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Original article

Influence on the oxidized protein hydrolase (OPH) activity of herbal tea extract

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Abstract

Glycative stress is a comprehensive concept meaning biological stress caused by the formation and accumulation of advanced glycation end products (AGEs). The increase of glycative stress leads to the risk of onset and development of aging, diabetic complications, Alzheimer's, and arteriosclerosis. The countermeasures against glycative stress are called anti-glycation, which includes inhibitions of postprandial hyperglycemia and glycation, the decrease of AGEs, and the lowering of dietary-derived AGEs. Oxidized protein hydrolase (OPH) is a kind of protease which is the decrease of aged protein and AGEs and is broadly distributed within living tissues. In this research, for the purpose of investigating components which decrease glycative stress, the effect of herbal tea extracts on OPH activity were verified. The extracts of 58 products (38 categories) of herbal teas on OPH activity and OPH activity enhancing action was recognized in 47 herbal tea products (81%). OPH activity was observed in 34 categories (89%) of herbal tea. On the other hand, the extracts of 12 products inhibited OPH activity. Green tea (unfermented tea) and black tea (fermented tea) derived from *Camellia sinensis* showed an inhibitory effect; however, roasted green tea (houji tea) and pu-er tea (late fermented tea) enhanced the activity. The comparison of utilized portions of herbal teas in which an OPH activity enhancing action was observed showed that seed tea had a stronger influence than leaf tea. The active components enhancing OPH activity are possibly included in various plants. It was found that the roasting and fermenting process for tea leaves have an influence on OPH activity. It is possible that herbal teas could prevent the accumulation of aged protein and AGEs by enhancing OPH activity, and as a result, contribute to the prevention of the progression of aging caused by glycative stress.

***KEY WORDS*:** oxidized protein hydrolase (OPH), herbal tea extract, glycative stress, anti-glycation

Introduction

The non-enzymatic reactions between reducing sugar such as glucose and proteins are called glycation. Glycation occurs *in vivo* and as a result, advanced glycation end products (AGEs) are accumulated in the tissues. The formation and accumulation of AGEs induces browning of proteins and the decline in function caused by stiffness of tissues by crosslinking. AGEs combine with the receptor for AGEs (RAGE), and causes various inflammatory changes in various tissues. Therefore, the comprehensive concept of biological stress caused by formation and accumulation of AGEs is called "glycative stress"[1,2]. The increase of glycative stress leads to the risks of the onset and development of aging, diabetic complications, Alzheimer's and arteriosclerosis. Countermeasures against glycative stress are called anti-glycation, which includes the inhibitions of postprandial hyperglycemia, generation and the dissolution of AGEs[3].

Oxidized or glycated proteins *in vivo* are called aged proteins. Oxidized protein hydrolase (OPH) is a kind of serine protease, which is called acylamino-acid releasing enzyme (AARE)[4,5] or acylaminoacyl-peptide hydrolase (APEH)[6] and release N-terminal acylated amino acid of aged protein. OPH is widely distributed in living tissues, such as liver[7,8], brain[9], blood[10], red cell membrane[11,12], and placenta[13].

Serum OPH activity of rats with diabetic mellitus

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remarkably increased and the blood carbonyl-modifying protein decreased[14]. OPH contributes to the breakdown of aged protein in cooperation with proteasome[15]. OPH decreased AGEs including CML and fluorescent AGEs. OPH also exists in the stratum corneum and has a weak negative correlation with aging. However, no components influencing OPH activity have been found.

In this research, for the purpose of investigating the components contributing to stress reduction, the effect of herbal tea extracts on OPH activity was verified.

Materials and Method

1) Reagents

Acylamino-acid releasing enzyme (AARE) (Takara Bio Inc. Shiga, Japan) was used. AARE was adjusted with phosphate buffer solution of 50 mmol/L (pH 7.2) to 0.025 U/mL, N-acetyl-L-alanine *p*-nitroanilide (AAPA) (Bachem; Bubendorf, Switzerland) was used as the oxygen substrate. The AAPA was adjusted with 50% ethanol water solution to 25 mmol/L. For other regents of Special Grade were purchased from Fujifilm Wako Pure Chemical (Osaka, Japan) and Nakarai Tesque (Kyoto, Japan)

2) Herbal teas

Fifty-eight commercially released dried herbal tea products (38 categories) were provided by Hikawa Co. Ltd. (Shimane, Japan).

3) Preparation of Sample Extract

A sample extract was obtained by putting 2g herbal tea powder into 40 mL purified water and incubating it in a water bath set at 80°C for 60 minutes. The extract was cooled to room temperature and filtered. The solid content of the sample solution was calculated by tea extract 5 mL placed in an aluminum tray, water being dried and evaporated at 120°C for two hours and the evaporation residue being weighed. The concentration of the sample extract was calculated from the solid content and dilution rate and it was shown as a final reaction concentration.

4) Measurement of OPH Activity and Calculation of OPH Activation Rate

Reaction mixture to which a sample extract for the measurement of OPH activity was quantitatively added at a rate of 1/25 and was composed as follows: 0.1 mol/L Tris (tris (hydroxymethyl) aminomethane)–hydrochloric acid buffer solution (pH 7.4), 2 mmol/L AAPA, 1 mU/mL OPH, sample extract. The reaction mixture of 250 µL was incubated at 37°C for 60 minutes and the amount of *p*-nitroanilide (*p*NA), which was released by AAPA and decomposed by OPH, was spectrophotometrically measured at 405 nm (S). For the reference of OPH activity, distilled water, instead of the sample, was added to the above-mentioned compound liquid (R). The OPH activation rate (%) was calculated using the following equation, presuming that the amount of *p*NA that was isolated and formulated during the 60 minutes immediately after the start of the reference reaction (0 minute) is 100%:

$$\text{OPH activation rate (\%)} = \{(S_{60} - S_0) / (R_{60} - R_0)\} \times 100$$

S: *p*NA concentration of reaction mixture added with sample extract
R: concentration of *p*NA of reference reaction mixture
60: 60 minutes
0: initial (0 time)

For the measurement of OPH activity, three concentrations were verified. The highest value out of the three was set as the representative value.

Statistics Analysis

The OPH activation rate was expressed as mean value ± standard deviation. For the test of the result of analysis, BellCurve for Excel (Social Information Service Co., Tokyo, Japan), a statistics software, was used and Tukey's multiple comparison test was conducted for the comparison among groups. As the result of a statistics analysis, a risk ratio of < 5% was regarded as significant.

Results

As the result, the OPH activity enhancing action was observed in 46 products out of the 58 hot water extracts of 58 herbal tea products (81%, ***Table 1***). Out of the 46 products in which the OPH activity enhancing action was recognized, the OPH activation rate of 24 products was over 50% (66.5 ± 12.9%), and that of 22 products was more than 0% or less than 50% (35.5 ± 13.4%, ***Table 2***). On the other hand, 12 products inhibited OPH activity, 8 products inhibited more than 0% and less than 50% (–23.7 ± 10.8) and 4 products inhibited over 50% (–58.7 ± 4.4%).

By categories, the OPH activity enhancing action was recognized in 34 categories (89%) out of 38 categories. High values of OPH activation rates were observed in brown rice (89.2%: average, n = 2), barley (83.6 ± 2.7%, n = 3), dandelion (78.3%) and rose hip (77.2%), all of which showed a higher value than 70% (***Fig. 1***). On the other hand, four categories inhibited OPH activity. The OPH activation rate of black tea showed the largest negative value (–55.7%), which indicates a strong inhibitory action. followed by green tea (–52.1 ± 9.3%, n = 3), meigui (–16.5%: average value, n = 2), and jasmine tea (–0.8%).

Regarding the effect of tea extracts from *Camellia sinensis* on OPH activity, green tea (unfermented tea) and black tea (fermented tea) showed inhibitory action of more than –40%, on the other hand, roasted green tea (houji tea) and pu-er tea (late fermented tea) enhanced the activity. Three products of oolong tea used in this experiment showed both activity enhancing action and inhibitory action.

The comparisons of utilized portions of the herbal teas in which OPH activity enhancing actions were recognized showed that their activation rates increased in the order of seed (72.7 ± 17.4%, n = 9), root (52.6 ± 17.8%, n = 4), and leaf (43.9 ± 17.8%, n = 29), and the activation rate of seed tea was significantly larger than that of leaf tea ($p < 0.01$, ***Fig. 2***).

Table 1. Influence on the OPH activity of 58 sample (38 category) herbal tea extract

Sample ID	Sample name	Japanese general tea category	Use for tea	Scientific name	Family name	Sample conc. (μg/mL)	*p*NA conc. (mmol/L)	Activation ratio (%)
41	Dokudami	Dokudami-cha	leaf	*Houttuynia cordata*	Saururaceae	24.8	0.120	48.9
1	Job's tears (non-caramel)	Hatomugi-cha	seed	*Coix lacryma-jobi var. ma-yuen*	Poaceae	0.9	0.123	53.5
4	Job's tears					387.7	0.120	48.8
22	Brown rice (crushed)	Genmai	seed	*Oryza sativa*		224.0	0.149	85.8
26	Brown rice (crushed)					163.8	0.155	92.7
21	Barley tea	mugi-cha	seed	*Hordeum vulgare*		93.1	0.150	86.5
55	Nijo Barley tea					116.6	0.147	83.0
56	Rokujo Barley tea					95.1	0.146	81.2
8	Lemon grass	Lemon grass	leaf	*Cymbopogon citratus*		384.1	0.118	47.0
46	Kuma bamboo grass	Kumazasa-cha	leaf	*Sasa veitchii*		165.3	0.134	67.4
38	Amacha	Amacha	leaf	*Hydrangea macrophylla var. thunbergii*	Saxifragaceae	63.7	0.113	41.0
49	Salacia	Salacia-cha	root	*Salacia sp.*	Celastraceae	74.7	0.109	37.4
13	Five-leaf ginseng	Amachazuru-cha	leaf	*Gynostemma pentaphyllum*	Cucurbitaceae	214.2	0.133	65.3
15	Cassia seed	Habu-cha	seed	*Senna obtusifolia*	Fabaceae	97.2	0.140	73.6
36	Candle bush	Candle bush	leaf	*Cassia alata*		273.1	0.110	36.3
40	Sword bean	Natamame-cha	seed	*Canavalia gladiata*		114.9	0.120	49.2
43	Rooibos	Rooibos-cha	leaf	*Aspalathus linearis*		99.6	0.129	60.3
16	Mulberry leaf	Kuwa-no-ha-cha	leaf	*Morus sp.*	Moraceae	50.3	0.125	55.7
20	Mulberry leaf (Shimane Pref.)					34.6	0.140	74.0
25	Rugosa rose	Meigui	flower	*Rosa rugosa spp.*	Rosaceae	22.3	0.058	**-27.4**
54	Rugosa rose					2.1	0.076	**-5.5**
28	Rose hip	Rose hip	fruit	*Rosa canina*		77.0	0.142	77.2
44	Tencha	Ten-cha	leaf	*Rubus suavissimus*		31.7	0.123	53.4
58	Tencha					59.9	0.100	24.9
59	Tencha (fermented)					53.0	0.108	34.9
45	Banaba	Banaba-cha	leaf	*Lagerstroemia speciosa*	Lythraceae	20.5	0.089	10.5
42	Guava leaf	Guava leaf-cha	leaf	*Psidium guajava*	Myrtaceae	8.9	0.089	10.2
6	Evening primrose	Tsukimiso-cha	leaf	*Oenothera sp.*	Onagraceae	2.5	0.088	9.6
52	Evening primrose					1.6	0.080	**-0.5**
37	Moringa	Moringa	leaf	*Moringa oleifera*	Moringaceae	61.0	0.125	55.2
47	Kaki leaf	Kaki-no-ha-cha	leaf	*Diospyros kaki*	Ebenaceae	208.0	0.124	53.8
33	Green tea (benifuki)	Ryoku-cha	leaf	*Camellia sinensis*	Theaceae	18.9	0.034	**-57.7**
34	Green tea (saemidori)					41.9	0.035	**-56.2**
32	Green tea (Kagosima Pref.)					25.1	0.046	**-42.9**
31	Green tea (autum-winter harvest)					56.6	0.047	**-41.2**
27	Green tea (2nd harvest)					45.8	0.041	**-49.3**
35	Green tea (3rd harvest)					63.5	0.028	**-65.1**
19	Coarse tea (roasted)	Ban-cha				43.3	0.108	34.5
3	Roasted green tea	Hoji-cha				6.1	0.093	15.4
29	Roasted green tea					51.9	0.125	55.2
5	Jasmine tea	Jasmine-cha (oolong tea scented with the aroma of jasmine blossoms)				6.2	0.080	**-0.8**
12	Black tea	Koh-cha				62.7	0.036	**-55.7**
14	Oolong tea (leaf)	Oolong-cha				51.7	0.063	**-21.9**
17	Oolong tea (tea bag)					38.4	0.104	29.2
24	Oolong tea (Taiwan)					43.9	0.108	34.9
7	Pu'er tea (tea bag)	Pu'er-cha				37.0	0.115	43.4
11	Pu'er tea (leaf)					4.0	0.114	42.2

Sample ID	Sample name	Japanese general tea category	Use for tea	Scientific name	Family name	Sample conc. (μg/mL)	*p*NA conc. (mmol/L)	Activation ratio (%)
9	Gymnema	Gymnema-cha	leaf	*Gymnema sylvestre*	Apocynaceae	6.7	0.133	65.5
48	Shiso	Shiso-cha	leaf	*Perilla frutescens var. crispa*	Lamiaceae	29.1	0.082	39.8
39	Olive leaf	Olive-ha-cha	leaf	*Olea europaea*	Oleaceae	51.3	0.121	50.7
2	chicory (raw)	Chicory	leaf	*Cichorium intybus*	Asteraceae	50.3	0.124	54.7
51	chicory					384.0	0.128	59.7
30	Burdock tea	Gobo-cha	root	*Arctium lappa*		1448.2	0.117	46.2
53	Burdock tea					714.7	0.119	48.4
18	Safflower	Benibana-cha	flower	*Carthamus tinctorius*		88.2	0.119	47.7
23	Dandelion	Tanpopo-ne-cha	root	*Taraxacum sp.*		936.0	0.143	78.3
50	Chrysanthemum flower	Kikuka-cha	flower	*Chrysanthemum morifolium*		35.7	0.126	56.9
10	Lingzhi mushroom	Lingzhi	mushroom	*Ganoderma lucidum*	Ganodermataceae	109.5	0.126	57.0
Ref						–	0.080	0.0

Sample conc, final sample concentration in reaction reagent; *p*NA conc, concentration of *p*NA separated in reaction liquid in 60 minutes; Activation ratio, activation ratio of OPH to reference; OPH, oxidized protein hydrolase; *p*NA, *p*-nitroanilide.

Table 2. OPH activity by processing of green tea

Tea category	Processing	n	Activation ratio (%)
Green tea	unfermented	3	**-52.1 ± 9.3**
Coarse tea	roasted	1	34.5
Roasted green tea		2	35.3
Black tea	fermented	2	**-55.7**
Oolong tea		3	14.1 ± 31.3
Pu'er tea		2	42.8

Results are expressed as mean ± SD or average values. Green tea, tea of *Camellia sinensis* source; activation ratio, activation ratio of OPH to reference; OPH, oxidized protein hydrolase; SD, standard deviation.

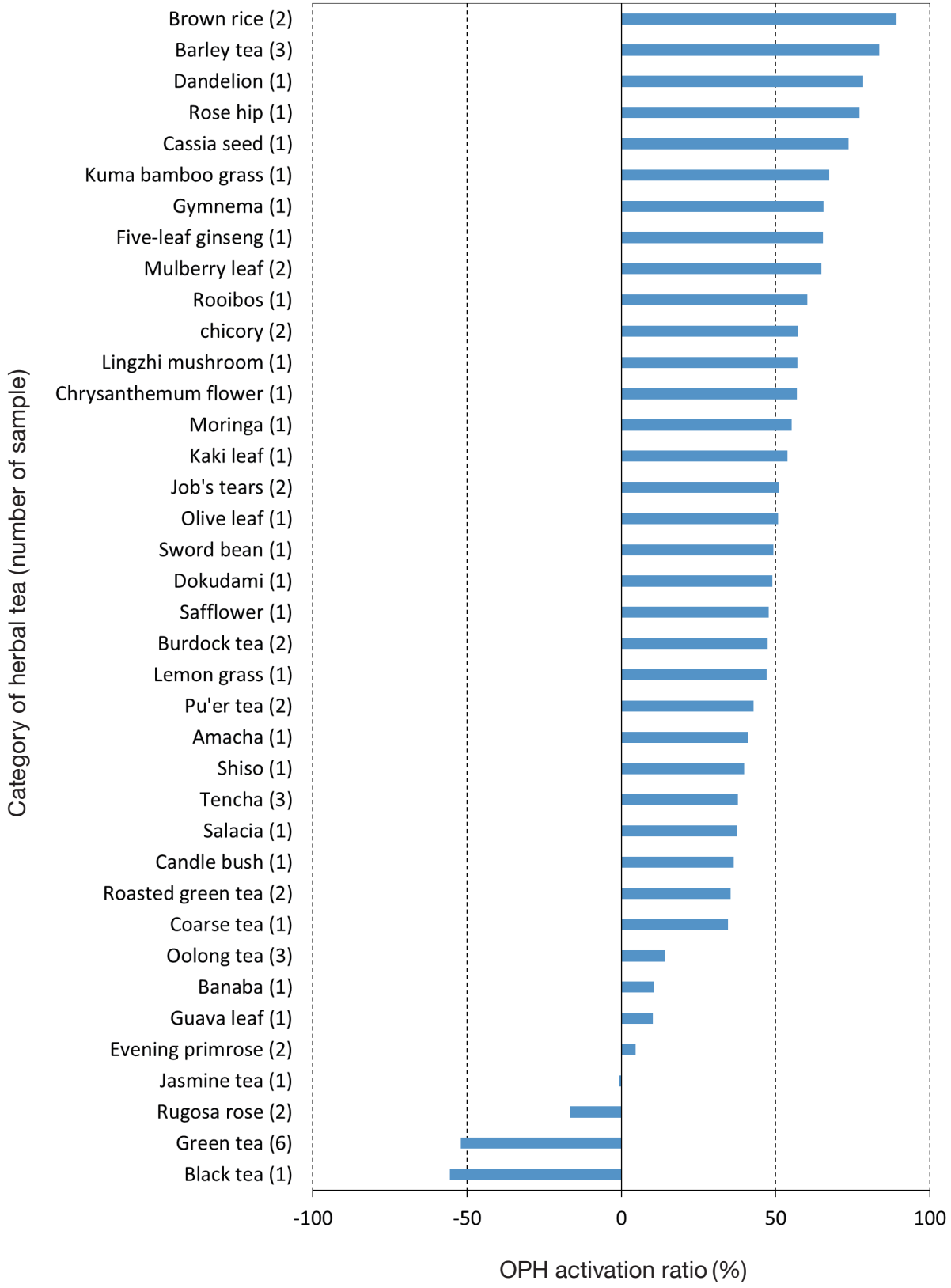

Fig. 1. **Influence on the OPH activity of 38 category herbal tea extract.**

OPH activation ratio, activation ratio of OPH to reference; Extract condition, dry herb powder 2g + 40 mL hot water (80°C), incubated for 60 min; OPH, oxidized protein hydrolase.

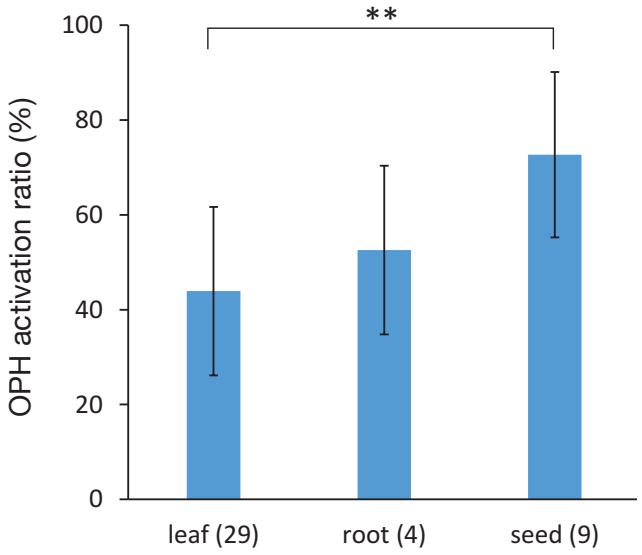

Fig. 2. **Influence on the OPH activity use for tea.**

Results are expressed as mean±SD. Parenthesis shows number of samples. **: $p < 0.01$, Tukey's multiple comparison test. OPH activation ratio, activation ratio of OPH to reference; Extract condition, dry herb powder 2 g + 40 mL hot water (80°C), incubated for 60min; OPH, oxidized protein hydrolase; SD, standard deviation.

Discussion

Components of Herbal Teas Affecting OPH Activity

Herbal tea extracts effect on OPH activity. Activity enhancing action was recognized in 46 products (81%) of 34 categories (89%) out of 58 samples of 38 categories verified. This result shows that compounds enhancing OPH activity are possibly included in various plants. The characteristic components at various family and genus levels have already been reported from the stand point of chemical taxonomy [17,18]. The OPH activation rate was higher in herbal teas using seeds over leaves; however, no relationship between strong and weak activities from the view point of plant taxonomy was observed.

The action inhibiting OPH activity was recognized in green tea (unfermented tea); however, the inhibitory action was reduced by the roasting or fermenting process. Green tea includes caffeine, theophylline and tannins [19]. It is also known that catechin changes to theaflavin by tea leaves being oxidatively fermented [20-22]. The roasting and fermenting processes of tea leaves is possibly associated with the changes of OPH activity inhibiting action caused by the change of the components of tea leaves.

OPH Activity Enhancing action from the Viewpoint of Inhibition of Glycative Stress

OPH is broadly distributed in living tissues [7-13]. Because OPH has the action of decreasing the amounts of AGEs and N^{ε}-(carboxymethyl) lysine (CML) [16], it is possible that the intake of herbal tea leads to the enhancement of OPH activity, which promotes the decrease of aged protein that was changed to AGEs *in vivo*. Furthermore, OPH-like activity is also recognized in the stratum corneum, so it is possible that it is involved in the decrease of CML in the stratum corneum. However, it has been observed that OPH activity in the stratum corneum has a weak negative correlation with aging, and the amount of CML in the stratum corneum and the increase of accumulation of AGEs in skin (skin autofluorescence; SAF) is observed [16]. The increase of AGEs in the skin is involved in lost texture [23], yellowing of skin, the lowing of barrier function [24], and progression of skin aging. The use of herbal teas as skin care medicine possibly inhibits reduction of OPH activity, contributing to the inhibition of SAF. Using herbal tea as both food and skincare medicine could prevent the accumulation of AGEs by enhancing OPH activity and it is possible that it could inhibit aging caused by glycative stress.

Conclusion

The effect of hot water extract of herbal teas on OPH activity was recognized. OPH activity enhancing action was recognized in 46 products (81%) out of 58 samples of which the activity was evaluated. It was found that active components enhancing OPH activity are included in various plants. The effect of the herbs on OPH activity differed by kinds of herbs, processing methods such as roasting and fermentation and utilized portions. The OPH activity enhancing action of herbal teas is expected for its effect on the prevention of the accumulation of aged protein and AGEs, and it could possibly contribute to the inhibition of aging caused by glycative stress

Statement of conflict of interest

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