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Anti-allergic activity of grapeseed extract (GSE) on RBL-2H3 mast cells

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ABSTRACT

Grapeseed extract (GSE) is a rich source of natural phenolic compounds and possesses various pharmacological activities, including antioxidant, anticarcinogenic and anti-inflammatory properties. However, effects of GSE on immunoglobulin (Ig) E-mediated allergic responses still remain elusive. In the present study, the effects of GSE on activation and degranulation of RBL-2H3 mast cells were investigated. GSE pretreatments (20–100 µg/ml) reduced IgE-antigen mediated release of β-hexosaminidase and histamine in RBL-2H3 cells. Additionally, GSE reversibly inhibited expression of FcεRI on RBL-2H3 cells. GSE treatments caused a significant elevation of intracellular cAMP levels, whereas the Ca²⁺ influx upon antigen stimulation was inhibited. Suppression on FcεRI expression together with decreased calcium uptake and increased cAMP level might be involved in attenuated degranulation of mast cells by GSE treatment. Our results suggest a possible pharmaceutical application of GSE in treating type I allergic diseases.

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1. Introduction

The grapevine (*Vitis vinifera*), indigenous to Western Asia and Southern Europe, is an important source for food and beverage. The leaves and seeds of the grapevine (*V. vinifera*) are commonly utilised in herbal medicine, whereas the grape fruits are used as a dietary supplement (Prieur, Rigaud, Cheynier, & Moutounet, 1994). Phenolics are important secondary metabolites in plants and are widely distributed in various vegetables and fruits. In addition to carbohydrates and fruit acids, phenols represent the third most abundant constituent in grapes (Shi, Yu, Pohorly, & Kakuda, 2003). Many commonly known natural phenolic compounds include flavonoids, lignans, stilbenes and phenolic acids. The mono- and polyphenolic substances in grapes can be categorised into phenolic acids and related compounds (e.g. caffeic acid, ferulic acid, vanillic acid and gallic acid), and flavonoids (e.g. colourless flavan-3-ols, coloured flavanones and anthocyanins), respectively (Shi et al., 2003). Grapeseeds are the major industrial by-products generated during

the production of beverage such as grape juice and wine. Grapeseed extract (GSE) is rich in various phenolics (ranging from 5% to 8% by weight) such as phenolic acids, stilbenes, ellagitannins, flavonols, flavan-3-ols, anthocyanins and proanthocyanidins (Forester & Waterhouse, 2009; Shi et al., 2003). It has been shown that catechins (catechin, epicatechin and procyanidins) and their polymers represent the most abundant phenolics isolated from grapeseeds (Prieur et al., 1994). Routine consumption of foods containing various phenolics has been supported to have many pharmacological and health-promoting benefits against chronic neurodegenerative, cardiovascular and cerebrovascular diseases (Chandrasekara & Shahidi, 2011; de la Rosa, Alvarez-Parrilla, & Shahidi, 2011; Forester & Waterhouse, 2009). In addition, GSE has been implicated in various biological activities, including antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activities (de la Rosa et al., 2011; Forester & Waterhouse, 2009; Nassiri-Asl & Hosseinzadeh, 2009).

Mast cells are granule-containing secretory cells in the immune system. They are widely distributed in the connective tissues throughout the body and mostly reside under the skin, respiratory and gastrointestinal tracts for the direct interaction with environmental stimuli (Theoharides et al., 2010). Mast cells are the important effector cells playing a pivotal role in both immediate and late-phase reactions of type I hypersensitivity. Activation of mast cells can be initiated by allergen-induced crosslinking of IgE bound by the high-affinity IgE receptors (FcεRI) on the cells (Abramson, Licht, & Pecht, 2006; Abramson & Pecht, 2007). Cross-linked FcεRI

Abbreviations: BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; DNP, dinitrophenyl; FcεRI, high-affinity IgE receptor; FITC, fluorescein isothiocyanate; IgE, immunoglobulin E; IL, interleukin; mIgE-DNP, murine anti-dinitrophenol IgE.

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then initiates a complex network of signalling events that lead to phosphorylation of tyrosine kinases and mobilisation of Ca^{2+} into cytosol (Pecht & Corcia, 1987). Within seconds to minutes of activation, mast cells degranulate to release various preformed mediators, including β -hexosaminidase, histamine and serotonin, that ultimately contribute to acute inflammation and chronic allergic disorders such as seasonal rhinitis, food allergy and asthma (Abramson et al., 2006). RBL-2H3 is a rat basophilic leukaemia cell line, a cultured tumour analogue of mast cells, sharing various similar characteristics with mucosal mast cells (Seldin et al., 1985). It has been considered an excellent model system for the study of mast cell secretion (Aketani, Teshima, Umezawa, & Sawada, 2001) and screening for agents with anti-allergic potential (Aketani et al., 2001; Huang et al., 2008).

Clinically, steroids, antihistamines and immunosuppressants are commonly used for treating allergic symptoms. However, major concerns are the undesirable side-effects caused by repeated application of such drugs (Passalacqua, Compalati, & Canonica, 2010). Therefore, there has been increasing interests in the search of natural products possessing anti-allergic potential. In the current study, the effects and mechanisms of GSE on IgE-mediated allergic reaction were investigated.

2. Materials and methods

2.1. Chemicals and reagents

The following chemicals and reagents were obtained or purchased from the indicated sources: Mouse anti-DNP IgE (mIgE-DNP) antibody (Dr. Daniel H. Conrad, Virginia Commonwealth University, Richmond, VA, USA); Foetal bovine serum (FBS) (Hyclone, Logan, UT, USA); dimethyl sulphoxide (DMSO), Dulbecco's modified Eagle's minimal essential medium (DMEM) powder, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), *o*-phthalaldehyde (OPT); calcium inophore A23187 and *p*-nitrophenyl-*N*-acetyl-D-glucosaminide (*p*-NAG) (Sigma-Aldrich, St. Louis, MO, USA); dinitrophenyl-conjugated bovine serum albumin (DNP-BSA) (Pierce, Rockford, IL, USA); FITC-conjugated rat anti-mouse IgE heavy chain IgG1 (AbD Serotec, Oxford, UK); Fura-2/AM (Invitrogen, Carlsbad, CA, USA); cyclic adenosine monophosphate (cAMP) ELISA kit (Cell Biolabs, Inc., San Diego, CA, USA); RBL-2H3 cell line (Bioresource Collection and Research Center, Hsin-Chu, Taiwan). The IH636 grapeseed extract (GSE) was purchased from InterHealth Nutraceuticals (Benicia, CA, USA), which contains approximately 75–80% oligomeric proanthocyanidins and 3–5% monomeric proanthocyanidins (Shi et al., 2003; Wren, Cleary, Frantz, Melton, & Norris, 2002). All other common chemicals and reagents were obtained in the purest possible forms for this study.

2.2. Cell culture

RBL-2H3 cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin G and 100 $\mu\text{g}/\text{ml}$ streptomycin. Cells were maintained in a humidified 5% $\text{CO}_2/95\%$ air atmosphere at 37 °C.

2.3. Cell viability assay

Cell viability was determined by the previously published methylthiazolotetrazolium (MTT) assay (Chen et al., 2009) with minor modifications. The method is based upon the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form impermeable crystals of a dark blue formazan, thus resulting in accumulation within healthy cells. Briefly, RBL-2H3 cells were first seeded into 96-well plates (1×10^5 cells/well) until reaching 70–80%

confluence. Cells were then either treated with solvent as controls or treated with various concentrations of GSE (ranging from 1 to 400 $\mu\text{g}/\text{ml}$) for 24 h. The final DMSO concentrations for control and treatments were 0.05%. Four hours before the end of incubation, MTT (prepared as stock solution at 10 mg/ml in PBS) was added at a final concentration of 0.1 mg/ml. At the end of the incubation, the medium was aspirated and replaced with 200 μl DMSO to dissolve the formazan crystals. The plates were gently shaken for 20 min in the dark to ensure maximal dissolution of formazan crystals. Aliquots of supernatant were transferred into a new 96-well plate and read at 595 nm on a multiwell scanning spectrophotometer. The optical density of the formazan produced by untreated cells was considered as representing 100% viability. The degree of cell viability by the test compounds was calculated as the percentage absorption of the control value. All experiments were repeated at least three times.

2.4. Degranulation assay

The release of β -hexosaminidase from activated RBL-2H3 cells was measured as previously reported (Chen et al., 2003). Briefly, RBL-2H3 cells (4×10^4 cells/well) were sensitised with mIgE-DNP (1 $\mu\text{g}/\text{ml}$) for 6 h. Cells were then treated with various GSE concentrations for 4 h, followed by thorough washing with pre-warmed (37 °C) Tyrode's buffer (135 mM NaCl, 5 mM KCl, 1 mM MgCl_2 , 1.8 mM CaCl_2 , 5.6 mM glucose, 20 mM HEPES and 0.1% (w/v) BSA, pH 7.4) before stimulation with the cross-linking antigen, DNP-BSA, at 100 ng/ml for 1 h. The reaction was stopped by cooling the plate on ice for 15 min. The supernatant from unstimulated cells was collected as the spontaneous release controls. To quantify the β -hexosaminidase contents, equal volumes (50 μl each) of supernatant and *p*-NAG (1 μM) prepared in 0.1 M citrate buffer (pH 4.5) were incubated at 37 °C for 1 h. The reaction was stopped by adding 100 μl stop buffer (0.1 M $\text{Na}_2/\text{NaHCO}_3$, pH 10.0), followed by measurement of the absorption at 405 nm on a microplate reader.

2.5. Histamine release assay

RBL-2H3 cells were seeded to 96-well plates (1×10^5 cells/well) and sensitised overnight with 1 $\mu\text{g}/\text{ml}$ mIgE-DNP. The cells were washed with 200 μl PBS followed by incubation with 200 μl pre-warmed Tyrode's buffer supplemented with indicated concentrations of GSE for 4 h. Then, cells were stimulated with 100 ng/ml DNP-BSA for 1 h before the reaction was terminated by cooling the plates in ice-bath and the supernatant was collected. The amount of released histamine was calculated by detecting the fluorophore formation between histamine and OPT in a fluorometric assay where the absorbance at 347 nm (λ_{ex}) and 442 nm (λ_{em}) was measured, respectively.

2.6. Flow cytometry

Surface expression of Fc ϵ RI was detected by flow cytometry. Briefly, 1×10^6 RBL-2H3 cells were incubated with GSE for indicated length of time, followed by extensive washes and resuspended in ice-cold 1X PBS containing 0.1% bovine serum albumin (PBS-BSA). Cells were then incubated with mIgE-DNP (1 $\mu\text{g}/\text{ml}$) for 60 min at 4 °C, washed twice with PBS-BSA and incubated with FITC-conjugated F(ab')₂ goat anti-mouse IgG for 1 h at 4 °C. As background control, untreated cells were stained with FITC-conjugated F(ab')₂ goat anti-mouse IgG and prepared as other experimental groups. After washes to remove unbound Ab, cells were resuspended in 1 ml PBS-BSA and subjected to analysis of Fc ϵ RI expression using an EPICS-XL flow cytometer (Beckman-Coulter). The data were analysed and plotted using the free software

WinMDI (v2.8). The level of FcεRI expression is expressed as the mean fluorescence intensity (MFI).

2.7. Measurement of calcium (Ca^{2+}) mobilisation

RBL-2H3 mast cells (5×10^5 cells/well) were pretreated or not with indicated GSE concentrations for 18 h and subsequently primed with mIgE-DNP (1 µg/ml) for 6 h. After treatments, cells were extensively washed with PBS and incubated with 1 ml of Tyrode's buffer containing Fura-2/AM (2 µM) at 37 °C for 1 h with periodic swirling. Then, cells were washed twice with PBS and incubated in Tyrode's buffer (900 µl) containing 1 mM CaCl_2 for 30 min. Cell suspension was equally divided and loaded onto 96-well black microplate equilibrated in a thermostated holder to 21 °C. After the addition of DNP-BSA (100 ng/ml), fluorescence was immediately monitored with excitation and emission wavelengths at 340 and 490 nm, respectively, at indicated time intervals.

2.8. Cyclic adenosine monophosphate (cAMP) assay

To measure the intracellular cAMP levels, 1×10^6 RBL-2H3 cells were separately resuspended in pre-warmed (37 °C) Tyrode's buffer containing varying concentrations of GSE. The reaction was allowed to proceed for discrete time intervals, terminated by adding ice-cold acidified ethanol (0.9 ml of 95% ethanol/1 M HCl, 99:1, v/v) with brief vortexing followed by snap frozen in liquid nitrogen. The supernatant was harvested by centrifugation and evaporated to dryness by vacuuming in a reduced pressure chamber. The samples were reconstituted in assay buffer and cAMP levels determined by the commercial cAMP Biotrak Enzyme Immunoassay (EIA) kit (GE Healthcare, Piscataway, NJ) according to manufacturer's instruction.

2.9. Statistical analysis

All data values are expressed as mean $\pm$ standard deviation (SD) of at least three independent experiments. Where appropriate, data were analysed by one-way analysis of variance (ANOVA) followed by the Dunnett's multiple comparison test between treatments and control. Values with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Cytotoxic effect of GSE on RBL-2H3 cells

The MTT assay was used to access the cytotoxicity of GSE on RBL-2H3 mast cells. GSE in the range of 1–100 µg/ml did not cause significant cytotoxicity after 24-h treatment as compared with the control (Fig. 1), whereas GSE used at higher concentration (400 µg/ml) caused a significant cytotoxicity.

3.2. Effect of GSE on antigen-induced degranulation of IgE-sensitised mast cells

The release of mediators by degranulation is one of the key phenomena observed during allergic inflammation (Theoharides et al., 2010). We investigated the effects of GSE on antigen-induced degranulation in IgE-sensitised RBL-2H3 cells. GSE reduced IgE-mediated β -hexosaminidase release in a dose-dependently manner (85.5% and 62.1% inhibition at 10 and 100 µg/ml, respectively) (Fig. 2A). Similarly, but less significantly, GSE dose-dependently inhibited IgE-mediated histamine release (90.1% and 73.2% inhibition at 10 and 100 µg/ml, respectively) (Fig. 3A). Kinetically, a significant inhibition on release of β -hexosaminidase and histamine was observed with pretreatment of GSE (100 µg/ml) for the length

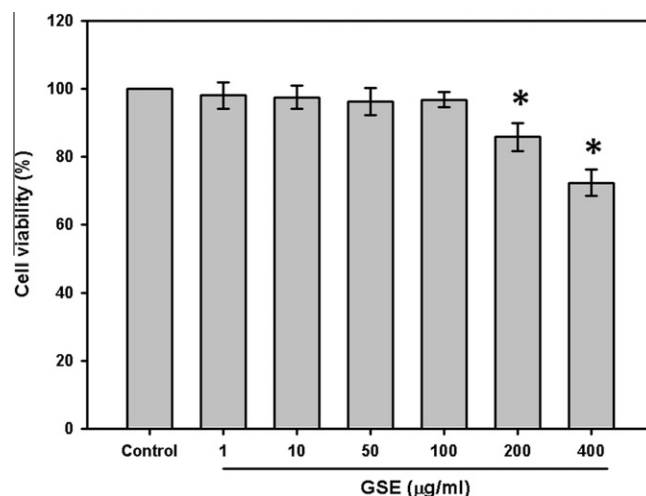


Fig. 1. Cytotoxic effect of GSE on RBL-2H3 cells. RBL-2H3 cells were treated with indicated concentrations of GSE for 24 h. Cytotoxicity was detected by the MTT assay as described in the Section 2. The percent cell viability was calculated relative to the untreated control as representing 100% viability. Each value represents the mean $\pm$ SD of three independent experiments. * $p < 0.05$ (vs. control by Dunnett's multiple comparison test).

of 12 and 24 h, respectively (Figs. 2B and 3B). This indicates that GSE is capable of inhibiting the degranulation of mast cells.

3.3. Effect of GSE on the expression of high affinity IgE receptor, FcεRI

The high affinity receptor for IgE, FcεRI, is crucial in the initiation of signalling events to activate mast cells. We investigated the effects of GSE on the surface expression of FcεRI in RBL-2H3 mast cells. Cells were cultured for 24 h with indicated concentrations of GSE, after which cell surface FcεRI was analysed by flow cytometry. Treatments with GSE caused suppression on FcεRI levels in a dose-dependent manner in RBL-2H3 mast cells (Fig. 4A). The relative cell surface FcεRI expression levels were decreased to 53.8%, 49.1% and 34.8% for GSE used at 1, 10 and 100 µg/ml, respectively (Fig. 4B). However, the inhibitory effect of GSE on FcεRI expression is reversible, as FcεRI expression on RBL-2H3 cells gradually returned to the normal levels upon the removal of GSE from culture medium (Fig. 4C).

3.4. Effect of GSE on cAMP levels

To investigate the mechanisms of GSE on the reduction of pro-inflammatory mediators, we assayed the influence of GSE on cAMP levels in RBL-2H3 cells. It has been known that agents elevating cAMP levels in basophils and mast cells can inhibit IgE-mediated histamine release and IL-4 production (Weston & Peachell, 1998). When RBL-2H3 cells were treated with varying GSE concentrations (10 and 100 µg/ml, respectively), the cAMP levels significantly increased at 20–40 s in a dose-dependent manner and then gradually started to decrease after 40 s until reaching to basal levels at 180 s (Fig. 5).

3.5. Effect of GSE on intracellular calcium level

Movement of calcium across membranes of mast cells and basophils is a crucial factor for histamine release (Choi et al., 1993). To further characterise the underlying mechanism involved in GSE-mediated inhibition of histamine release, we measured and compared the changes of calcium influx between untreated and GSE-treated IgE-sensitised RBL-2H3 cells. In untreated control

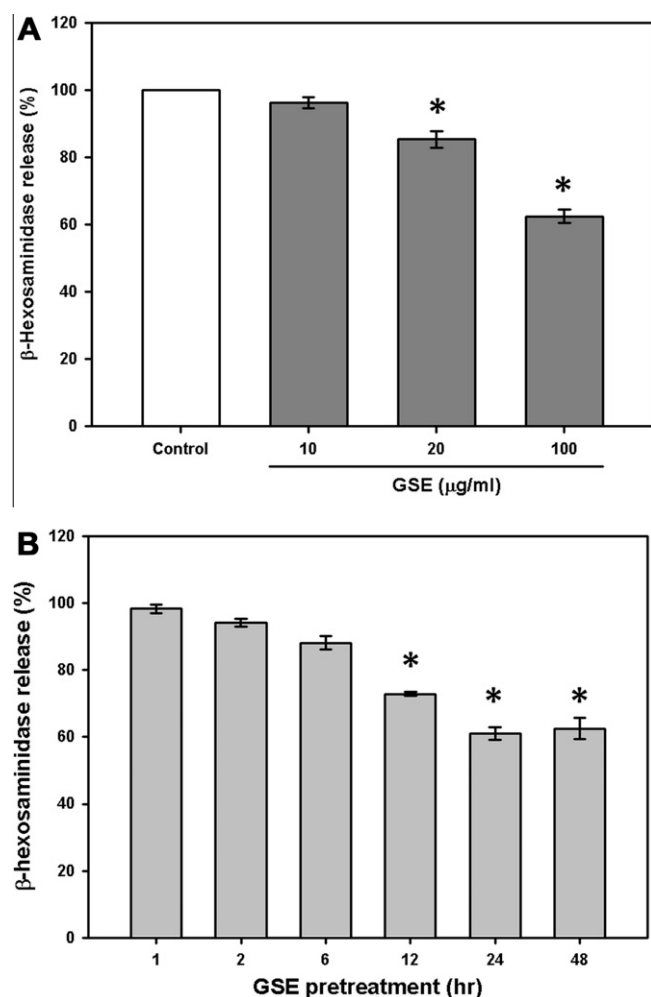


Fig. 2. The inhibitory effect of GSE on degranulation of β -hexosaminidase from RBL-2H3 cells. (A) RBL-2H3 cells were either left untreated or incubated with various doses of GSE (10, 20 and 100 μ g/ml) for 24 h followed by sensitisation with mlgE-DNP (1 μ g/ml) for 6 h. After addition of DNP-BSA (100 ng/ml) for 1 h, supernatant was harvested and assayed for β -hexosaminidase levels as described in the Section 2. (B) RBL-2H3 cells were treated with GSE (100 μ g/ml) for indicated time intervals, followed by sensitisation and stimulation with mlgE-DNP and DNP-BSA, respectively. The β -hexosaminidase levels were measured as described earlier. Results are expressed as mean $\pm$ SD ($n = 3$). * $p < 0.05$ vs. control.

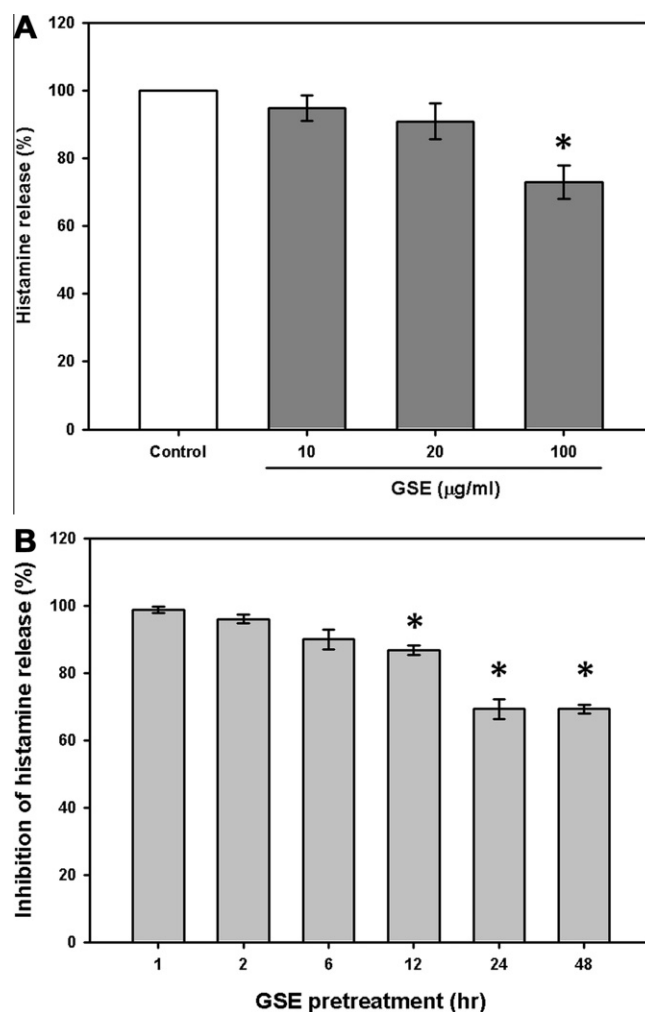


Fig. 3. The inhibitory effect of histamine release from RBL-2H3 cells. (A) RBL-2H3 cells were either left untreated or incubated with various doses of GSE (10, 20 and 100 μ g/ml) for 24 h followed by sensitisation with mlgE-DNP (1 μ g/ml) for 6 h. After addition of DNP-BSA (100 ng/ml) for 1 h, supernatant was harvested and assayed for histamine levels as described in the Section 2. (B) RBL-2H3 cells were treated with GSE (100 μ g/ml) for indicated time intervals, followed by sensitisation and stimulation with mlgE-DNP and DNP-BSA, respectively. The histamine levels were measured as described earlier. Results are expressed as mean $\pm$ SD ($n = 3$). * $p < 0.05$ vs. control.

cells, the elevation of intracellular calcium influx was observed and plateaued in 30–60 s after the addition of DNP-BSA (Fig. 6, open triangles). Preincubation of GSE (at either 10 or 100 μ g/ml) with RBL-2H3 cells significantly decreased antigen-induced intracellular calcium levels in a dose-dependent manner (Fig. 6, closed triangles and circles).

4. Discussion

Polyphenolic compounds from various agricultural food sources (e.g. pecans, millets, green tea and GSE) have been implicated in their antioxidation, anti-inflammatory and anti-cancer activities (Chandrasekara & Shahidi, 2011; de la Rosa et al., 2011; Forester & Waterhouse, 2009). For example, flavonoids and proanthocyanidins harvested from *Carya illinoensis* have been shown to exhibit free radical-scavenging activities (de la Rosa et al., 2011). Phenolic extracts of commonly consumed millets have been shown with inhibitory activities on the generation of free radicals and reactive oxygen species (Chandrasekara & Shahidi, 2011). In addition, proanthocyanidin gallate has been shown to inhibit proliferation and

induce apoptosis in human breast adenocarcinoma MCF-7 cells (Kuo, Hsu, Lin, Lin, & Lin, 2004).

Among four allergic disease types, the type I (or immediate type) hypersensitivity is mediated by allergen cross-linking of Fc ϵ R1-bound IgE on surface of mast cells. Activated mast cells degranulate to release various mediators, including histamine, β -hexosaminidase and other proinflammatory cytokines such as IL-4 and TNF α (Abramson et al., 2006). Therefore, reduction or inhibition of mast cell-mediated inflammatory responses is crucial to avoid immediate hypersensitivity responses in atopic individuals. Although applications of GSE, such as anti-tumour, anti-inflammatory and antimicrobial activities have been reported (Bralley, Hargrove, Greenspan, & Hartle, 2007; Kao et al., 2010; Li, Zhang, Wu, & Tian, 2001; Veluri et al., 2006), the anti-allergic effects of GSE still remain elusive. In the current study, we investigated the inhibitory effects of GSE on the activation of IgE-sensitised mast cells. The granule-associated β -hexosaminidase is an exoglycosidase and is released in parallel with other chemical mediators (e.g. histamine and serotonin) upon antigen crosslinking of Fc ϵ R1. Therefore, the release of β -hexos-

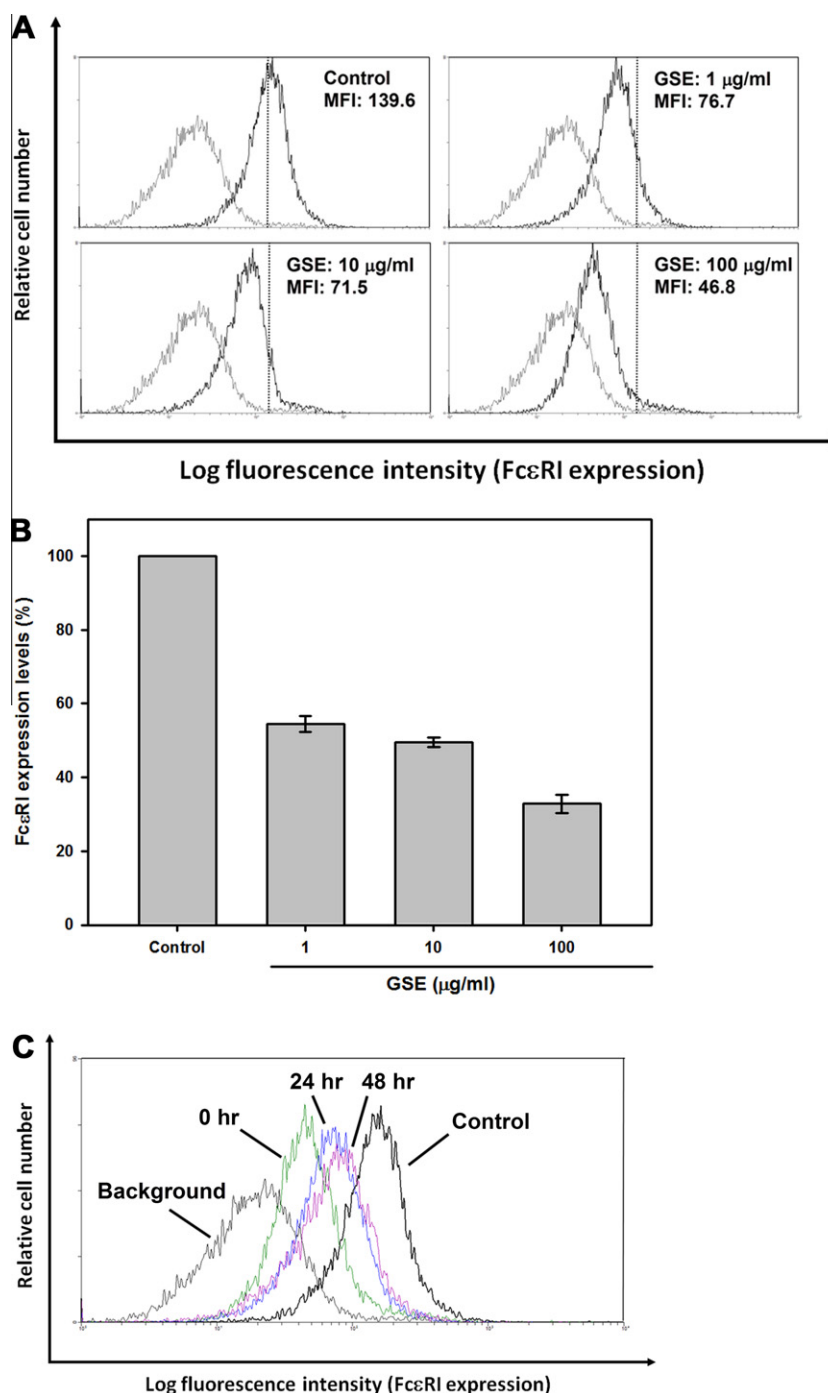


Fig. 4. Effect of GSE on cell surface expression of FcεRI. (A) RBL-2H3 cells were cultured with indicated GSE concentrations for 24 h. Cell surface FcεRI levels were examined by immunostaining using mIgE-DNP followed by FITC-conjugated rat IgG1 against mouse-IgE. Mouse IgG2b antibody was used as the isotype-matched negative control. Data acquisition was performed by flow cytometry. (B) Quantitative comparison of FcεRI expression levels between control and GSE-treated RBL-2H3 cells from three independent experiments. (C) RBL-2H3 cells were cultured with GSE (100 μg/ml) for 24 h, followed by measuring FcεRI levels after the removal of GSE and replenishment with free medium at indicated time intervals. The control was untreated cells whose FcεRI levels were measured at the same time as the 0-h treatment group.

aminidase and histamine has been commonly regarded as useful indicators to evaluate the activation of mast cells in various acute allergic and inflammatory responses (Abramson et al., 2006; Pecht & Corcia, 1987). This assay is a convenient tool for studying signal transduction pathways leading to exocytosis of mast cells as well as screening potential drugs for the blockade of mast cell degranulation (Granberg, Fowler, & Jacobsson, 2001). Result from Fig. 1 indicates that GSE used in between 1–100 μg/ml is not cytotoxic to RBL-2H3 mast cells, as it has been shown that GSE concentrations

exhibited variable cytotoxicity to different cells types (Ye et al., 1999). In Figs. 2 and 3, we showed that release of both β-hexosaminidase and histamine from RBL-2H3 cells was significantly inhibited in a dose-dependent manner by pretreatments with GSE without affecting viability of the cells. The reduction in both β-hexosaminidase and histamine levels upon GSE treatments indicates that GSE is capable of inhibiting the degranulation process of mast cells.

The high affinity IgE receptor, FcεRI, is expressed on surface of mast cells and basophils. Multivalent allergens can crosslink

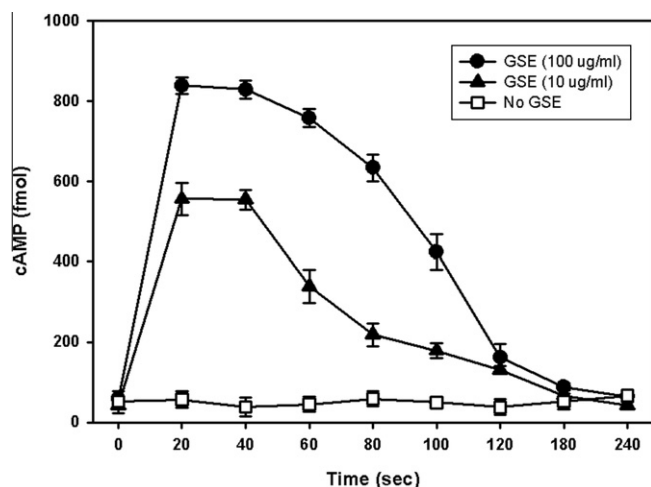


Fig. 5. Effect of grapeseed extract on cAMP in mast cells. Aliquots of RBL-2H3 cells were separated treated with varying concentrations of GSE at 37 °C for indicated time intervals. Cells were then lysed and supernatant was subjected to determination of cAMP levels by commercial EIA kit. Results represent the mean $\pm$ SD ($n = 3$). *Statistically significant from the control at $p < 0.05$.

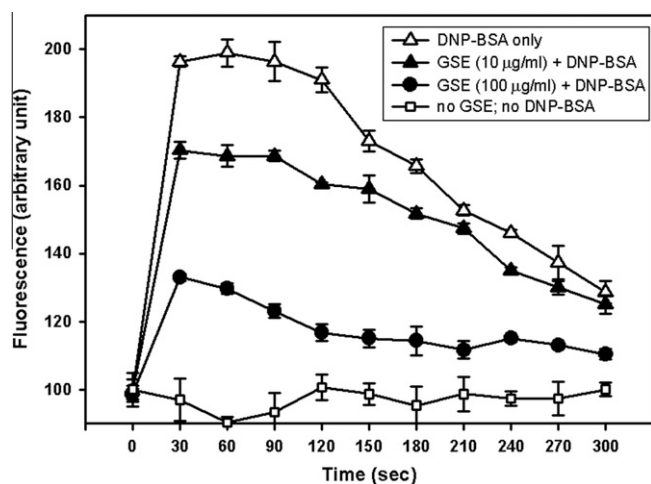


Fig. 6. Effect of grapeseed extract on Ca^{2+} influx in mast cells. RBL-2H3 cells were pretreated with indicated concentrations of GSE (filled symbols), followed by sensitization with mlgE-DNP (1 $\mu\text{g}/\text{ml}$) for 18 h and loading with Fura-2/AM (2 μM). For positive control, untreated cells were solely sensitized with mlgE-DNP. All cells, except the negative control group, were activated with DNP-BSA (100 ng/ml). Fura-2 fluorescence intensity was immediately monitored at $\lambda_{\text{ex}} = 340 \text{ nm}$ and $\lambda_{\text{em}} = 490 \text{ nm}$, respectively. Results are expressed as mean $\pm$ SD ($n = 3$).

allergen-specific IgE bound by Fc ϵ R1 and initiate signalling events that ultimately lead to mast cell activation, resulting in the secretion of allergic mediators and the induction of gene transcription of various inflammatory cytokines (Abramson et al., 2006). Since signal transduction via Fc ϵ R1 plays an essential role in the IgE-mediated allergic responses, expression of Fc ϵ R1 is therefore critical for antigen-dependent activation of mast cells. In this study, we examined the effect of GSE on surface Fc ϵ R1 expression in RBL-2H3 mast cells. Cells pretreated with GSE at the non-toxic concentration ranges (1–100 $\mu\text{g}/\text{ml}$) effectively inhibited the Fc ϵ R1 levels in a dose-dependent manner (Fig. 4A and B). However, the inhibition of GSE on Fc ϵ R1 expression was a temporary effect because expression of Fc ϵ R1 on RBL-2H3 gradually resumed as GSE was removed from culture medium (Fig. 4C).

Intracellular cAMP levels are controlled by the balancing action between two enzymes, adenylate cyclase and cAMP-hydrolysing

phosphodiesterase (Fantidis, 2010). In mast cells, the increased cAMP concentrations can lead to cAMP-dependent activation of protein kinase A (PKA), which in turn regulates various biological activities by phosphorylating its substrates and decreases the availability of cytosolic Ca^{2+} required for various enzymatic activities involved in the allergic responses (Abramson et al., 2006; Abramson & Pecht, 2007; Pecht & Corcia, 1987). Additionally, it has been reported that agents capable of inducing and sustaining prolonged cAMP levels in mast cells are potent inhibitors of mediator release from activated mast cells (Brown, Spina, & Page, 2008). In our study, incubation of GSE caused a significantly dose-dependent increase of cAMP levels in RBL-2H3 cells (Fig. 5), suggesting that the inhibitory effects of GSE on mast cell degranulation may be associated with an elevation of intracellular cAMP concentrations.

In order to further investigate the mechanism underlying the inhibitory effects of GSE on mast cell degranulation, we examined whether GSE elicits effect on the free cytoplasmic calcium levels in RBL-2H3 cells. It has been shown that the movement of Ca^{2+} across the membrane into the cytosol, either from intracellular store such as the endoplasmic reticulum or from the external environment, is critical to IgE-antigen mediated mast cell activation and degranulation (Pecht & Corcia, 1987). The increase in cytosolic Ca^{2+} levels often precedes the release of histamine and β -hexosaminidase, the expression of cytokines (e.g. IL-4 and TNF α) and the production of inflammatory mediators, including prostaglandins and leukotrienes (Theoharides et al., 2010). Treatments of mast cells with substances allergenic in nature, such as the environmental hormone di(2-ethylhexyl) phthalate (DEHP), causes the increased cytosolic Ca^{2+} levels along with other allergic parameters (Lee, Oh, & Lim, 2011). Therefore, inhibition of Ca^{2+} movement into the cytosol has been one of the targets of designing effective anti-allergic drugs. Our results showed that, when RBL-2H3 mast cells were pretreated with GSE, a dose-dependent inhibition on the cytosolic Ca^{2+} levels was observed (Fig. 6). Thus, GSE possibly elicits its inhibitory effects on degranulation of RBL-2H3 mast cells at least in part via the inhibition of Ca^{2+} influx from the environment into the cells. Our finding is the first report demonstrating the underlying mechanism by which GSE inhibits the degranulation process downstream of the Ca^{2+} influx in mast cells.

Grape phenolics have been long gained remarkable interest based on the promising results of their antioxidant properties and abilities to act as free radical scavengers. Studies have shown that grapeseed phenolics may help inhibit enzyme systems responsible for the production of free radicals associated with inflammatory reactions (Shi et al., 2003). Additionally, intervention of procyanidins on both synthesis and release of inflammation-promoting substances (such as serine protease, histamine, prostaglandins and leukotrienes) (Shi et al., 2003) that are present in the chronic stage of allergy has been documented. In the current study, we examined various effects of GSE on the early stage of mast cell-dependent allergic responses. Our results indicate that GSE inhibits degranulation and reduces expression of high-affinity IgE receptor in antigen-activated RBL-2H3 mast cells. The anti-allergic effects of GSE could be partly mediated through the reduction of Ca^{2+} influx and elevation of cAMP in the mast cells. Our current findings offer insight into the immunomodulatory functions of GSE and potential regimens for treating mast cell-mediated type I hypersensitivity.

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