

Isolation of 5-(Hydroxymethyl)Furfural from *Lycium chinense* and its Inhibitory Effect on the Chemical Mediator Release by Basophilic Cells

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Key words

- anti-allergic
- basophile
- β -hexosaminidase
- 5-(hydroxymethyl)furfural
- *Lycium chinense*
- Solanaceae
- RBL-2H3

Abstract

The effect of hot water extracts of *Lycium chinense* fruits (LCF) on the β -hexosaminidase (β -hexo) release by IgE sensitized BSA stimulated rat basophilic leukemia (RBL-2H3) cells was investigated. The ethylacetate (EtOAc) layer of the extract has shown an inhibitory effect on β -hexo release from RBL-2H3 cells at the antigen antibody binding stage. The water (H₂O) fraction (EFW) of the chloroform (CHCl₃) extract from the EtOAc layer also inhibited β -hexo release at the same stage in a dose-dependent manner. With column chromatography preparation, proton and carbon nuclear magnetic resonance (¹H and ¹³C NMR) spectra, electron ionization mass spectrometer (EI-MS) spectra, and high-performance liquid chromatography (HPLC) analysis, the active component was determined to be 5-(hydroxymethyl)furfural (5-HMF). Thus, the 5-HMF showed an inhibitory effect

on β -hexo release at the antigen-antibody binding stage and the antibody-receptor binding stage. Furthermore, 5-HMF suppressed [Ca²⁺]_i influx in the IgE-sensitized BSA-stimulated RBL-2H3 cells. Our results show that 5-HMF may be useful for the treatment or prevention of type I allergic diseases.

Abbreviations

β -hexo:	β -hexosaminidase
5-HMF:	5-(hydroxymethyl)furfural
Keto:	ketotifen fumarate
KU812:	human basophilic cells
LCF:	<i>Lycium chinense</i> fruits
MTT:	3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide
RBL-2H3:	rat basophilic leukemia cells
ROS:	reactive oxygen species

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Introduction

Wolfberry, commercially gouqi berry, is the common name for the fruit of two very closely related species of boxthorn in the family Solanaceae: *Lycium barbarum* L. (*L. barbarum*) and *Lycium chinense* (LC). The fruit is native to southeastern Europe and Asia [1] and widely used in traditional Chinese herbal medicine. There have been several investigations of the different organs of the plant and the components of wolfberries, but studies have been performed mostly with *L. barbarum* and fewer investigations on LC. On the other hand, studies on other organs, in particular the roots and leaves, have focused on LC. As reviewed by Olivier, the composition of the fruits of LC is similar to that of *L. barbarum*, and many biological activities are reported [2,3]. The bioactive components of the *L. barbarum* fruits have been mainly attributed to its polysaccharide–protein

complex (LBP), generally consisting of 6 monosaccharides and 18 amino acids. The β -glycan structural aspect of a polysaccharide is thought to correlate with its biological function, and LBP enhances immune function, decreases the side effects of chemotherapy and radiotherapy, induces apoptosis, reduces blood glucose levels, protects liver damage and exhibits antiaging, antitumor, cytoprotective, and neuromodulative effects [2, 4]. Considerable data on the biological activity of LCF have been reported [2,4–6], but their anti-allergic effects have not been studied or defined. Mast cells and basophiles play essential roles in the pathogenesis of allergic reactions such as atopic dermatitis, allergic rhinitis, and asthma. RBL-2H3 cells are mucosal mast cells that express the immunoglobulin Fc epsilon receptor I (Fc ϵ RI). Stimulation of IgE-sensitized RBL-2H3 cells with a specific antigen triggers a cascade of events leading to degranulation, mediator release, activation

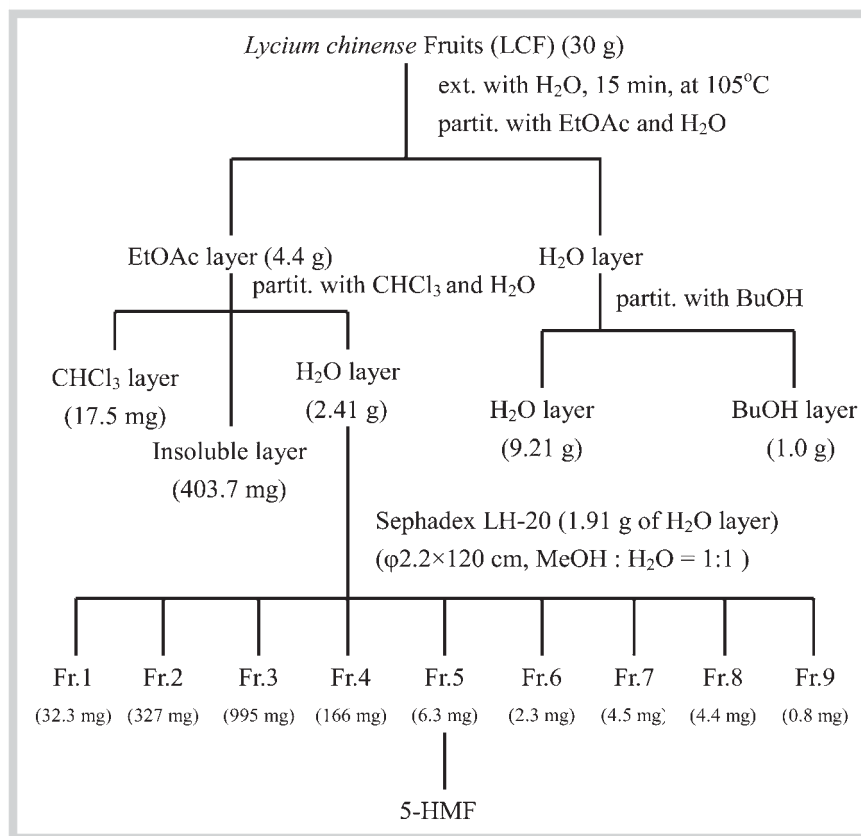


Fig. 1 Isolation procedure of 5-HMF from LCF.

of mitogen activated protein kinase (MAPK), increase in reactive oxygen species (ROS) production, calcium influx, and cytokine production [7–9]. This cascade suggests that mast cells are ideal targets for controlling various allergic diseases [7], thus the β -hexo activity in the medium is used as a marker of mast cell degranulation [10].

Hydroxymethyl furfural, HMF or 5-HMF, is an aldehyde and a furan compound formed during the thermal decomposition of sugars and carbohydrates. 5-HMF has been identified in a wide variety of heat-processed foods including milk, fruit juices, spirits, honey, breakfast cereals, etc. 5-HMF is one of the major intermediates of the Maillard reaction or caramelization and is formed during the heating or preservation of foods [11, 12]. 5-HMF is also considered as an indicator of the browning reaction and quality parameters of foods, especially for flavor deterioration of alcoholic drinks such as wine, whisky, rum, and sake as well as fruit juice and caramel [12, 13]. Therefore, studies on the toxicity and metabolism of 5-HMF in animals and cells were emphatically done, and there are a number of results concerning its small cytotoxic activity at low concentrations and apoptotic effect at high concentrations [12, 14]. However, the biological activity of 5-HMF has not received much attention until Abdulmalik et al. [15] reported that 5-HMF has an inhibitory effect on sickling red blood cells (RBC) *in vitro*. Recently, antioxidant [6] and 3T3-L1 differentiation activities of 5-HMF have been reported [16], but there is no experimental evidence available regarding 5-HMF-mediated inhibition of chemical mediator release by immediate-type allergic model cells.

In this study, we focused on the effect of LCF extract on type I allergy, investigated its *in vitro* antiallergic activity by using RBL-2H3 cells and identified its active compound. This is the first report that LCF extract has antiallergic activity and that its active component is 5-HMF.

Materials and Methods



Plant materials and chemicals

LCF were purchased from Shinwa Bussan Co., Ltd. and identified by a botany expert. Their voucher specimens (UT-LC060825) were deposited in the Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan. Dinitrophenylated bovine serum albumin (DNP-BSA) was purchased from Cosmo Biotechnology Co. Anti-DNP-IgE, Ketotifen fumarate (>99%), L-glutamine, and 5-HMF ($\geq 99\%$, W501808) were purchased from Sigma. Fetal bovine serum (FBS) was purchased from Hyclone Co., Ltd. Eagle's minimum essential medium (MEM) was purchased from Nissui Pharmaceutical Co. Ltd.

Extraction from LCF

Dried LCF (30 g) were submitted to extraction with Mili Q (300 mL) at 105 °C for 15 min. The hot H₂O extract of LCF was concentrated with an evaporator, lyophilized and dissolved in H₂O, then partitioned with EtOAc (200 mL $\times$ 3) to obtain an EtOAc-soluble fraction (EF, 4.4 g). The H₂O-soluble fraction was then partitioned with butanol (BuOH; 200 mL $\times$ 3) to give fractions of H₂O (WF, 9.21 g) and BuOH (BF, 1.0 g). The EF was partitioned with CHCl₃ (200 mL $\times$ 3) and H₂O (200 mL $\times$ 3) to give fractions of CHCl₃ (EFCH, 17.5 mg), H₂O (EFW, 2.41 g), and an insoluble (EFI, 403.7 mg) layer. After removal of the solvent, the entire fraction was dissolved in PBS (–) just before being added to a releasing mixture of RBL-2H3 cells (● Fig. 1).

Identification of the active component in LCF

The active fraction EFW of LCF was subjected to Sephadex LH-20 (ϕ 2.2 $\times$ 120 cm, MeOH:H₂O = 1:1) to yield nine fractions (Fr. 1–9). All the fractions were also applied to a β -hexo release assay

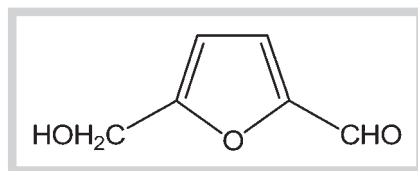


Fig. 2 Structure of 5-HMF from LCF.

with the IgE-mediated RBL-2H3 cells. The chemical structure of the main active compound was identified as 5-HMF on the basis of UV 280 nm, ^1H NMR, ^{13}C NMR, EI MS, retention times of HPLC by comparison of spectral and elemental analyses of standard 5-HMF, and previous reported data (The Aldrich Library of ^{13}C and ^1H FT-NMR spectra Volume I, 297–300) (● Fig. 2).

Cells and cell culture

RBL-2H3 cells were purchased from JCRB Cell Bank, Japan, and maintained in MEM supplemented with 10% FBS and 2 mM L-glutamine. KU812 cells were purchased from Riken Cell Bank, maintained in a RPMI 1640 medium supplemented with 10% FBS and incubated at 37 °C in a 5% CO_2 incubator.

MTT assay

Cell viability was assessed by MTT assay, which is a measure of the mitochondrial respiration of the cells [17]. RBL-2H3 and KU812 cells were harvested at approximately 60–80% confluence and seeded on to 96-well plates at 5.0×10^4 cells/well in 100 μL of medium. After an overnight incubation, all medium was removed followed by addition of 100 μL of 5-HMF (final 0, 0.3, 0.5, 1.0, 5.0, 10.0 $\mu\text{g}/\text{mL}$ with medium) and ketotifen fumarate (Keto, final 214 μM and 300 μM with medium). The cells were then incubated for 24 h before 10 μL of 5 mg/mL of MTT was added. After 24 h of incubation, 100 μL of 10% sodium dodecyl sulfate (SDS) was added, followed by another 24 h of incubation to completely dissolve the formazan produced by the cells. The absorbance was then determined at 570 nm with a microplate reader (Power Scan HT; Dainippon Pharmaceutical Co., Ltd). Blanks were prepared at the same time to correct for the absorbance caused by sample color and by the inherent ability of a sample to reduce MTT in the absence of cells. The optical density of the formazan produced by the untreated control cells represented 100% viability.

β -Hexosaminidase release assay at antigen-antibody binding stages

The β -hexo release inhibition assay using RBL-2H3 cells was performed as previously described [18]. RBL-2H3 cells were seeded onto 96-well plates at 5.0×10^4 cells/well in 100 μL of medium. Cells were incubated for 24 h at 37 °C and sensitized with 0.3 $\mu\text{g}/\text{mL}$ anti-DNP-IgE, then washed twice with PBS (–) to eliminate free IgE. After incubating at 37 °C for 10 min in 60 μL per well of releasing mixture containing 5 μL of EF, BF, and WF layer (1/10000–1/10, dilution, respectively), Fr. 5 (final 0.01, 0.1, and 0.3 $\mu\text{g}/\text{mL}$), and a 5-HMF (final 0, 0.01, 0.1, 0.3 $\mu\text{g}/\text{mL}$ with PBS [–]), the cells were exposed to 0.3 $\mu\text{g}/\text{mL}$ DNP-BSA in PBS (–), followed by incubation at 37 °C for 1 h. Three mM Keto and PBS (–) were used as positive and negative controls, respectively. The β -hexo released into the supernatant was measured as in the previous study [18].

β -Hexosaminidase release assay at the antibody-receptor binding stage

To determine the effect of 5-HMF at the antibody-receptor binding stage, the test sample was added to the cells before the IgE sensitization. RBL-2H3 cells were seeded on to 96-well plates at 0.5×10^5 cells/well in 50 μL of the medium, and 5 $\mu\text{L}/\text{well}$ of a test sample (final 0.01, 0.1, and 0.3 $\mu\text{g}/\text{mL}$ of 5-HMF) was then added before incubating at 37 °C for 1 h. Then 50 μL of the medium with 0.6 $\mu\text{g}/\text{mL}$ anti-DNP-IgE was added to each well, before being incubated at 37 °C for 24 h. The cells were washed twice with PBS (–) to eliminate free IgE. After incubating the cells at 37 °C for 10 min in 60 $\mu\text{L}/\text{well}$ of the releasing mixture, the cells were exposed to 0.3 $\mu\text{g}/\text{mL}$ DNP-BSA in PBS (–) before being incubated at 37 °C for 1 h. The β -hexo released to the supernatant was measured as in the previous section.

Determination of intracellular $[\text{Ca}^{2+}]_i$ level

$[\text{Ca}^{2+}]_i$ measurement was performed by the method of Aase and Arna [19] with some modifications. RBL-2H3 cells (5.0×10^4 cells per well in 100 μL of medium) were precultured at 37 °C for 24 h in 96-well plates with medium and 0.3 $\mu\text{g}/\text{mL}$ anti-DNP IgE. The cells were washed twice with 200 μL of PBS (–) to eliminate free IgE and then incubated with 100 μL per well of loading buffer containing Fluo3-AM (Calcium Kit-Fluo3; Dojindo Co., Ltd.) at 37 °C for 1 h. The cells were then washed twice with 200 μL of PBS (–) to eliminate free Fluo3-AM, followed by incubation with 60 μL per well of recording buffer and 5-HMF (final 0.01, 0.1, and 0.3 $\mu\text{g}/\text{mL}$) at 37 °C for 1 h in a 5% CO_2 incubator. For positive and negative controls, 3 mM Keto and PBS (–) were used, respectively. The fluorescence intensity (FI) was determined 100 s after adding 0.3 $\mu\text{g}/\text{mL}$ of DNP-BSA antigen. FI was measured at an excitation wavelength of 490 nm and emission wavelength of 530 nm using a microplate reader (Infinite™ 200 Series; Tecan Group, Ltd.).

Statistical analysis

Results are expressed as mean $\pm$ standard deviation. Statistical comparisons were carried out using Student's t-test for paired values.

Results

Effects of the LCF extract on IgE-mediated β -hexo release from RBL-2H3 cells at the antigen-antibody binding stage were examined; results are shown in ● Fig. 3. The EF and BF at a treatment range of 1/10000–1/10 dilutions significantly inhibited the IgE-mediated β -hexo release from RBL-2H3 cells compared with controls in a dose-dependent manner (except 1/500 BF). The EF exhibited a more potent inhibition on β -hexo release than WF and BF. Inhibitory activity of EF at 1/500 dilution was $51.3 \pm 0.9\%$ ($p < 0.001$) and WF at 1/500 dilution was $85.8 \pm 0.2\%$ ($p < 0.01$) of the LCF extract. Furthermore, using CHCl_3 to partition the active EF into CHCl_3 layer (EFCH), H_2O layer (EFW), and insoluble layer (EFI), also determined their activity at the same condition. The EFCH and EFW showed inhibitory effects on β -hexo release at the same stage, and the effect was significant compared with control. The inhibitory effect of the EFW was higher than that of the EFCH (data not shown).

The EFW was fractionated into nine fractions (Fr. 1–9) by Sephadex LH-20 gel column chromatography. All the fractions were applied to the β -hexo release assay with the IgE-mediated RBL-2H3 cells. The fifth fraction of EFW (Fr. 5) had the most potent inhibi-

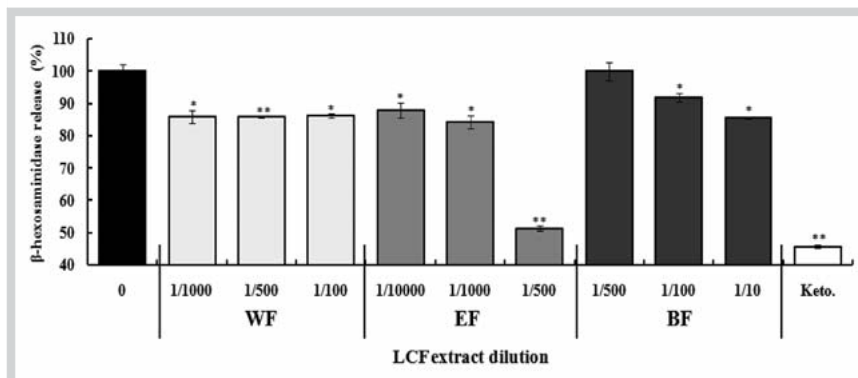


Fig. 3 Inhibitory effect of water extract and the fraction from LCF on the β -hexo release from RBL-2H3 cells at the antigen-antibody binding stage. The cells (5.0×10^4 cells/well) were preincubated with each extract at 37°C for 10 min prior to their incubation with DNP-BSA. Results represent one trial ($n = 6$). Three additional trials show similar results. *Significantly different from the negative control (PBS [-]) (*: $p < 0.05$, **: $p < 0.01$, Student's *t*-test). EF: EtOAc layer of LCF extract; BF: BuOH layer of LCF extract; WF: H₂O layer of LCF extract.

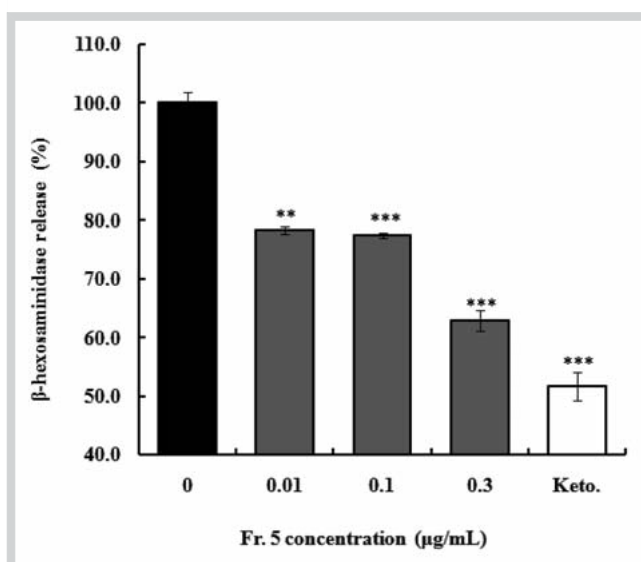


Fig. 4 Inhibitory effect of Fraction 5 of EtOAc extract from LCF on the β -hexo release from RBL-2H3 cells at the antigen-antibody binding stage. The cells (5.0×10^4 cells/well) were preincubated with each fraction at 37°C for 10 min prior to their incubation with DNP-BSA. Results represent one trial ($n = 6$). Three additional trials show similar results. *Significantly different from the negative control (PBS [-]) (*: $p < 0.01$, ***: $p < 0.001$, Student's *t*-test). Fr. 5: Fraction No. 5 of EFW.

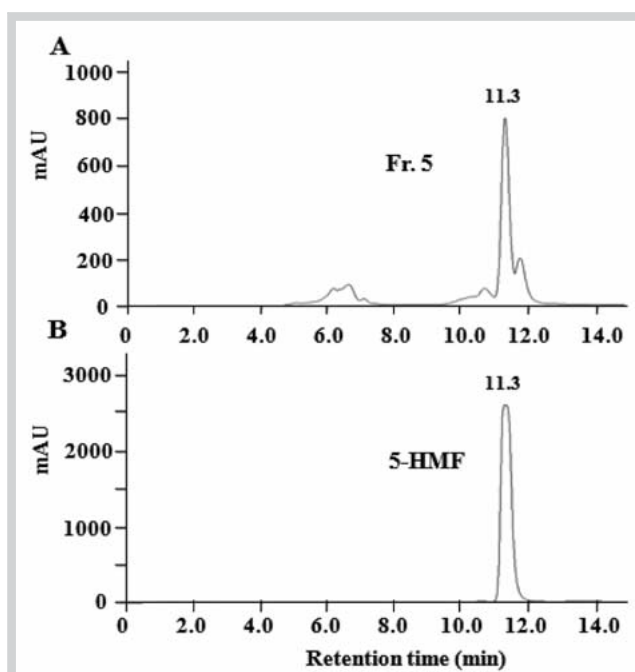


Fig. 5 HPLC chromatograms of Fraction 5 (A) and the 5-HMF (B). Column: ODS-120A, $\phi 7.8 \times 300$ mm, TOSOH; mobile phase, 50% methanol; flow rate, 1.0 mL/min; detected with UV at 283 nm.

tion of β -hexo release (● **Fig. 4**; data for other fractions are not shown). Fr. 5 was identified as 5-HMF on the basis of UV, ^1H NMR, ^{13}C NMR, EIMS, and HPLC. The ^1H NMR spectrum showed characteristic signals for an aldehyde proton at δ_{H} 9.60 (s), two aromatic methine protons at δ_{H} 7.40 (d, $J = 3.6$ Hz) and 6.70 (d, $J = 3.6$ Hz), and an oxymethylene at δ_{H} 4.64 (m). In the ^{13}C NMR data, signals due to δ_{C} 110.6, 159.6, 122.4, and 153.0 showed a furan ring. HREIMS analysis of Fr. 5 revealed the molecular formula to be $\text{C}_6\text{H}_6\text{O}_3$ (m/z 126.0326 [M^+], $\Delta + 1.0$ mmu). HPLC retention time of Fr. 5 and 5-HMF was common at 11.3 min (● **Fig. 5**). The results show that 5-HMF in the range of 0.3–5.0 $\mu\text{g/mL}$ (2.4–39.7 μM) did not cause any cytotoxicity after treating the RBL-2H3 and KU812 cells for 24 h. But the viability of KU812 cells treated with 10.0 $\mu\text{g/mL}$ (79.4 μM) of 5-HMF was $93.2\% \pm 3.3\%$, a significant effect compared with control ($p < 0.05$ vs. the negative control). It should be noted that Keto, a clinically available anti-allergic drug, which is known as a mast cell stabilizer, H1-receptor antagonist, and eosinophil inhibitor [17], also had no signifi-

cant cytotoxic effect at a concentration of 214 μM (● **Fig. 6**), but 300 μM of Keto was severely cytotoxic (data not shown).

To confirm the inhibition of β -hexo release by 5-HMF on IgE-mediated RBL-2H3 cells at the antigen-antibody binding stage, we used commercially available 5-HMF for the assay. The 5-HMF in a dose-dependent fashion inhibited β -hexo release from RBL-2H3 cells, a significant effect at 0.01–0.1 $\mu\text{g/mL}$ and 0.3 $\mu\text{g/mL}$ ($p < 0.05$ and $p < 0.01$ vs. the negative control). 0.3 $\mu\text{g/mL}$ of 5-HMF was inhibited $41.6 \pm 2.3\%$, and the inhibitory effect was lower than that of 3 mM keto (final 214 μM , $\text{IC}_{50} = 200\text{--}300$ μM) in this stage (● **Fig. 7**).

From these results, the active compound of Fr. 5 is probably 5-HMF. One gram of LCF extract was found to contain 3.8 mg of 5-HMF.

Inhibition of β -hexo release by 5-HMF on IgE-mediated RBL-2H3 cells at the antibody-receptor binding stage was examined. 5-HMF at 0.01–0.3 $\mu\text{g/mL}$ inhibited β -hexo release at this stage, and the effect was significant at 0.1 $\mu\text{g/mL}$ and 0.3 $\mu\text{g/mL}$ ($p < 0.05$ and $p < 0.01$ vs. the negative control). 0.3 $\mu\text{g/mL}$ of 5-HMF inhibited

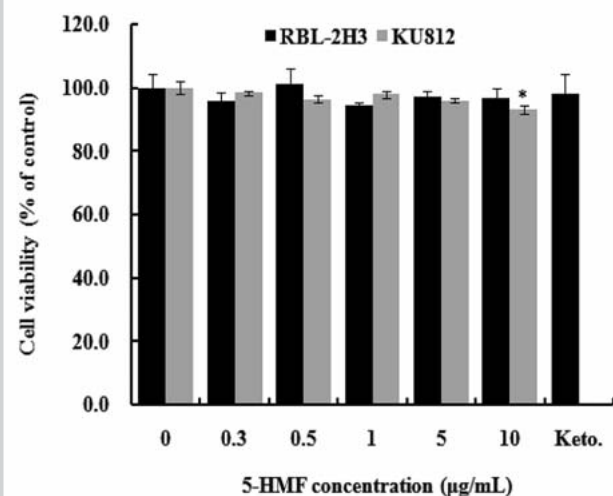


Fig. 6 Cytotoxicity effect of 5-HMF on RBL-2H3 cells and KU812 cells by MTT assay. The percent cell viability was calculated relative to the untreated control. The cells (5.0×10^4 cells/well) were incubated with 5-HMF at 37 °C for 24 h in 5% CO₂. *Significantly different from the negative control (PBS [-]) (*: $p < 0.05$, Student's t-test).

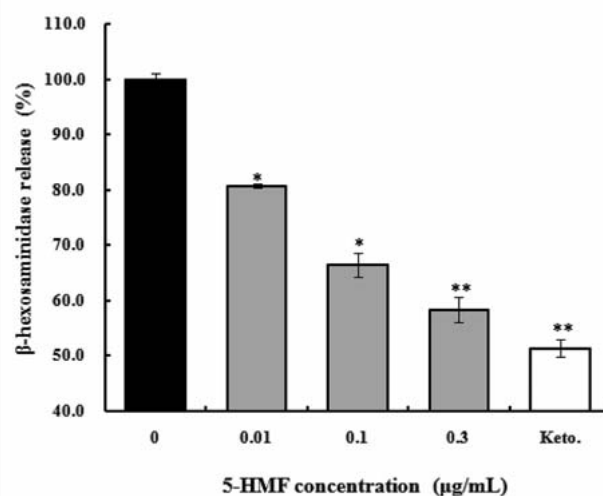


Fig. 7 Inhibitory effect of 5-HMF on the β -hexo release from RBL-2H3 cells at the antigen-antibody binding stage. The cells (5.0×10^4 cells/well) were preincubated with 5-HMF at 37 °C for 10 min prior to their incubation with DNP-BSA. Results represent one trial ($n = 6$). Three additional trials show similar results. *Significantly different from the negative control (PBS [-]) (*: $p < 0.05$, **: $p < 0.01$, Student's t-test).

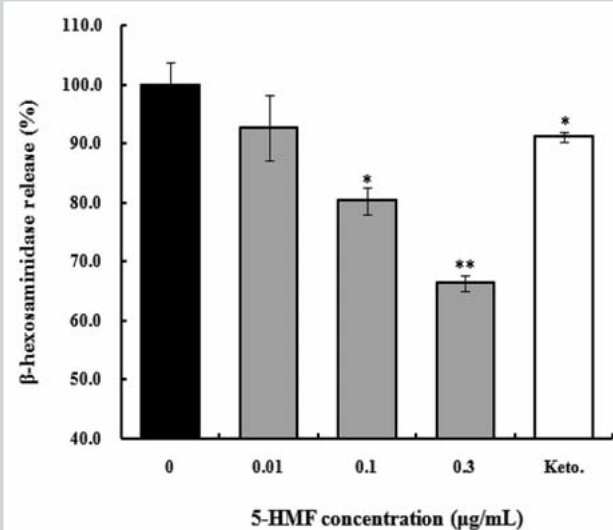


Fig. 8 Inhibitory effect of 5-HMF on the β -hexo release from RBL-2H3 cells at the antibody-receptor binding stage. The cells (0.5×10^5 cell/well) were preincubated with 5-HMF at 37 °C for 1 h prior to their IgE sensitization, and then preincubated with 5-HMF at 37 °C for 24 h prior to their DNP-BSA stimulation. Results represent one trial ($n = 6$). Three additional trials show similar results. *Significantly different from the negative control (PBS [-]) (*: $p < 0.05$, **: $p < 0.01$, Student's t-test).

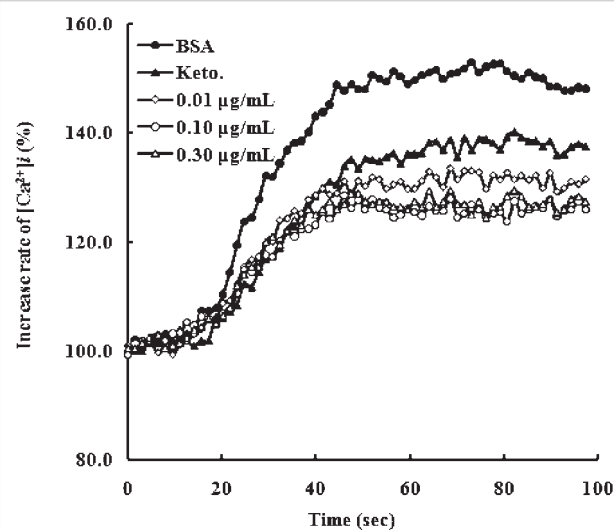


Fig. 9 Effect of 5-HMF on intracellular [Ca²⁺]_i levels 100 sec after stimulation with the DNP-BSA antigen. The cells (5.0×10^4 cells/well) were preincubated with 5-HMF at 37 °C for 1 h prior to their incubation with DNP-BSA. Two additional trials show similar results.

33.6% $\pm$ 1.2% of the β -hexo release, and the inhibition was higher than that of 3 mM keto at this stage (● Fig. 8). This result suggests that the inhibitory effect of 5-HMF on chemical mediator release is different than that of the Keto.

To determine the effect of 5-HMF on calcium influx, intracellular [Ca²⁺]_i levels were assayed. The increased levels of [Ca²⁺]_i in IgE-mediated RBL-2H3 cells caused by 5-HMF are shown in ● Fig. 9. As expected, stimulation of cells with DNP-BSA induced calcium influx, keto decreased calcium influx, and the addition of 5-HMF

to IgE-sensitized RBL-2H3 cells resulted in a reduction of calcium influx (● Fig. 9).

Discussion



In this study, 5-HMF did not show cytotoxicity at the concentration of 0.3–5.0 µg/mL but significantly decreased cell viability at the 10.0 µg/mL treatment on KU812 cells (● Fig. 6). This agrees

with Li et al. [6] who have shown that 5-HMF displayed no cytotoxic effects on RAW 264.7, HL-60, and MRC-5 cells at 1–10 μ M, while the highest concentration (100 μ M) decreased cell viability. The induction of cell damage by 5-HMF at a high concentration may be due to its production of H_2O_2 [20,21] and affected by its apoptotic activity [12].

Many studies indicate that different cells have different sensitivity towards the cytotoxicity of the 5-HMF [22,23]. Orally administered 5-HMF is less toxic to rats than human [14] and, after oral application to rats, rapidly absorbed from the gastrointestinal tract [12]. The susceptibility of cells to 5-HMF may correlate with expression levels of receptors [24], metabolism [23,25], chemical structure [26,27], and enzyme activity of the compound [28]. The different sensitivity between KU812 and RBL-2H3 cells may be explained through their different metabolisms. Our result provides scientific evidence that 5-HMF can be used safely in subsequent investigations in cultured cell systems.

Cross-linking of Fc ϵ RI induced by complex formation of IgE with an antigenic protein is an essential event in the IgE-mediated allergic reaction, so inhibition of the binding between IgE and Fc ϵ RI has been a target for the development of antiallergic drugs. Interfering with the binding of IgE to Fc ϵ RI is considered a straightforward strategy for preventing the IgE mediated allergic reaction [29]. To confirm the blocking effect of 5-HMF on antigen binding to IgE cross-linking Fc ϵ RI and IgE binding to Fc ϵ RI receptors, the two-stage binding was investigated. Our results indicated that 5-HMF inhibited β -hexo release at both stages. These suggest that 5-HMF might be useful for treating and preventing allergic diseases by blocking the cross-linking of IgE and IgE binding to the Fc ϵ RI receptor. On the other hand, recent studies indicated that the furanic compound readily pass through serum albumin and RBC membrane [26]. The membrane penetration ability of 5-HMF may be related to its inhibition effect on β -hexo release.

An increase in cytosolic calcium [Ca^{2+}]_i level is considered to be an essential and ubiquitous mechanism in the process of mast cell degranulation [30]. Our results indicate that the intracellular [Ca^{2+}]_i level was lower in the 5-HMF-treated cells (● Fig. 9). From these observations, we consider that the decrease in intracellular [Ca^{2+}]_i is involved in the inhibitory effect of 5-HMF on β -hexo release (● Figs. 7, 8). Nitric oxide (NO) and H_2O_2 are two major ROS known to regulate calcium signaling and degranulation of mast cells. And nicotinamide adenin dinucleotide phosphate (NADPH) oxidase is mainly responsible for ROS production in the IgE-mediated RBL-2H3 cells [5,31]. The sustained elevation of cytosolic calcium through store-operated calcium entry was totally abolished when ROS production was blocked. Furthermore, there was a significant correlation between inhibition of histamine release and DPPH or superoxide anion radical scavenging activities [32]. On the other hand, 5-HMF has free radical scavenging properties on DPPH, hydroxyl, alkyl, and superoxide anion radical and also had free radical scavenging properties on intracellular ROS in RAW 264.7 cells [6], some enzymatic activity in U-937 cells [28], and an inhibitory effect on NO release from LO2 cells [21]. The structure of 5-HMF contains interesting functional reactive groups such as double bonds, an aldehyde oxygen atom, and another oxygen existing in the furan ring, which can attract electrons easily [14], and substitution type at the 5-position of the central furan ring is important to biological activities [26]. One of the possibilities is that the inhibition effect of 5-HMF on intracellular calcium release may be affected by its free radical scavenging activities and decrease enzymatic activities. Other factors may also be responsible for its inhibition effect.

Recent research showed that 5-HMF is the main active component in other traditional medicines, such as *Rehmanniae*, *Cornus*, and others [6]. 5-HMF can be obtained by acid-catalyzed dehydration of hexoses [33] and prepared from the fructose portion of the sugar molecule [34] and conversion of 1-amino-1-deoxyfructose by heating milk with added oxalic acid [35], also easily created in conventional heating processes of foods [36].

In conclusion, LCF extract was found to have antiallergic properties in type I allergy model basophilic cells. We also found that 5-HMF is the main antiallergy agent of LCF extract, and it significantly inhibits IgE-mediated chemical mediator release from basophilic cells at different stages. These results suggest that 5-HMF may be a good candidate for therapeutic treatment of type I allergic diseases. The detailed mechanism behind the antiallergic affect of 5-HMF is the subject of a future study. We expect that this research will play a significant role in the effective use of 5-HMF in the future.

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