °C in 5% CO₂. The cells were then rinsed twice with Tyrode's buffer containing 10 mM HEPES and 1 g/L BSA, and incubated in Tyrode's buffer containing different concentrations of Quillaja saponin for 1 h. The buffer was removed and replaced with 250 μ L of DNP-HSA at 100 ng/mL in Tyrode's solution, and cells were incubated for 1 h at 37 °C in 5% CO₂. Degranulation of the cells was monitored by both β -hexosaminidase secretion assay and histamine ELISA.

2.5. Beta-hexosaminidase secretion assay

In order to evaluate the effects of potential inhibitory activity of Quillaja saponin on degranulation response in mast cells, the beta-hexosaminidase secretion was used as an indicator

to measure the chemical mediator release. Aliquots (50 μ L) of the culture supernatant or cell lysate, which was obtained by the addition of 250 μ L Tyrode's solution containing 1 g/L Triton X-100, were incubated with 50 μ L of 2 mM 4-nitrophenyl N-acetyl- β -D-glucosaminide in 0.2 M citrate buffer (pH 4.5) at 37 $^{\circ}$ C for 3 h. The reaction was stopped by adding 150 μ L/well of 1 M Tris-HCl (pH 9.0) and the absorbance was measured at a wavelength of 405 nm. A controls without Quillaja saponin was used to measure spontaneous release. The release of β -hexosaminidase was calculated using the following formula: % total enzyme activity = [(OD secreted)/(OD secreted + OD lysate)] $\times$ 100, in which secreted OD was equal to the activity of enzyme in the extracellular fluid; and in the lysate was equal to the enzyme activity in cells.

2.6. Histamine ELISA

Histamine concentrations in cell culture supernatants were measured using a histamine ELISA kit (Labor Diagnostika, Nord GmbH & Co. KG, Nordhorn, Germany) according to the manufacturer's instructions. The reaction product was measured at 450 nm and the amount of histamine was expressed as ng/mL from the standards generated as the protocol guidelines.

2.7. Cytokine mRNA expression analysis

In order to analyze the mRNA expression of cytokines released, the IgE-sensitized RBL-2H3 cells were pretreated with Quillaja saponin (8 μ g/mL) for 1 h and then treated with DNP-BSA for 1 h. Total RNA from the treated cells was prepared using Aurum total RNA mini kit (Bio-Rad) according to the manufacturer's protocol and was stored at -80° C until use. RNA concentration and integrity were determined using Nanodrop 8000 (Nanodrop products, Wilmington, DE). The cDNA was synthesized from 1.0 μ g of the total RNA using iScript cDNA synthesis kit (Bio-Rad) following the manufacturer's instruction. The real-time PCR reactions were performed using an iCycler iQ Real Time PCR System (Bio-Rad). The temperature profile was 95 $^{\circ}$ C for 15 min, then 15 s at 95 $^{\circ}$ C, 56 $^{\circ}$ C for 15 s and 72 $^{\circ}$ C for 30 s, and the cycles were repeated for 40–50 times. PCR product formation was continuously monitored during the PCR reaction using an iCycler iQ

software (ver. 1.7; Applied Biosystems, Foster City, CA). Accumulated PCR products were detected directly by monitoring the increase of the reporter dye (SYBR). The target gene expression was normalized with rat β -actin and relative gene expression was determined using $R = 2^{(Ct(\text{reference}) - Ct(\text{test}))}$ (Kleta et al., 2004). Threshold cycle (C_t) values were obtained at the cycle number at which the gene is amplified beyond the threshold of 30 fluorescence units. The efficiencies of all primers used in this study (listed in Table 1) were between 96% and 105%.

2.8. IgE receptor mRNA expression analysis

For IgE receptor mRNA expression, RBL-2H3 cells were pre-treated with indicated concentrations of Quillaja saponin for 3 h at 37 $^{\circ}$ C in 5% CO_2 followed with the RNA extraction and RT-PCR analysis, and the assay methods were the same as the above mentioned. The sequences of the primers used are listed in Table 1.

2.9. Measurement of intracellular calcium ion concentration

Intracellular Ca^{2+} concentration was measured using the fluorescence indicator Fluo-4/AM (Molecular ProbesTM, Invitrogen, Burlington, ON, USA) (Sakai, Sugawara, Matsubara, & Hirata, 2009). Briefly, RBL-2H3 cells were seeded into culture plate-96 well black opaque (PerkinElmer, Waltham, MA) at a density of 0.5×10^5 cells/well and allowed to adhere for 3 h at 37 $^{\circ}$ C in 5% CO_2 . The culture medium was then replaced with 125 μ L of fresh medium containing 0.5 μ g/mL of anti-DNP IgE and incubated overnight at 37 $^{\circ}$ C in 5% CO_2 . The cells were then rinsed twice with Tyrode's buffer containing 10 mM HEPES and 1 g/L BSA, and pre-incubated with indicated concentrations of Quillaja saponin in Tyrode's buffer for 1 h. The buffer was removed and loaded with Fluo-4 NW dye mix (Molecular ProbesTM, Invitrogen) at 37 $^{\circ}$ C for 45 min. After the removal of Fluo-4 NW dye mix, cells were challenged for 3 min with 125 μ L of 100 ng/mL DNP-HSA in Tyrode's buffer. The fluorescence intensity (FI) was immediately determined at an excitation wavelength of 485 nm and emission wavelength of 535 nm at 37 $^{\circ}$ C with Perkin Elmer Victor2 TM 1420 Multiple Counter (PerkinElmer).

Table 1 – Prime sequences used for cytokine and IgE receptor mRNA expression analysis.

Gene	Direction	Sequence (5' $\rightarrow$ 3')
IL-4	Sense	ACC TTG CTG TCA CCC TGT TC
	Antisense	TTG TGA GCG TGG ACT CAT TC
IL-6	Sense	TGG AGT TCC GTT TCT ACC TG
	Antisense	TTC ATA TTG CCA GTT CTT CG
β -Actin	Sense	TTG CTG ACA GGA TGC AGA AG
	Antisense	ACA TCT GCT GGA AGG TGG AC
Rat Fc ϵ RI- α	Sense	ATC GTC ACA TTG GGT CAT TG
	Antisense	CAC GTC AGC AGA AGA TTG GA
Rat Fc ϵ RI- β	Sense	GTT GTC TGC TCC ACA CTC CA
	Antisense	GGC TGC CTC TCA CCA GAT AC
Rat Fc ϵ RI- γ	Sense	CTC AGC TGC ATT CCT TTT CC
	Antisense	TTG GTG GAT ATG GGG ACA TT

2.10. Statistics analysis

All data were analyzed with Student's t-test and *post hoc* comparisons were made using the Duncan's test. Data are presented as means $\pm$ SEM ($n = 3$). Significance was determined using the $P < 0.05$ levels. Statistical calculation was performed using the GraphPad Prism® package (GraphPad Software Inc., San Diego, CA).

3. Results

3.1. Cytotoxicity effect of Quillaja saponin on RBL-2H3 cells

We used the WST-1 to assess the cytotoxicity of Quillaja saponin on RBL-2H3 cells which were treated for 1 h at final concentrations of 0.5, 1.0, 5.0, 8.0 and 10.0 $\mu\text{g/mL}$. The results showed that the cell viabilities were all above 90% within the treated concentration range except 10.0 $\mu\text{g/mL}$ after 1 h treated (Fig. 1; $P < 0.05$). Therefore, for all subsequent experiments we used Quillaja saponin ranging from 0.5 to 8.0 $\mu\text{g/mL}$ to DNP-IgE-sensitized cells.

3.2. Inhibition of β -hexosaminidase release from RBL-2H3

To examine potential anti-allergic activity of Quillaja saponin, an *in vitro* model of IgE-mediated allergy and mediator release was established using rat basophil cell lines. Cells, stimulated with antigen, released β -hexosaminidase which is a valid biomarker for mast cell degranulation.

Quillaja saponin suppressed the β -hexosaminidase release *in vitro* with RBL-2H3 in a dose-dependent manner (Fig. 2a), which was significant in the range of 1.0–8.0 $\mu\text{g/mL}$ ($P < 0.05$ as compared to that of the positive control).

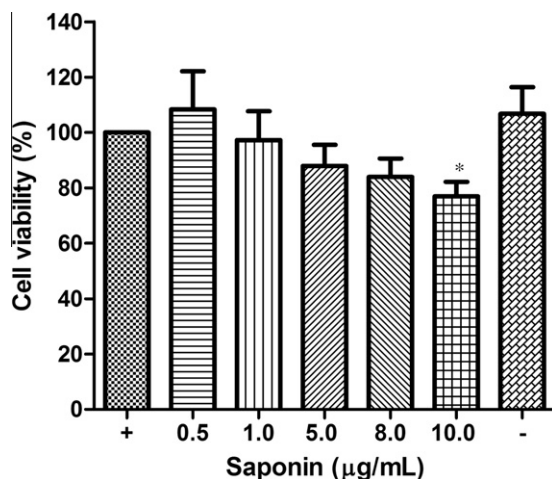


Fig. 1 – Water-soluble tetrazolium-1 (WST-1) cell viability assay for Quillaja saponin at various concentrations (0–10 $\mu\text{g/mL}$). Values represent mean $\pm$ SEM, $n = 3$. $P < 0.05$ indicates significant difference as compared to that of the positive control.

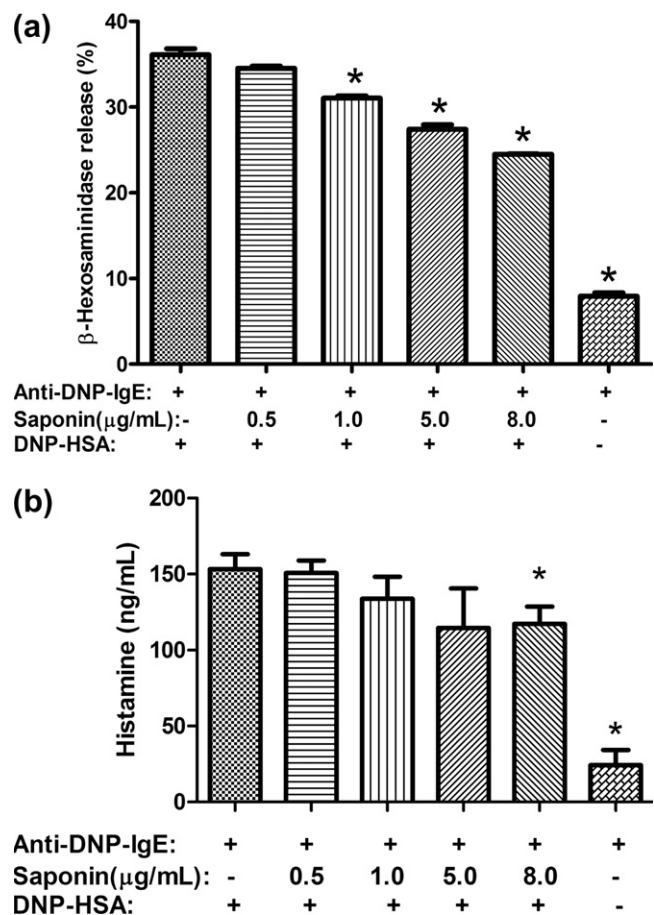


Fig. 2 – Degranulation of RBL-2H3 cells sensitized by anti-DNP IgE. RBL-2H3 cells were incubated with anti-DNP IgE prior to pre-incubation with Quillaja saponin at the indicated concentrations for 1 h at 37 °C respectively, followed by addition of DNP-HSA. (a) Degranulation was measured by β -hexosaminidase assay. (b) Degranulation was measured by histamine ELISA assay. Values represent mean $\pm$ SEM, $n = 3$. * $P < 0.05$ indicates significant difference as compared to that of the positive control.

3.3. Inhibition of histamine release from RBL-2H3

The release of various mediators, including histamine, stored in cytoplasmic granules is a characteristic and critical function of mast cells. We assessed the ability of histamine release from RBL-2H3 cells treated with or without Quillaja saponin upon stimulation with anti-DNP-IgE. In nontreated cells with the Quillaja saponin, the values of histamine release was 153.3 ng/mL. The inhibition of histamine secretion with Quillaja saponin from RBL-2H3 cells was performed in a dose-related manner (Fig. 2b). With the Quillaja saponin concentration increasing from 0.5 to 8.0 $\mu\text{g/mL}$, the histamine release trends to decrease from 154.7 to 107.5 ng/mL. The effect was significantly different at the concentration of 8.0 $\mu\text{g/mL}$ compared to the positive control (with no Quillaja saponin). Also, the results suggest that β -hexosaminidase release assay did positively correlate with the histamine assay in the sense that with increasing concentrations of saponin

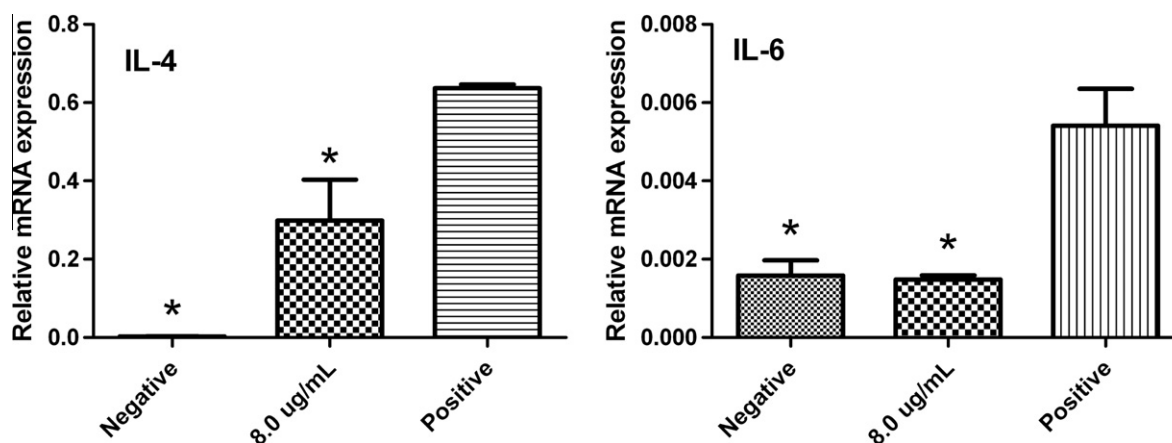


Fig. 3 – Measurement of mRNA expression of cytokines in RBL-2H3 cells. The total RNA was extracted from the cells post incubation with Quillaja saponin (8.0 $\mu\text{g/mL}$) for 1 h at 37 °C. Values represent mean $\pm$ SEM, $n = 3$. *Different from the negative control cells at $P < 0.05$.

the enzyme activity decreased. Further investigations need to be conducted to elucidate underlying molecular mechanism.

3.4. Effect of Quillaja saponin on cytokines mRNA expression

The cytokines IL-4 and IL-6 are involved in allergic reactions (Bruhns, Fremontm, & Daeron, 2005). The IL-4 has a critical role in the allergic reaction and promotes the switching of naïve T cells to allergic-type Th2 cells (Anderson & Coyle, 1994; Hines, 2002) and the IL-6 is a major inflammatory cytokine released from mast cells in the late phase of the allergic response. The RT-PCR results in this study showed that Quillaja saponin at 8.0 $\mu\text{g/mL}$ concentration reduced antigen-induced mRNA expression of IL-4 and IL-6 (Fig. 3), thereby markedly inhibiting the gene expression related to allergic reactions.

3.5. Effect of Quillaja saponin on the intracellular Ca^{2+} level

Allergen stimulation induces Ca^{2+} influx and elicits the secretion of inflammatory mediators from mast cells. Calcium signals have been implicated to play a role in the induction of Fc ϵ RI-mediated mast cell activation and allergic reaction (Baba et al., 2008). There seems to be a possibility that saponin regulate these signals, thereby modulating mediator release. To test this possibility, we determined whether saponin affected calcium signals. Sustained calcium effects were observed after Fc ϵ RI cross-linking by decrease in intracellular Ca^{2+} level by the antigen, thereby causing histamine and β -hexosaminidase to be released. The effects of Quillaja saponin on calcium level in mast cells were examined using Fluo4-AM, a calcium-specific fluorescent probe. As shown in Fig. 4, the RBL-2H3 cells after DNP-HSA stimulation showed a decreased in intracellular Ca^{2+} level (expressed with fluorescence intensity) at each Quillaja saponin treated concentration relative to positive control cells (with no Quillaja saponin), and the suppression effects were significant ($P < 0.05$) at 0.5–

8.0 $\mu\text{g/mL}$. This suggests that Quillaja saponin can inhibit Ca^{2+} mobilization.

3.6. Effect of Quillaja saponin on Fc ϵ RI α , β and γ mRNA expression

To examine the effects of Quillaja saponin on Fc ϵ RI mRNA expression, the mRNA expression levels of Fc ϵ RI α , β and γ chains were evaluated. The results showed that mRNA expression of Fc ϵ RI α chain was significantly suppressed by Quillaja saponin at 1.0, 5.0, and 8.0 $\mu\text{g/mL}$ ($P < 0.05$) when compared to negative cells (with no Quillaja saponin) (Fig. 5). However, the amounts of β and γ chain mRNA expression detected in Quillaja saponin treated mast cells stayed at almost the same level as that of the untreated cells. The ratio of signal intensity of β chain to β actin was at 0.36 in Quillaja saponin untreated cells and were at 0.39, 0.36, 0.43 and 0.3 in Quillaja saponin treated cells at concentration ranging from

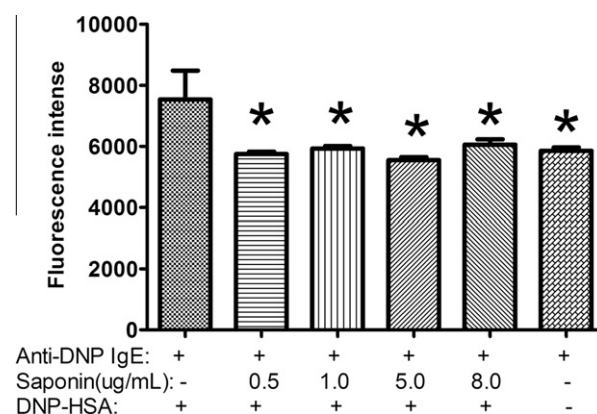


Fig. 4 – Effect of Quillaja saponin on the intracellular Ca^{2+} level. IgE-sensitized RBL-2H3 cells were loaded with Ca^{2+} indicator Fluo-4/AM post treatment with indicated concentrations of Quillaja saponin and the cells were stimulated with DNP-HSA for 3 min, and fluorescence was measured. Values represent mean $\pm$ SEM, $n = 3$. * $P < 0.05$.

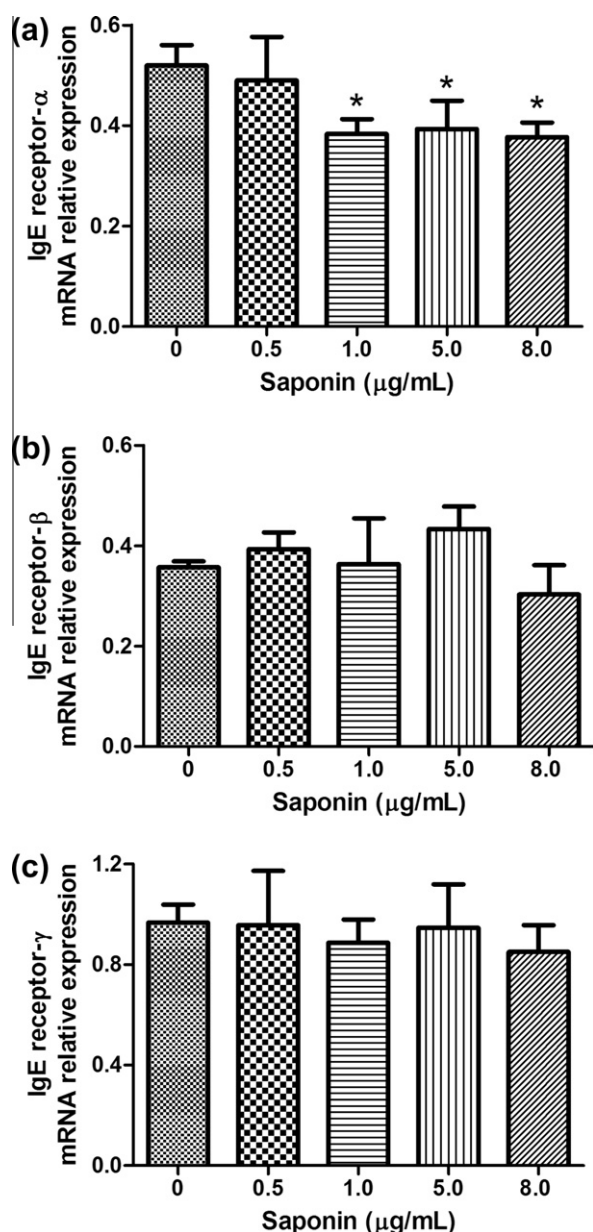


Fig. 5 – Effect of Quillaja saponin on the IgE receptor mRNA expression of RBL-2H3 cells. (A) The mRNA expression of IgE receptor α chain. (B) The mRNA expression of IgE receptor β chain. (C) The mRNA expression of IgE receptor γ chain. Total RNA was extracted after incubated with Quillaja saponin at the indicated concentrations (0–8 $\mu\text{g/mL}$) for 3 h at 37 °C. Values represent mean $\pm$ SEM, $n = 3$. * $P < 0.05$.

0.5 to 8.0 $\mu\text{g/mL}$, respectively. The ratio of signal intensity of γ chain to β actin was at 0.97 in Quillaja saponin untreated cells and it was at 0.96, 0.89, 0.95 and 0.85 in Quillaja saponin treated cells at concentrations ranging from 0.5 to 8.0 $\mu\text{g/mL}$, respectively.

4. Discussion

Different compounds may be involved in different mechanisms with regards to suppression of the allergic response.

For example reported a lipopeptide P3CSK4 predominantly enhanced type 1 response, whereas the saponin enhanced both the type 1 and type 2 response (Huber, Baier, Bessler, & Heinevetter, 2002). Some compounds inhibited the degranulation of mast cells in the early phase of the allergic response, however some suppressed cytokine secretion on the late phase (Chen, Shih, Hsu, & Chiang, 2010). Here, our results showed that Quillaja saponin not only has the ability to inhibit degranulation but also was able to decrease the secretion of cytokines IL-4 and IL-6.

To better understand the anti-allergic mechanism of Quillaja saponin, further studies on molecular mechanism were necessary. It is important to understand the mechanisms by which Fc ϵ RI cross-linking is suppressed by saponin. The IL-4 cytokine acts as an eosinophil chemoattractant that induces endothelial cells to produce eosinophil chemotactic factor and eotaxin (Rothenberg, Luster, & Leder, 1995) and that enhances endothelial cell surface adhesion molecules such as vascular cell adhesion molecule-1 (Schleimer et al., 1992) and promotes the switch from naïve T cells to allergic type Th2 cells (Hines, 2002). The IL-6 is critical for allergic inflammation. The results from this study showed that Quillaja saponin can suppress both the mRNA expression of IL-4 and IL-6, which indicated that Quillaja saponin suppressed the Th2 as well as inflammatory cytokines. In the present study we have described inhibitory effects of Quillaja saponin on the activation of IgE-sensitized mast cells. The hypostasis of allergic reaction initiating is mediated by cross-linking of cell surface high-affinity IgE receptors (Fc ϵ RI) with polyvalent antigens. The Fc ϵ RI receptor is a key molecule in triggering IgE-mediated allergic reaction, and it has been suggested that the down-regulation of its expression in mast cells and basophils may lead to the attenuation of IgE-mediated allergic symptoms (Fujimura et al., 2001), therefore Fc ϵ RI and IgE sensitization may be important as therapeutic targets in the treatment of allergy. Thus, activation of mast cells through Fc ϵ RI is necessary to initiate allergic reactions.

The expression of Fc ϵ RI has been shown to be up-regulated by IL-4 (Hara, Yamada, & Tachibana, 1998), which is known to transfer a signal to the signal transducers and activators of transcription (STAT) molecule STAT6 motif. Fc ϵ RI is a tetrameric structure consisting of one α chain, one β chain and two disulfide-linked γ chains (Blank et al., 1989; Fujimura et al., 2001; Ra, Jouvin, & Kinet, 1989). The expression levels of Fc ϵ RI are functionally important since mast cells with a high level of Fc ϵ RI on their surface demonstrate enhanced sensitivity and a stronger allergic response to antigenic stimulation. We have demonstrated in this study that Quillaja saponin suppressed the amounts of Fc ϵ RI α chain mRNA, but not β and γ chains. Previous rat and mouse studies have demonstrated that high affinity cell surface IgE binding is dependent on the co-expression of the three subunits (Blank et al., 1989; Hakimi et al., 1990; Ra et al., 1989). However, many researches confirmed that α chain plays a crucial role in the binding of Fc ϵ RI with IgE (Blank, Ra, & Kinet, 1991; Hakimi et al., 1990). The importance of Fc ϵ RI α chain for allergic reactions has been also demonstrated by the observation that Fc ϵ RI α chain deficient mice completely failed to exhibit IgE-mediated anaphylaxis (Dombrowicz, Flamand, Brigman, Koller, & Kinet, 1993). Therefore, down regulation of Fc ϵ RI α chain

appears to be an effective strategy to prevent allergic diseases. Also, the granule-associated enzyme β -hexosaminidase is released in parallel with other chemical mediators including histamine and serotonin upon antigen crosslinking of Fc ϵ RI. Therefore, the release of β -hexosaminidase and histamine has both been commonly regarded as useful indicators to evaluate the activation of mast cells in various allergic inflammatory responses.

Cross linking of Fc ϵ RI on the cell membrane induces elevation of intracellular Ca^{2+} concentration, which is closely related to the degranulation of mast cells. An increase in cytosolic calcium level Ca^{2+} is considered to be an essential and ubiquitous mechanism in the process of mast cell degranulation (Oka, Sato, Hori, Ozaki, & Karaki, 2002; Vo, Kong, & Kim, 2011). However, the intracellular Ca^{2+} level decreased from RBL-2H3 treated with Quillaja saponin at certain concentrations after antigen stimulation.

Our results indicate that Quillaja saponin inhibits degranulation and reduces expression of high-affinity IgE receptor in antigen-activated RBL-2H3 mast cells. We conclude from these results that the down regulation of intracellular Ca^{2+} influx is related to the inhibitory effect of Quillaja saponin demonstrated via the β -hexosaminidase and histamine release assays. The decrease of IL-4, IL-6, and Fc ϵ RI α chain mRNA expression by Quillaja saponin also concludes the possibility that it could be a valid candidate acting as a functional food ingredient or a nutraceutical for the therapeutic treatments of various allergic diseases.

5. Conclusion

We conclude that the anti-allergic effects of Quillaja saponin could be partly mediated through the reduction of Ca^{2+} influx and decrease in cytokine expression in mast cells. Our current findings offer insights into the immunomodulatory functions of Quillaja saponin and possible potential regimens for treating mast cell-mediated type I-hypersensitive allergic reactions. The detailed cell signaling pathways involved behind the antiallergic effects of Quillaja saponin will be the subject for further investigations.

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