Original Article

Therapeutic effects of isoflavones on impaired salivary secretion

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Dry mouth, which is characterized by decreased salivation, has a number of causes; the involvement of estrogen has been suggested as symptoms typically develop in middle-aged females. However, there is a lack of consensus regarding the treatment of this condition. Soy isoflavones, a subgroup of flavonoids, are abundantly found in the soy germ. They are thought to exert a number of effects by specifically binding to estrogen receptors due to their structural similarity to estrogen. Recently, soy isoflavones have been found to exert antioxidant effects, ameliorating disorders caused by reactive oxygen/free radicals. Based on these observations, the effects of soybean isoflavones on impaired salivary secretion were studied in patients with dry mouth. Soy isoflavone aglycones were administered at 25 mg per day to 15 subjects with an average age of 67.9 ± 8.0 years for 2 months, and salivary secretion was analyzed. The results showed a significant improvement based on the saliva flow rate and self-completed questionnaire, thus suggesting the usefulness of isoflavones in improving the symptoms of salivary gland hypofunction.

***Key Words*: isoflavones, dry mouth, estrogen, salivary secretion, reactive oxygen species**

Studies have shown that dry mouth is a multi-factorial disease in which the saliva flow decreases, thereby resulting in not only mouth dryness, feelings of thirst, and difficulty in food ingestion but also dysgeusia, dental caries, periodontal disease, burning mouth, and reduced quality of life (QOL).[1] Although the pathogenesis of this disease is not yet fully elucidated, its incidence increases with age. The disease has been reported to be more common in postmenopausal women.[2] In addition, an *in vivo* mouse experiment demonstrated that ovariectomy resulted in decreased salivary secretion and that estrogen replacement ameliorated this decrease.[3] Although feelings of thirst have been reported to improve upon estrogen replacement therapy (ERT) in patients with menopausal disorders,[4] an alternative method to ERT has been sought due to concerns of the Women's Health Initiative (WHI) regarding the link between ERT and tumorigenesis.[5]

In recent years, oxidative stress has been proposed to play a role in accelerated aging[6] and in the pathogenesis of Sjogren's syndrome (SS), which is characterized by severe mouth dryness in middle-aged females.[7] Particular oxygen radicals, such as superoxides and peroxides, mediate apoptosis,[8] thus causing oxidative damage to membrane lipids and proteins and reducing their function.[9] Furthermore, pathophysiological mechanisms involving oxidative damage have been reported.[10,11]

In addition, the various physiological effects of soybeans and a variety of soybean bioactive compounds have been investigated. Specifically, research on soy isoflavones has been undertaken, and estrogenic effects on bone metabolism and anti-cancer activity have been reported.[12–15]

Isoflavones are a subclass of flavonoids that are abundantly found in the pulse family, including soybeans, soy foods, and Japanese arrowroot, and exist in the glycoside or aglycone form. A total of 12 types of soy isoflavones are contained in soybean and soy germ (hypocotyl), including the aglycones (genistein, daidzein, and glycitein), three types of glycosides, and their acetyl and malonyl glycosides.[16] Although many aglycones are contained in fermented soybean products, such as miso and fermented soybeans, the isoflavones found in soybean or soybean products are typically glycosides. After ingestion, isoflavone glycosides are hydrolyzed by salivary enzymes,[17] enzymes found in the small intestinal mucosa,[18] and β-glucosidases from *Enterobacteriaceae*, thereby generating isoflavone aglycones[19,20] that are absorbed in the intestine to exert their effects.

Although the binding capacity of aglycones to estrogen receptors (ERs) differs according to the test method employed, it is reported to be weaker than that of estrogen.[21,22] Because of structural similarities to estrogen, aglycones specifically combine with ERs (α and β) and demonstrate estrogenic effects.[23,24] ER-α, which is abundant in the female genitalia, is also abundant in mammary glands, the hypothalamus, endothelial cells, and vascular smooth muscle. ER-β is abundant in the prostate gland, ovary, lung, brain, blood vessels, bone,[25] and salivary glands.[26–28]

In addition, isoflavones exhibit antioxidative properties and ameliorating effects against free radical-induced damage of cell membrane lipids, lipoproteins,[29–33] and DNA.[34–37] In this study, the effects of soy isoflavones on dry mouth patients are examined.

Materials and Methods

Evaluation of isoflavone effects in subjects. Tablets containing 25 mg of isoflavones extracted from soy (aglycone equivalent, 23.6 mg) were provided by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Fifteen patients of the Dry Mouth Clinic of Tsurumi University Dental Hospital were enrolled in this study. The subjects included one male and fourteen females, ranging in age from 49 to 81 years (67.9 ± 8.0 years; mean ± SD). All fifteen subjects with dry mouth had a salivary secretion of less than 10 ml, as determined by the stimulated saliva flow rate (gum test). Ten patients were assigned to the SS group, and the remaining five to the non-SS group (Table 1). The diagnosis of SS was based on the diagnostic criteria.[38] Patients who had a medical history of significant cardiovascular disease, significant pulmonary obstructive disease, gastrointestinal obstructive disease, epilepsy, or

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Table 1. Characteristics for the subjects in this study

	SS	Non-SS
Number of Subjects	10	5
Sex (male/female)	1/9	0/5
Age	66.9 ± 9.4	69.8 ± 4.6
Gum test (ml/10 min)	3.59 ± 2.31	6.28 ± 2.05
Saxon test (g/2 min)	1.84 ± 1.56	2.01 ± 1.53

Values represent the mean ± SD. SS, Sjogren's syndrome; non-SS, dry mouth group.

Parkinson's disease were excluded from the study. The enrolled subjects had subjective dry mouth that was attributable to various factors (e.g., SS, adverse medication effects, mental stress, and depression). All 15 subjects provided informed consent. Each participant consumed one test tablet per day. The evaluation of the effects was performed by measuring salivary secretion and by a self-completed questionnaire (15 questions about oral and eye conditions) before intake and after 1 and 2 months of isoflavone intake.

Preparation of saliva. Tests were performed before intake and 1 and 2 months after oral intake of the isoflavone tablet. A piece of sterilized gauze was weighed before and after being chewed by a participant for 2 min (Saxon test). The difference between the two measurements (dry weight before chewing and wet weight after chewing) was regarded as the salivary secretion. The saliva samples were centrifuged at 10,000 rpm for 30 min and then passed through an ultrafiltration membrane (pore size, 0.22 μm).

Measurement of oxidative stress. The filtered samples were subjected to an enzyme-linked immunosorbent assay for the measurement of the oxidative stress marker 8-hydroxy-2'-deoxyguanosine (8-OHdG) and the lipid peroxidation markers hexanoyl-lysine (HEL) and propanoyl-lysine (PRL) using an anti-8-OHdG monoclonal antibody (N45.1; Institute for the Control of Aging, Shizuoka, Japan), an anti-HEL monoclonal antibody (Institute for the Control of Aging), and an anti-PRL monoclonal antibody (Healthcare Systems Inc., Aichi, Japan), respectively. The marker 8-OHdG in the sample was analyzed by an antibody chip[39] that was developed by Healthcare Systems Inc. As an antigen, 8-OHdG-BSA was spotted and immobilized onto the chip. The sample was then applied to the chip with a specific monoclonal antibody against 8-OHdG.[40] The chip was further treated with anti-mouse immunoglobulin antibody alkaline phosphatase labelled. The binding of the antibody was evaluated by chemiluminescence using CDP-Star. The amount of 8-OHdG was estimated by comparison with the standard curve of authentic 8-OHdG. HEL and PRL were tested in the same manner as 8-OHdG.

Measurement of isoflavones. The frozen saliva samples were thawed on ice. Three aliquots of saliva were transferred to sample tubes. The internal standard solution (genistein-d_4, daidzein-d_4, and equol-d_4) was added to each saliva sample (400 μl) to obtain a concentration of 125 pmol/ml. After the addition of ascorbic acid (50 μl of 0.1% phosphoric acid), the samples were hydrolyzed in the presence of the enzyme (60 μg) in phosphate buffer (pH 5.3, 500 μl) for 2 h at 37°C. After hydrolysis, the isoflavones were extracted with ethyl acetate (1 ml) twice and concentrated using a centrifugal evaporator. The dried extract was redissolved in 20 μl of 20% acetonitrile containing 0.1% acetic acid. A portion (5 μl) of the solution was subjected to LC-MS/MS analysis. An HPLC system (SI-2, Shiseido, Tokyo, Japan) connected to a quadruple MS/MS system API 4000 Qtrap (AB SCIEX, Santa Clara, CA) was used, and data acquisition and mass spectrometric evaluation were conducted using Analyst 1.5.1 software (AB SCIEX). The HPLC gradient conditions were as follows: the ratio of methanol containing 0.1% acetic acid (solution B) was increased linearly against the 0.1% acetic acid (solution A) after 4 min from 20% to 60% over 11 min and then to 90% over 5 min with a flow rate of 0.3 ml/min on a Zorbax Eclipse XDB column (2.1 × 150 mm, 5 μm, Agilent Technologies, city, CA) at 40°C. Selected reaction monitoring (SRM) was used to perform mass spectrometric quantification of isoflavones (precursor ion to product ion transitions from *m/z* 241/119 for equol, *m/z* 245/123 for equol-d_4, *m/z* 253/133 for daidzein, *m/z* 257/136 for daidzein-d_4, *m/z* 269/133 for genistein, and *m/z* 273/136 for genistein-d_4). The column eluent was introduced into the mass spectrometer using electrospray ionization in the negative-ion mode with a declustering potential of −90 V and ion spray voltage of −4,400 V. The temperature of the gas was 500°C. Nitrogen was used as the collision gas.

Statistical analysis. The results are expressed as the mean ± SD. Two-way repeated measures ANOVA was performed to test for the main effects of group ("SS" or "non-SS"), time ("before intake" and "after intake"), and their interaction. These analyses were performed using IBM SPSS (Statistical Package for the Social Sciences) Statistics ver. 19 (IBM Japan Inc., Tokyo, Japan). The data were analyzed for statistical significance, and the significance level was set at $p<0.05$.

Ethics. Informed consent was obtained from all subjects, and the Ethical Committee of Tsurumi University approved this study.

Results

Saliva flow rate. The saliva flow rate results are shown in Table 2. The Saxon test results showed a significant increase ($p = 0.005$) after 1 month (2.47 ± 1.66 g) and after 2 months (2.34 ± 1.65 g) compared with before intake (1.90 ± 1.50 g). No significant difference was found between the SS and non-SS groups.

Oxidative stress markers. The oxidative stress results for the 8-OHdG, HEL, and PRL levels are shown in Table 3. No significant differences were found among the measured data. For 8-OHdG, the comparison of the amount before intake (6.12 ± 7.79 ng/ml) with the amounts after 1 month (3.82 ± 3.21 ng/ml) and 2 months (3.87 ± 3.48 ng/ml) revealed an insignificant decrease. Meanwhile, HEL showed an insignificant decrease when the amount before intake (7.12 ± 8.35 ng/ml) was compared with that after 1 month (4.53 ± 5.83 ng/ml) or 2 months (4.71 ± 4.43 ng/ml). In general, the levels of 8-OHdG and HEL tended to decrease following intake. In contrast, for PRL, the comparison of the amount before intake (5.61 ± 9.92 ng/ml) with the amounts 1 month (8.86 ± 14.20 ng/ml) and 2 months (8.21 ± 13.36 ng/ml) after intake revealed an insignificant increase.

Table 2. Score for saliva flow rate pre- and post-intake of isoflavones for dry mouth patients

Item		Before	After 1 month	After 2 months	Results of two-way repeated measures ANOVA				
					Source of variation	SS (Type III)	DF	MF	F	*p* value
Saxon	Total	1.90 ± 1.50	2.47 ± 1.66	2.34 ± 1.65	Time	3.779	2.000	1.890	6.529	0.005*
	SS	1.84 ± 1.56	2.10 ± 1.59	2.10 ± 1.72	Time × SS or non-SS	1.495	2.000	0.747	2.583	0.095
	non-SS	2.01 ± 1.54	3.20 ± 1.71	2.84 ± 1.55	SS or non-SS	4.507	1.000	4.507	0.619	0.445

Results of two-way repeated measures ANOVA. Values represent the mean ± SD. Total, all subjects; SS, Sjogren's syndrome group; non-SS, dry mouth group; *Time, $p < 0.05$.

Table 3. Score for detection of oxidative stress in saliva pre- and post-intake of isoflavones for dry mouth patients

Item		Before	After 1 month	After 2 months	Results of two-way repeated measures ANOVA				
					Source of variation	SS (Type III)	DF	MF	F	*p* value
8-OHdG (ng/ml)	Total	6.12 ± 7.79	3.82 ± 3.21	3.87 ± 3.48	Time	61.042	2.000	30.521	1.628	0.216
	SS	5.77 ± 7.03	4.19 ± 3.07	4.16 ± 3.79	Time × SS or non-SS	9.474	2.000	4.737	0.253	0.779
	non-SS	6.83 ± 10.03	3.07 ± 3.73	3.29 ± 3.06	SS or non-SS	0.961	1.000	0.961	0.019	0.893
HEL (nmol/L)	Total	7.12 ± 8.35	4.53 ± 5.83	4.71 ± 4.43	Time	40.953	2.000	20.476	1.522	0.237
	SS	7.19 ± 9.11	3.79 ± 3.46	4.21 ± 3.46	Time × SS or non-SS	10.621	2.000	5.310	0.395	0.678
	non-SS	6.96 ± 7.56	6.01 ± 9.37	5.72 ± 6.32	SS or non-SS	13.617	1.000	13.617	0.131	0.723
PRL (nmol/L)	Total	5.61 ± 9.92	8.86 ± 14.20	8.21 ± 13.36	Time	42.960	2.000	21.480	0.280	0.758
	SS	7.82 ± 11.66	12.82 ± 16.17	11.73 ± 15.35	Time × SS or non-SS	49.170	2.000	24.585	0.320	0.729
	non-SS	0.35 ± 0.55	0.64 ± 1.21	0.31 ± 0.64	SS or non-SS	938.030	1.000	938.029	3.282	0.093

Results of two-way repeated measures ANOVA. Values represent the mean ± SD. Total, all subjects; SS, Sjogren's syndrome group; non-SS, dry mouth group; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; HEL, hexanoyl-lysine; PRL, propanoyl-lysine.

Table 4. Score for isoflavone concentrations in saliva pre- and post-intake of isoflavones for dry mouth patients

Item		Before	After 1 month	After 2 months	Results of two-way repeated measures ANOVA				
					Source of variation	SS (Type III)	DF	MF	F	*p* value
Equol (μmol)	Total	0.27 ± 0.45	0.48 ± 1.00	0.23 ± 0.53	Time	1.148	2.000	0.574	0.418	0.664
	SS	0.35 ± 0.55	0.64 ± 1.2	0.31 ± 0.64	Time × SS or non-SS	1.278	2.000	0.639	0.465	0.634
	non-SS	0.13 ± 0.05	0.18 ± 0.10	0.07 ± 0.05	SS or non-SS	0.131	1.000	0.131	0.086	0.774
Daidzein (μmol)	Total	32 ± 54	11 ± 9.8	45 ± 55	Time	8775.315	2.000	4387.658	3.452	0.047*
	SS	41 ± 65	12 ± 11	40 ± 55	Time × SS or non-SS	2960.434	2.000	1480.217	1.165	0.328
	non-SS	12 ± 18	9.7 ± 7.4	54 ± 61	SS or non-SS	383.663	1.000	383.663	0.102	0.755
Genistein (μmol)	Total	47 ± 70	20 ± 22	64 ± 72	Time	14901.865	2.000	7450.932	3.861	0.034*
	SS	63 ± 82	22 ± 25	59 ± 69	Time × SS or non-SS	6863.201	2.000	3431.601	1.778	0.189
	non-SS	13 ± 15	15 ± 13	73 ± 84	SS or non-SS	1933.870	1.000	1933.870	0.285	0.603

Results of two-way repeated measures ANOVA. Values represent the mean ± SD. Total, all subjects; SS, Sjogren's syndrome group; non-SS, dry mouth group; *Time, $p < 0.05$. Most of the equol scores were under the limit of detection.

Isoflavone concentrations in saliva. The concentration of each isoflavone (daidzein, genistein, and equol) is shown in Table 4. A significant main effect of time (after intake vs before intake) was noted for daidzein and genistein. Daidzein showed a significant increase when the amount before intake (32 ± 54 μmol) was compared with the amounts after 1 month (11 ± 9.8 μmol) and 2 months (45 ± 55 μmol), ($p = 0.047$). For genistein, a comparison of the amount before intake (47 ± 70 μmol) with the amounts 1 month (20 ± 22 μmol) and 2 months (64 ± 72 μmol) after intake revealed a significant increase ($p = 0.034$). For equol, a comparison of the amount before intake (0.27 ± 0.45 μmol) with the amounts after 1 month (0.48 ± 1.0 μmol) and 2 months (0.23 ± 0.53 μmol) revealed no significant difference. We speculate that the smaller than anticipated changes were due to the limits in the sensitivity of the employed tests.

Subjective measurements. The changes in the patient oral conditions are shown in Table 5. The results of the two-way repeated measures ANOVA showed the interaction between two main factors (time and SS vs non-SS) on "dry mouth" ($p = 0.031$) and "need water during eating" ($p = 0.020$). The condition of the eye regarding eyestrain, blurriness, dryness, and eye ache is shown in Table 6. No significant difference was found in any of the measured data.

Discussion

In a human intervention clinical trial, isoflavones were shown to be effective in the prevention and relief of menopausal symptoms.[41] Menopausal women with rapidly declining estrogen levels were reported to show decreased salivary secretion and intraoral discomfort.[42–44] In addition, considering reports that ERs are found in the salivary glands and that estrogen itself is secreted in the saliva, decreasing estrogen levels are assumed to affect salivary secretion. Furthermore, menopausal women on hormone replacement therapy showed improvements in a number of oral health-related complaints, such as dry mouth, glossalgia, periodontal disease, oral stickiness, and dysgeusia.[44,45] In this study, a significant effect was observed in the amount of saliva; oxidative stress levels showed a decreasing trend, and the interaction between isoflavone intake and the presence of SS was confirmed regarding the items "dry mouth" and "need water when eating" by the intake of 25 mg of soybean isoflavones (23 mg as aglycone) per day for two months in 15 subjects who recognized dry mouth symptoms. Therefore, the intake of isoflavones was thought to be effective in the relief of dry mouth that occurs in menopause and SS and of the general physical complaints of SS-affected individuals who presented with serious dry mouth.

As a result of measuring these three oxidative stress items before and after intake, 8-OHdG and HEL showed decreased levels and indicated the possibility that oxidative stress was reduced and the amount of saliva was increased by the intake of isoflavones. With regard to the antioxidant effect of isoflavones, the potential that the saliva secretional capacity was activated by eliminating oxidative stress and that the amount of saliva increased by activating the water secretion mechanism can be considered as an effect is expected in the improvement of the salivary secretion capacity by the continuous intake of isoflavones.

Furthermore, in a human study of isoflavone supplementation, a significant improvement in blood flow was found.[46] The components of saliva are derived from the blood and salivary glands, and the increase in salivary secretion was proposed to be due to "the increased blood flow". Thus, the possibility that the intake of isoflavones is useful for the recovery of salivary glands impaired by oxidative stress and for the improvement of blood flow is

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Table 5. Score for the subjective measurements of the oral condition pre- and post-intake of isoflavones for dry mouth patients

Item		Before	After 1 month	After 2 months	Results of two-way repeated measures ANOVA					
					Source of variation	SS (Type III)	DF	MF	F	*p* value
Dry mouth	Total	4.07 ± 1.44	4.00 ± 1.13	3.87 ± 1.06	Time	0.067	2.000	0.033	0.178	0.838
	SS	4.60 ± 1.26	4.30 ± 1.25	4.10 ± 1.20	Time × SS or non-SS	1.489	2.000	0.744	3.977	0.031*
	non-SS	3.00 ± 1.22	3.40 ± 0.55	3.40 ± 0.55	SS or non-SS	11.378	1.000	11.378	3.292	0.093
Have a cough or phlegm	Total	2.33 ± 1.05	2.53 ± 1.19	3.47 ± 4.96	Time	23.022	2.000	11.511	1.506	0.240
	SS	2.30 ± 1.16	2.50 ± 1.18	2.30 ± 1.06	Time × SS or non-SS	25.689	2.000	12.844	1.681	0.206
	non-SS	2.40 ± 0.89	2.60 ± 1.34	5.80 ± 8.56	SS or non-SS	15.211	1.000	15.211	1.420	0.255
Difficulty in chewing	Total	2.67 ± 1.40	2.73 ± 1.16	2.60 ± 1.30	Time	0.089	2.000	0.044	0.100	0.906
	SS	2.80 ± 1.62	3.00 ± 1.25	2.80 ± 1.48	Time × SS or non-SS	0.267	2.000	0.133	0.299	0.744
	non-SS	2.40 ± 0.89	2.20 ± 0.84	2.20 ± 0.84	SS or non-SS	3.600	1.000	3.600	0.860	0.371
Difficulty in swallowing	Total	3.00 ± 1.41	3.07 ± 1.16	2.80 ± 1.37	Time	0.467	2.000	0.233	1.000	0.382
	SS	3.30 ± 1.57	3.40 ± 1.17	3.10 ± 1.52	Time × SS or non-SS	0.022	2.000	0.011	0.048	0.954
	non-SS	2.40 ± 0.89	2.40 ± 0.89	2.20 ± 0.84	SS or non-SS	8.711	1.000	8.711	1.935	0.188
Difficulty in speaking	Total	2.67 ± 1.35	2.80 ± 1.08	2.53 ± 1.19	Time	0.200	2.000	0.100	0.239	0.789
	SS	2.90 ± 1.52	3.00 ± 1.05	2.50 ± 1.27	Time × SS or non-SS	1.267	2.000	0.633	1.515	0.239
	non-SS	2.20 ± 0.84	2.40 ± 1.14	2.60 ± 1.14	SS or non-SS	1.600	1.000	1.600	0.436	0.521
Worried about mouth or tooth condition	Total	3.40 ± 1.30	3.27 ± 1.49	2.87 ± 1.30	Time	1.867	2.000	0.933	2.220	0.129
	SS	2.60 ± 1.52	2.60 ± 1.82	2.20 ± 0.84	Time × SS or non-SS	0.089	2.000	0.044	0.106	0.900
	non-SS	2.60 ± 1.52	2.60 ± 1.82	2.20 ± 0.84	SS or non-SS	11.378	1.000	11.378	2.648	0.128
Have tooth or mouth sensitivity	Total	3.13 ± 1.46	3.07 ± 1.28	2.60 ± 1.40	Time	1.800	2.000	0.900	2.265	0.124
	SS	3.40 ± 1.26	3.20 ± 1.14	2.70 ± 1.42	Time × SS or non-SS	0.467	2.000	0.233	0.587	0.563
	non-SS	2.60 ± 1.82	2.80 ± 1.64	2.40 ± 1.52	SS or non-SS	2.500	1.000	2.500	0.485	0.498
Need water when eating	Total	3.87 ± 1.25	3.87 ± 1.30	3.67 ± 1.18	Time	0.022	2.000	0.011	0.056	0.945
	SS	4.20 ± 1.32	4.20 ± 1.40	3.70 ± 1.34	Time × SS or non-SS	1.800	2.000	0.900	4.558	0.020*
	non-SS	3.20 ± 0.84	3.20 ± 0.84	3.60 ± 0.89	SS or non-SS	4.900	1.000	4.900	1.203	0.293
Mouth feels pasty	Total	3.40 ± 1.30	3.33 ± 1.11	3.00 ± 1.31	Time	1.089	2.000	0.544	1.755	0.193
	SS	2.70 ± 1.49	2.90 ± 1.29	2.70 ± 1.34	Time × SS or non-SS	0.556	2.000	0.278	0.895	0.421
	non-SS	3.60 ± 1.34	3.20 ± 1.10	3.20 ± 1.30	SS or non-SS	0.178	1.000	0.178	0.041	0.842
Painful tongue	Total	2.80 ± 1.57	2.87 ± 1.41	2.80 ± 1.37	Time	0.000	2.000	0.000	0.000	1.000
	SS	2.70 ± 1.49	2.90 ± 1.29	2.70 ± 1.34	Time × SS or non-SS	0.356	2.000	0.178	0.937	0.405
	non-SS	3.00 ± 1.87	2.80 ± 1.79	3.00 ± 1.58	SS or non-SS	0.278	1.000	0.278	0.044	0.838
Worried about bad breath	Total	2.40 ± 1.06	2.53 ± 0.99	2.47 ± 0.99	Time	0.089	2.000	0.044	0.234	0.793
	SS	2.30 ± 0.95	2.50 ± 1.08	2.50 ± 1.08	Time × SS or non-SS	0.267	2.000	0.133	0.703	0.504
	non-SS	2.60 ± 1.34	2.60 ± 0.89	2.40 ± 0.89	SS or non-SS	0.100	1.000	0.100	0.034	0.856

Results of two-way repeated measures ANOVA. Values represent mean ± SD. Total, all subjects; SS, Sjogren's syndrome group; non-SS, dry mouth group; *Time × SS or non-SS, $p < 0.05$.

Table 6. Score for subjective measurements of the eye condition pre- and post-intake of isoflavones for dry mouth patients

Item		Before	After 1 month	After 2 months	Results of two-way repeated measures ANOVA					
					Source of variation	SS (Type III)	DF	MF	F	*p* value
Eyestrain	Total	3.13 ± 1.30	2.93 ± 1.39	3.00 ± 1.41	Time	0.622	2.000	0.311	1.411	0.262
	SS	3.10 ± 1.29	3.10 ± 1.52	3.10 ± 1.60	Time × SS or non-SS	0.622	2.000	0.311	1.411	0.262
	non-SS	3.20 ± 1.48	2.60 ± 1.14	2.80 ± 1.10	SS or non-SS	0.544	1.000	0.544	0.099	0.758
Blurred vision	Total	2.87 ± 1.46	2.87 ± 1.60	2.87 ± 1.46	Time	0.067	2.000	0.033	0.183	0.834
	SS	3.00 ± 1.63	2.90 ± 1.73	3.10 ± 1.60	Time × SS or non-SS	0.600	2.000	0.300	1.648	0.212
	non-SS	2.60 ± 1.14	2.80 ± 1.48	2.40 ± 1.14	SS or non-SS	1.600	1.000	1.600	0.236	0.635
Dry eye	Total	3.60 ± 1.40	3.47 ± 1.55	3.33 ± 1.45	Time	0.422	2.000	0.211	1.614	0.218
	SS	3.80 ± 1.48	3.70 ± 1.64	3.50 ± 1.51	Time × SS or non-SS	0.067	2.000	0.033	0.255	0.777
	non-SS	3.20 ± 1.30	3.00 ± 1.41	3.00 ± 1.41	SS or non-SS	3.600	1.000	3.600	0.560	0.468
Eye pain	Total	2.60 ± 1.64	2.47 ± 1.55	2.33 ± 1.54	Time	0.600	2.000	0.300	0.727	0.493
	SS	2.70 ± 1.77	2.60 ± 1.58	2.50 ± 1.72	Time × SS or non-SS	0.067	2.000	0.033	0.081	0.923
	non-SS	2.40 ± 1.52	2.20 ± 1.64	2.00 ± 1.22	SS or non-SS	1.600	1.000	1.600	0.225	0.643

Results of two-way repeated measures ANOVA. Values represent the mean ± SD. Total, all subjects; SS, Sjogren's syndrome group; non-SS, dry mouth group.

assumed in this study. Furthermore, the function of the salivary gland has likely been improved by the antioxidant effect of isoflavones against the oxidative stress of salivary glands, thereby improving the effect on blood flow. This potential mechanism may explain the significant promotion of saliva flow observed in our study. In the future, we aim to verify these results by analyzing the degree of the damage to salivary glands by oxidative stress and the direct effect of isoflavones on salivary glands.

With respect to the human intervention clinical trial, many effective cases found that the volume of isoflavone aglycones that

should be ingested per day is at least 30 mg and that the ingestion period should be at least two weeks; in this study, the ingested amount was 25 mg, and the ingestion period was two months.[47–50]

The study by NIH reported that that "Isoflavone aglycone decreases the vasomotor symptom of menopause by 10–20%".[51] With regard to this study, menopause symptoms, with a focus on "hot flash" symptoms, are thought to be improved by the intake of 30–60 mg of soy isoflavone aglycone per day for at least two weeks in the United States and Europe. A study evaluating the effects of isoflavones reported that differences in the metabolism of isoflavones by *Enterobacteriaceae* affected isoflavone potency.[52,53] Because intestinal bacterial flora differs among individuals as shown in previous studies and the average age of the subjects was older than 60 years, the potential change in bacterial flora is thought to be affected by increasing age and a decrease in the number of bacteria. Furthermore, approximately 50% of Asians are equol producers and contain the intestinal bacteria required to convert daidzein into equol, which may have affected the efficacy of this evaluation of isoflavones.[54–58] Thus, we are planning to conduct the analysis with consideration of the equol-producing ability of individuals.

In this study, isoflavone intake is thought to act as an antioxidant in salivary glands impaired by oxidative stress, and the salivary function was likely improved by the increased blood flow. As the salivary secretion amounts of the subjects showed an increasing trend without any accompanying side effects, such as sweating and polyuria, which may be caused by salivary secretion promoters, an improvement in the QOL was confirmed. We aim to study the detailed mechanism in future studies.

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Abbreviations

ERs	estrogen receptors
HEL	hexanoyl-lysine
HRT	hormone replacement therapy
LDL	low-density lipoprotein
8-OHdG	8-hydroxy-2'-deoxyguanosine
PRL	propanoyl-lysine
QOL	quality of life
ROS	reactive oxygen species
SS	Sjogren's syndrome

Conflicts of Interest

No potential conflicts of interest were disclosed.

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