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ORIGINAL ARTICLE

Drug interaction between Shoseiryuto Extract or Catechins and Fexofenadine through Organic-Anion-transporting Polypeptide 1A2 *In vitro*

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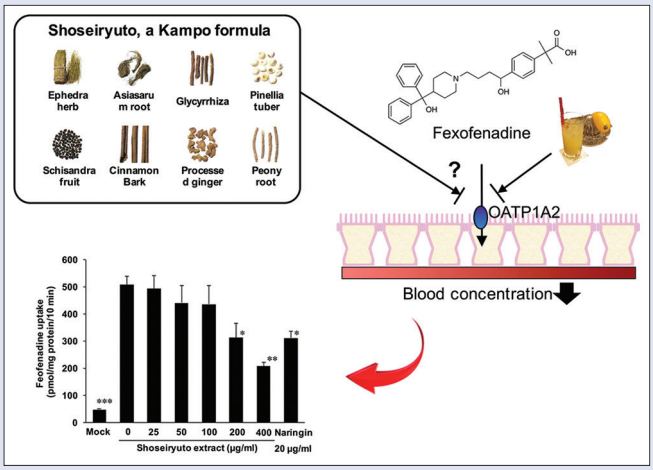
ABSTRACT

Objective: Fexofenadine is an anti-allergy drug frequently used to treat rhinitis, which is absorbed through organic-anion-transporting polypeptide (OATP) 1A2 in the intestine. Clinical studies have revealed that grapefruit juice inhibits OATP1A2 to reduce the absorption of fexofenadine. In Japanese traditional Kampo medicine, shoseiryuto, a formula composed of eight crude drugs, is frequently used to treat allergic rhinitis, especially cases of pollinosis. The objective of this study is to present the drug information regarding the interaction between shoseiryuto and fexofenadine through OATP1A2. **Materials and Methods:** We established human embryonic kidney 293 cells stably expressing OATP1A2 and evaluated the inhibitory effects of the extracts of shoseiryuto and its herbal components on the uptake of fexofenadine into the cells. **Results:** Shoseiryuto extract inhibited fexofenadine uptake in a concentration-dependent manner with an IC_{50} value of 238 μg/ml. The inhibitory titer of shoseiryuto was much lower than that of grapefruit juice, and it is predicted that shoseiryuto could not pharmacokinetically interact interaction with fexofenadine through OATP1A2. Among the eight herbal components of shoseiryuto, the extracts of the terrestrial stem of *Ephedra sinica* and the root and stolon of *Glycyrrhiza uralensis* inhibited fexofenadine uptake with IC_{50} values of 102 and 89 μg/ml, respectively. We isolated catechin as the active ingredient from the extract of the terrestrial stem of *E. sinica*. Catechins inhibited the uptake of fexofenadine in a concentration-dependent manner, and the IC_{50} values of epicatechin (EC) gallate, epigallocatechin (EGC) gallate, EGC, catechin, and EC were 11, 26, 41, 52, and 96 μM, respectively. **Conclusion:** These results would contribute to the safe and effective use of shoseiryuto in clinics.

Key words: Catechins, fexofenadine, herb-drug interaction, Japanese traditional Kampo medicine, organic-anion-transporting peptide, shoseiryuto

SUMMARY

- Regular dosage of shoseuryuto does not cause drug interaction through organic-anion-transporting polypeptide 1A2 in clinics.



Abbreviations used: CYP: Cytochrome P450; JPXVII: The 17th Edition of the Japanese Pharmacopoeia; EC: Epicatechin; ECG: Epicatechin gallate; EGC: Epigallocatechin; EGCG: Epigallocatechin gallate; HBSS: Hanks' balanced salt solution; HEK: Human embryonic kidney; OATP: Organic-anion-transporting polypeptide; PBS: Phosphate-buffered saline; PHB: p-hydroxybenzoic acid butyl ester.

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INTRODUCTION

Shoseiryuto is an herbal formula in Japanese traditional Kampo medicine. It is used to treat acute upper respiratory inflammation, bronchitis, bronchiectasis, pulmonary edema and allergic rhinitis in Japan.[1] A full-scale, national, large randomized clinical trial demonstrated the efficacy, and safety of shoseiryuto in the treatment of perennial nasal allergy.[2] According to the 17th Edition of the Japanese Pharmacopoeia (JPXVII),[3] the daily dosage of shoseiryuto for humans comprises the extract of the mixture of eight crude drugs: 3.0 g each of ephedra herb (dried terrestrial stem of *Ephedra sinica*), cinnamon bark (dried bark of the trunk of *Cinnamomum cassia*), peony root (root of *Paeonia lactiflora*), processed ginger (dried rhizome of *Zingiber officinale* after steaming), asiasarum root (dried root of *Asarum sieboldii*), glycyrrhiza (dried root and stolon of *Glycyrrhiza uralensis*), schisandra fruit (dried fruit of *Schisandra chinensis*), and 6.0 g of pinellia tuber (dried tuber of *Pinellia ternata*).

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Recently, the drug information between herbal medicines and other pharmaceutical drugs is needed in the clinics.[4] In Japan, using Japanese traditional Kampo medicines becomes popular in general clinics, and Kampo medicines are well combined with Western medicines. Grapefruit juice inhibits not only CYP3A4 through its component furanocoumarins to augment the absorption of several drugs that are metabolized by this enzyme,[5] but it inhibits organic-anion-transporting polypeptide (OATP) 1A2 to reduce the absorption of fexofenadine.[6-8] Bailey *et al*. found that naringin, flavonoid glycosides contained in grapefruit juice, can inhibit OATP1A2.[9] Flavonoids are widely distributed in various plant species as secondary metabolites and some crude drugs used in Kampo medicines contain flavonoid glycosides.[10,11] It is possible that Kampo medicines could inhibit the activity of OATP1A2 expressed in the intestinal epithelium, thereby reducing the blood concentrations of other drugs concomitantly prescribed with Kampo formulas in integrative medicine.

Since fexofenadine is an anti-allergy drug frequently used in the treatment of rhinitis – particularly in case of pollinosis – in Japan, there is a demand for drug information regarding the interaction between this drug and shoseiryuto, which is also frequently used to treat rhinitis and pollinosis. In the present study, we evaluated the inhibitory effects of shoseiryuto extracts on the uptake of fexofenadine into human embryonic kidney 293 (HEK293) cells stably expressing OATP1A2 and attempted to identify the active ingredients to allow prediction of potential interaction between shoseiryuto and fexofenadine.

MATERIALS AND METHODS

Preparation for the extracts of shoseiryuto and each component

The shoseiryuto extract used in this study was the same as that used in our previous study,[12] which also showed a fingerprint pattern. Asiasarum root (lot #, 5G08M), glycyrrhiza (#1I10), cinnamon bark (#9H04M), ephedra herb (#9D03M), pinellia tuber (#8I04M), and peony root (#0C27M) were purchased from Daiko Shoyaku (Nagoya, Japan) and processed ginger (#25034831) and schisandra fruit (#23021581) were obtained from Tsumura (Tokyo, Japan). These crude drugs met the grade standards of JPXVII,[3] and were used as small pieces prepared by cutting the whole crude drugs. Voucher specimens were deposited in the Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University. Each 5 g of crude drug was boiled in 150 ml of distilled water for 30 min, filtered and lyophilized to yield the extract. The extract ratios yielded were 12% for asiasarum root, 27% for glycyrrhiza, 9% for cinnamon bark, 15% for ephedra herb, 40% for pinellia tuber and 32% of peony root and 14% for processed ginger and 37% for schisandra fruit. Each extract was suspended in distilled water at a concentration of 100 mg/ml and maintained at −20°C until use.

Fractionation of ephedra herb

Ephedra herb (200 g, lot # 0I15M, Daiko) was boiled in 3 L of distilled water for 30 min, filtered and lyophilized to yield the extract (35 g, extract ratio yielded was 18%). The extract was suspended in water, partitioned with ethyl acetate and water-saturated butanol and the ethyl acetate (1.2 g, 3.4%), butanol (5.3 g, 15%), and water (30 g, 86%) fractions were yielded. Since the ethyl acetate fraction had an inhibitory effect on fexofenadine uptake, this fraction (1.0 g) was subjected to silica gel open column chromatography (3.3 cm × 30 cm) eluted with hexane/acetone/acetic acid 6:4:1, 5:5:1, 3:7:0, and 0:10:0, stepwise and fractions 1–10 were yielded. Since the fraction 6 (0.20 g) had activity, this fraction (0.15 g) was subjected to preparative silica gel thin layer chromatography expanded with chloroform/acetone 4:6 and the spot named compound 6-2 (35 mg), which had an Rf value of 0.59, was collected as the active ingredient. By the comparison of the ^{1}H-NMR spectrum and optical rotation data to those of the authentic compound (Nacalai Tesque, Kyoto, Japan), compound 6-2 was identified as (+) catechin.

Grapefruit juice and nagingin content

Grapefruit juice (200 ml, Lot # EHMF03, Megmilk Snow Brand, Tokyo, Japan) was purchased from a supermarket in Nagoya City, and naringin was obtained from Sigma-Aldrich (St. Louis, MO, USA). A 10 ml aliquot of the juice was diluted with water and lyophilized to determine the concentration (w/w %). The concentration of naringin in this juice was measured using a high-performance liquid chromatography system consisting of a Shimadzu SPD-M10A$_{VP}$ with photodiode array detector (Shimadzu, Kyoto, Japan), under the following conditions: column, Inertsil ODS-3 (4.6 mm × 150 mm, GL Science, Tokyo, Japan); mobile phase, 30% acetonitrile containing 0.7% of formic acid; flow rate, 1.2 ml/min; column temperature, 40°C; detection, 285 nm; and retention time of naringin, 18.0 min. Linear regression of the concentration range of naringin (300 ng–3 μg in 10 μl) was calibrated by the peak area using the least-squares method ($r^2 > 0.999$).

Uptake study of fexofenadine through organic-anion-transporting polypeptide 1A2

Uptake experiments were conducted according to our previous study[13] except for the kind of transporters and its substrate. In brief, we developed HEK293 cells stably expressing OATP1A2 and were maintained in Dulbecco's Eagle medium containing 10% fetal bovine serum and 250 μg/ml of hygromycin B (Nacalai Tesque, Kyoto, Japan). The cells were seeded onto poly-L-lysine (Sigma)-coated 24-well plates (2.0×10^5 cells/well) and were incubated for 2 days. After pre-incubation of the cells for 15 min at 37°C with 0.22 ml of Hanks' balanced salt solution (HBSS; pH 7.2), they were incubated with 0.22 ml of HBSS containing 25 μM fexofenadine (Tokyo Chemical Industry, Tokyo, Japan) with or without the samples for 10 min at 37°C. The extracts of shoseiryuto or its components, catechin, epicatechin (EC, Nacalai), EC gallate (ECG, Nacalai), epigallocatechin (EGC, Nacalai) and EGC gallate (EGCG, LKT Laboratories, St. Paul, MN, USA) were used as samples and grapefruit juice or naringin were used as a positive control. After the cells were washed three times with ice-cold phosphate-buffered saline with Ca^{2+} and Mg^{2+} (PBS; 0.15 M, pH 7.2), they were incubated in 100 μl of ethanol containing 5 μg/ml p-hydroxybenzoic acid butyl ester (PHB, Nacalai) used as an internal standard and 1% acetic acid for 10 min at room temperature. The ethanol extracts of the cells were mixed with 100 μl water and the concentration of fexofenadine was measured by LC-MS/MS (Waters Quattro Premier XE, Milford, MA, USA) with an electrospray ionization source in the positive ion mode and multiple reaction monitoring under the following conditions: column, Inertsil ODS-4 (2.1 mm × 75 mm; GL Science); mobile phase, a linear gradient elution system, 0.05% acetic acid in water (solvent A): 0.05% acetic acid in acetonitrile (solvent B) (A/B) =90/10-80/20 for 0–0.5 min; 80/20-80/20 for 0.5–1 min; 80/20-20/80 for 1–2.8 min; 20/80-90/10 for 2.8–2.9 min at a flow rate of 0.23 ml/min. Both quadrupoles were maintained at the unit resolution, and the transitions (precursor to daughter) monitored were 502.3–171.3 m/z for fexofenadine (retention time, 2.0 min) and 195.2–139.0 m/z for PHB (2.6 min). Linear regression of the concentration range of fexofenadine (16 nM–1.0 μM) was calibrated by the peak area ratio of these compounds to PHB using the least squares method ($r^2 > 0.98$). The remaining cells were incubated with 0.15 ml of 1 M NaOH overnight at room temperature. After the addition of 0.15 ml of 1 M HCl, the protein content of the solutions was measured using a BCA™ Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). The uptake amount of fexofenadine into the cells was normalized by the amount of protein

and the uptake by OATP1A2, expressed as a percentage of the control for uptake, was calculated as follows: uptake of fexofenadine (% of control) = ([uptake into sample-added HEK293-OATP1A2 cells]–[uptake into mock cells])/([uptake into control HEK293-OATP1A2 cells]–[uptake into mock cells]) ×100. The half maximal inhibitory concentration (IC_{50}) was calculated using the logarithmic regression of the concentration and % of control uptake made from three points that crossed 50% of control values using the least squares method.

Measurement of catechins in ephedra herb extract

The concentrations of catechins in ephedra herb extract were measured by LC-MS/MS with an electrospray ionization source in the negative ion mode and multiple reaction monitoring using baicalin (final concentration, 5 μg/ml, Wako) as an internal standard, under the following conditions: column, Inertsil ODS-4 (2.1 mm × 75 mm); mobile phase, a linear gradient elution system, solvent A/solvent B = 90/10-70/30 for 0–8 min; 70/30-70/30 for 8–9 min at a flow rate of 0.2 ml/min. Both quadrupoles were maintained at the unit resolution and the transitions (precursor to daughter) monitored were 288.9–245.1 m/z for catechin (retention time, 3.9 min), 288.9–245.1 m/z for EC (4.6 min), 305.2–124.8 m/z for EGC (3.2 min), 441.1–168.9 m/z for ECG (6.7 min), 457.0–124.9 m/z for EGCG (5.0 min), and 445.1–269.1 m/z for baicalin (8.7 min). Linear regressions of the concentration range of the catechins (5–80 μg/ml) were calibrated by the peak area ratio of these compounds to baicalin using the least squares method ($r^2 > 0.98$).

Statistics

Statistical analysis was carried out by one-way analysis of variance and Dunnett's multiple comparison *t*-test using PASW Statistics software (version 18, SPSS; IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

We constructed HEK293 cells stably expressing human OATP1A2. We first confirmed the functionality of the HEK293-OATP1A2 cells by measuring the uptake of fexofenadine and assessing the inhibitory effects of grapefruit juice and naringin, which were used as a representative substrate and inhibitor of OATP1A2, respectively. The grapefruit juice used in the present study was 0.10 dried weight g/ml, and the naringin concentration was 5.1 mg/ml. The uptake of fexofenadine into HEK293-OATP1A2 cells was markedly greater than that into mock cells and was significantly suppressed by grapefruit juice in a concentration-dependent manner, with an IC_{50} value of 1:106 dilution rate (1.0 mg dried weight of grapefruit juice/ml), the concentration of which was comparable to 48 μg/ml naringin. Naringin significantly inhibited the uptake of fexofenadine via OATP1A2 to approximately 59% of control values at 20 μg/ml [Figure 1a]. These results suggest that naringin may be a great contributor to the inhibitory effects of grapefruit juice on OATP1A2. In this regard, while many flavonoid glycosides found in fruit juices have inhibitory effects on OATP2B1, most of the inhibitory effects of grapefruit juice on OATP2B1 appear to be medicated by naringin.[14] In clinical studies, the intake of grapefruit juice (300 ml) reduced the area under the plasma concentration (0–∞) for fexofenadine to approximately 64% of the control value,[7] and 1:200-diluted grapefruit juice inhibited the uptake of fexofenadine via OATP1A2 to approximately 64% of the control value in the present study. We suggest that to extrapolate the *in vitro* result here to the clinical context with respect to the inhibition of OATP1A2, the dilution rate of 1:200 can be a useful conversion factor. The concentration of naringin in grapefruit juice varies greatly depending on the area of cultivation or season and has ranged from 0.17 to 6.5 mM.[15] The grapefruit juice used in the present study contained 5.1 mg/ml (=8.7 mM) of naringin, which was a slightly higher concentration than in the previous studies. Therefore, the dilution rate of 1:200 calculated using data obtained from experiments employing this particular grapefruit juice may be a relatively extreme value compared to what may have been observed with common grapefruit juice.

Shoseiryuto extract inhibited fexofenadine uptake through OATP1A2 in a concentration-dependent manner, with an IC_{50} value of 238 μg/ml [Figure 1b]. The one-time dosage of shoseiryuto extract in human is approximately 1.9 g (three times a day). When a patient takes this dose in a cup of water (100 ml), the concentration is 19 mg/ml; when this solution is diluted by 1:200, the final concentration is 95 μg/ml. Since the lowest concentration that appeared to have a significant inhibitory effect on OATP1A2 was 200 μg/ml in the present study, shoseiryuto extract may not inhibit the absorption of fexofenadine in a clinical context except in the cases of excessive dosage. Patients can, therefore, use the regular dosages of fexofenadine and shoseiryuto at the same time with the expectation of a synergistic effect of these two drugs on the prevention of rhinitis.

We evaluated the inhibitory effects of the eight crude drugs that comprise shoseiryuto on OATP1A2. Among these, the extracts of ephedra herb, glycyrrhiza, cinnamon bark, asiasarum root, and peony root had significant inhibitory effects on the uptake of fexofenadine via OAPT1A2

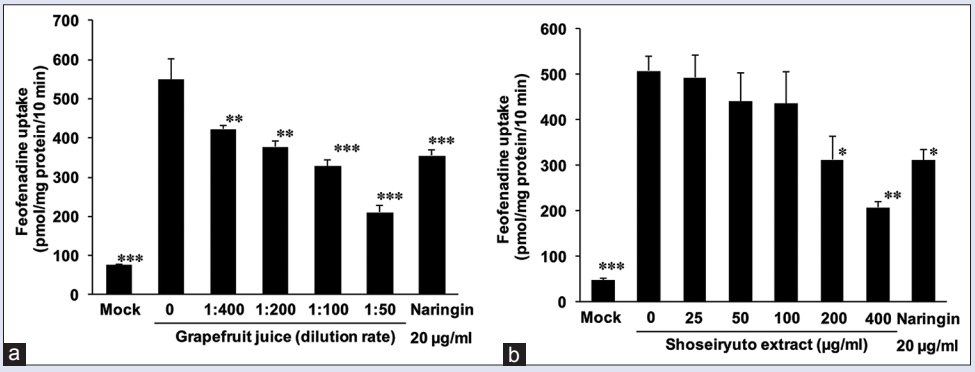

Figure 1: Effects of grapefruit juice (a) and shoseiryuto extract (b) on organic-anion-transporting polypeptide 1A2-mediated fexofenadine uptake. Human embryonic kidney 293 cells transfected with mock plasmid or organic-anion-transporting polypeptide 1A2 were treated with fexofenadine (25 μM) with or without grapefruit juice or shoseiryuto extract for 10 min and the uptake of fexofenadine was measured. Each column represents the mean ± standard error ($n = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus the group without grapefruit juice or shoseiryuto assessed by one-way analysis of variance and Dunnett's multiple comparison *t*-test

at 50 μg/ml [Table 1]. We then evaluated the concentration-dependent inhibitory effects of the two most effective crude drug extracts, ephedra herb, and glycyrrhiza. These extracts inhibited the uptake of fexofenadine through OATP1A2 in a concentration-dependent manner, with IC_{50} values of 102 and 89 μg/ml, respectively [Figure 2a and b]. The one-time dosages of the extracts of ephedra herb and glycyrrhiza in human are about 0.15 and 0.27 g (three times a day), respectively. Using the calculations mentioned above, these extracts may not inhibit the absorption of fexofenadine in a clinical context except in cases of excessive dosage.

We attempted to isolate the active ingredients responsible for the inhibition of fexofenadine uptake through OATP1A2 from the extract of ephedra herb that was most effective in this regard. When this was partitioned into ethyl acetate, butanol, and water fractions, the inhibition titers at the concentrations comparable to 400 μg/ml of the original ephedra herb extract (23% ± 6% of control) were 35% ± 6%, 95% ± 17%, and 61% ± 16% of the control, respectively, suggesting that the most of the active ingredients in the ephedra herb extract had moved to the ethyl acetate fraction. It is possible that flavonoid glycosides such as herbacetin 7-*O*-glucoside, pollenitin B, vicenin-2, and isovitexin 2-*O*-rhamnoside[16,17] may not contribute to the inhibitory effect of ephedra herb on OATP1A2 because these flavonoid glycosides usually move to butanol fraction. By the activity-guided fractionation using silica gel chromatography, 35 mg of catechin was isolated from 1.0 g of the ethyl acetate fraction. LC-MS/MS analysis revealed that the extract of ephedra herb contained 0.23% (w/w) of catechin, 0.014% (w/w) of EC, and 0.039% (w/w) of EGC, while ECG and EGCG were not detected.

Table 1: Effects of the extract of each single crude drug component of shoseiryuto on OATP1A2-mediated fexofenadine uptake

Crude drug	Percentage of control
Ephedra herb	52±2***
Cinnamon bark	63±3***
Peony root	74±4***
Processed ginger	115±12
Asiasarum root	64±2***
Glycyrrhiza	53±1***
Schisandra fruit	109±6
Pinellia tuber	123±15

Human embryonic kidney 293 cells transfected with mock plasmid or organic-anion-transporting polypeptide 1A2 were treated with fexofenadine (25 μM) and each crude drug extract (50 μg/ml) for 10 min and uptake was measured. Data are expressed as the percentage of control; that is, the ratio of (the uptake of sample-treated cells-that of mock cells) to (that of control cells-that of mock cells) and as mean ± standard error (n=3). ***P<0.001 versus control group without shoseiryuto as assessed by one-way analysis of variance and Dunnet's multiple comparison t-test

Figure 3 shows the inhibitory effects of the catechins on the uptake of fexofenadine through OATP1A2. All of the catechins evaluated inhibited fexofenadine uptake in a dose-dependent manner, and the IC_{50} values were 52 μM for catechin, 96 μM for EC, 11 μM for ECG, 41 μM for EGC, and 26 μM for EGCG. Roth *et al.* demonstrated the inhibitory effect of catechins on OATP1A2 using estrone 3-sulfate as a substrate, and the IC_{50} values were 10 μM for ECG and 55 μM for EGCG; EC and EGC did not exhibit a significant inhibitory effect at 100 μM.[18] Abe *et al.* described that the IC_{50} values of EGCG on OATP1A2 using sulfobromophthalein as a substrate was 19 μM.[19] Although the order of the inhibitory titer on OATP1A2 among the catechins was the same, fexofenadine is more sensitive than estrone 3-sulfate and has similar titer to sulfobromophthalein, that are the representative substrate of OATP1A2. In another study, we measured the IC_{50} values of EGCG, ECG, (+)-catechin, EC and EGC on OATP2B1, another related transporter expressed in intestinal epithelium, using estrone 3-sulfate as 7.1 μM, 14 μM, 57 μM, 0.11 mM, and more than 0.52 mM, respectively.[13] The inhibitory titers of (+)-catechin and EC on OATP1A2 and 2B1 exhibited similar values, however, the inhibitory tier of EGCG on OATP1A2 was weaker than that on OATP2B1 and those of ECG and EGC on OATP1A2 were stronger than those on OATP2B1. Those differences may depend on the affinities of catechins on both transporters, and further investigation is required.

Comparison between the IC_{50} values of ephedra herb extract or the catechins and the catechins' contents in the extract shows that catechin, EC and EGC contributed only approximately 6.6%, 0.17%, and 1.1% of the inhibitory effects of ephedra herb extract on fexofenadine uptake through OATP1A2. Therefore, this extract contains other active ingredients that moved to the ethyl acetate fraction, other than the catechins, that have an inhibitory effect on OATP1A2.

CONCLUSION

The present study provides drug information about interactions between shoseiryuto and fexofenadine, both of which are frequently used for the treatment of allergic rhinitis. Although the present study focused on *in vitro* experimentation, it is predicted that the possibility of a pharmacokinetic drug interaction occurring between shoseiryuto and fexofenadine would be low and that the patients can use the two drugs

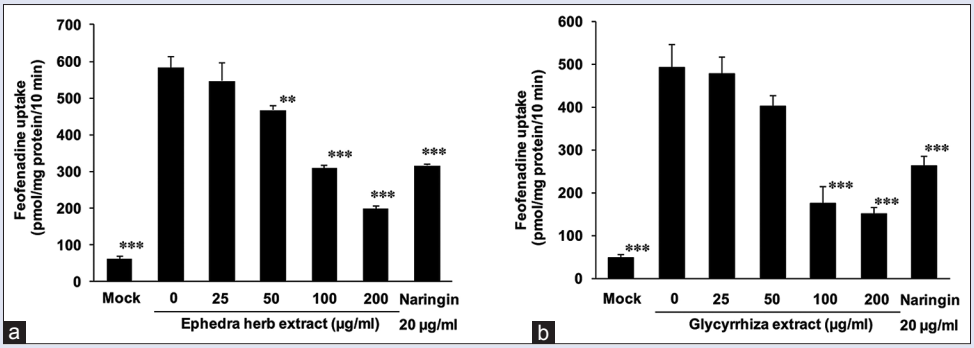

Figure 2: Effects of the extracts of ephedra herb (a) and glycyrrhiza (b) on organic-anion-transporting polypeptide 1A2-mediated fexofenadine uptake. Human embryonic kidney 293 cells transfected with mock plasmid or organic-anion-transporting polypeptide 1A2 were treated with fexofenadine (25 μM) with or without the extracts for 10 min and the uptake of fexofenadine was measured. Each column represents the mean ± standard error (n = 3). **P < 0.01, ***P < 0.001 versus the group without samples as assessed by one-way analysis of variance and Dunnett's multiple comparison t-test

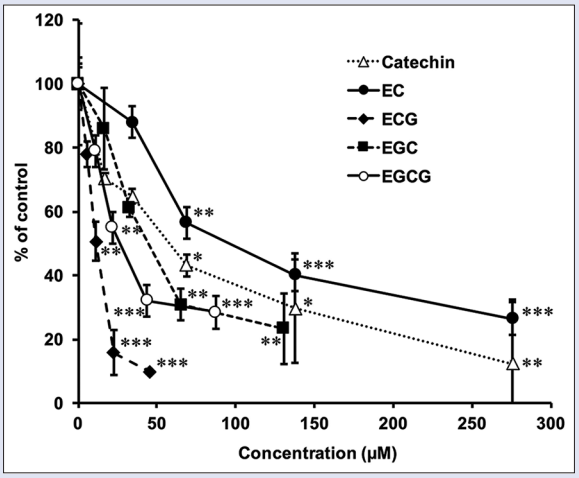

Figure 3: Effects of catechins on organic-anion-transporting polypeptide 1A2-mediated fexofenadine uptake. Human embryonic kidney 293 cells transfected with mock plasmid or organic-anion-transporting polypeptide 1A2 were treated with fexofenadine (25 μM) with or without catechin, epicatechin, epicatechin gallate, epigallocatechin, or epigallocatechin gallate for 10 min and the uptake of fexofenadine was measured. Each data point represents the mean ± standard error (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 vs. each group without catechins as assessed by one-way analysis of variance and Dunnett's multiple comparison t-test

simultaneously to have a synergistic effect on the prevention of allergic rhinitis.

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Conflicts of interest

Takashi Matsumoto and Junko Watanabe employed by Tsumura and Co. Toshiaki Makino received the research grants from Tsumura and Co, Kobayashi Pharmaceutical Co. Ltd., JPS Pharmaceutical Co. Ltd., and Kuki Sangyo Co.

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