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Inhibitory effect of prenylflavonoid in *Euchresta japonica* on copper-induced protein oxidative modification *in vitro*

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Abstract

Objective: To investigate the inhibitory effect of prenylflavonoid, 5,7,4'-trihydroxy-6,8-diprenylisoflavone isolated from *Euchresta japonica*, on oxidative stress. Materials and methods: The inhibitory effect of 5,7,4'-trihydroxy-6,8-diprenylisoflavone was tested on copper-induced protein oxidative modification *in vitro*. Result: 5,7,4'-trihydroxy-6,8-diprenylisoflavone inhibited copper-induced protein oxidative modification. Its inhibitory effect was stronger than that of genistein as a non-prenylated isoflavone. Conclusion: The results demonstrated that prenylflavonoid, 5,7,4'-trihydroxy-6,8-diprenylisoflavone isolated from *Euchresta japonica*, has inhibitory effect on oxidative stress as copper-induced protein oxidative modification *in vitro*.

Key words: 5,7,4'-trihydroxy-6,8-diprenylisoflavone, *Euchresta japonica*, copper, protein oxidative modification.

1. Introduction

Copper induces to generation of oxygen free-radical species. The very-low-density lipoprotein (VLDL) oxidation catalyzed by copper leads the lipid peroxidation, the formation of aggregates, and covalent modification of apolipoprotein E in VLDL [1]. It has been shown that the flavonoids, morin, genistein, apigenin and biochanin, prevent *in vitro* low-density lipoprotein oxidation [2].

We showed that some isoflavones, biochanin A, daidzein, formononetin and genistein, have antioxidative effects [3]. There are no prenyl groups in their chemical structures. Shirataki *et al* isolated prenylflavonoid, 5,7,4'-trihydroxy-6,8-diprenylisoflavone from *Euchresta japonica* which has been used as a substitute for Chinese medicine [4]. In this paper, we investigated the inhibitory effect of 5,7,4'-trihydroxy-6,8-

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diprenyli-soflavone on copper-induced protein oxidative modification *in vitro*.

2. Materials and methods

2.1 Plant material

The dried roots of *Euchresta japonica,* which were collected in Mt. Ichifusa, Kumamoto prefecture, Japan in April 1981. A voucher specimen was deposited in Department of Pharmaceutical Science, Josai University, Japan, by Dr. Yoshiaki Shirataki.

2.2 Preparation of 5,7,4'-trihydroxy-6,8-diprenylisoflavone

The dried root of *Euchresta japonica* were extracted with methanol. The methanol extract were fractioned repeatedly by silica gel column chromatography, with benzene-ethyl acetate mixture as the eluent. They afforded 5,7,4'-trihydroxy-6,8-diprenylisoflavone (ratio of yield: 0.05 %) following Shirataki's previous paper. [4]

2.3 Assay of inhibitory effects on protein oxidative modification

Protein oxidative modification by copper was performed by the following method. The test sample was added to the reaction mixture containing albumin (10 μg/ml) and 100 μM $CuCl_2$ in 50 mM Tris-HCl (pH 7.4) buffer. The reaction mixture was incubated at 37°C for 2h and then 1.6 ml of 0.125 M, pH 8.0 phosphate buffer containing 12.5 mM ethylendiaminetetra- acetic acid, 10.0 M urea and 0.1 ml of pH7.0 phosphate buffer containing 10 mM 5,5′-dithiobis(2-nitrobenzoic) acid were added.

The resulting solution was allowed to stand at room temperature for 5 min, and then the absorbance of the cystein–SH (Cys-SH) residue was read at 412 nm [5]. The inhibitory ratio (I.R.) of the test sample was evaluated by the following equation:

$$I.R.\% = (\Delta Cys\text{-}SH / \Delta Cys\text{-}SH^{\circ})$$

Where I.R.% is the inhibitory ratio, (%). ΔCys-SH is the Cys-SH residue in treated sample – Cys-SH residue in reaction blank, and ΔCys-SH° is Cys-SH residue before incubation – Cys-SH residue, untreated blank.

2.4 Calculation of 50 % inhibitory concentration (IC_{50})

The inhibitory ratio was plotted against the log [F1] ([F1] = concentration of sample). The dose-response data were used to corresponding IC_{50}.

2.5 Statistical analysis

The value were expressed as the mean± standard error of five experiments. The results were analyzed by the non-parametric ANOVA-Scheffe f-test.

Table 1.
Inhibitory effect of 5,7,4′-trihydroxy-6,8-diprenylisoflavone on copper-induced protein oxidative modification.

Test sample	Concentration (μM)	I.R. (%)
5,7,4′-trihydroxy-6,8-diprenylisoflavone	0.25	24.27 ± 5.29
	2.5	58.25 ± 1.77
	25	73.30 ± 3.30
	250	92.42 ± 2.61
genistein	0.25	3.85 ± 0.38
	2.5	32.66 ± 3.18
	25	65.40 ± 1.96
	250	92.66 ± 6.88
mannitol	0.25	18.93 ± 3.21
	2.5	36.89 ± 4.04
	25	75.73 ± 2.51
	250	94.66 ± 3.18
α-tocopherol	0.25	1.94 ± 0.94
	2.5	28.64 ± 1.66
	25	37.86 ± 2.80
	250	63.59 ± 5.73

Values are means ± S.E, (n=5).

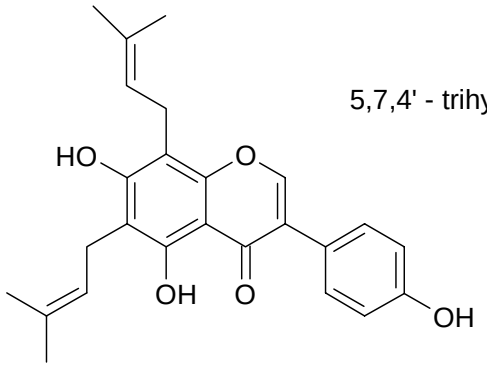

Fig. 1.
Chemical structures of 5,7,4'-trihydroxy-6,8-diprenylisoflavone and genistein

3. Results and discussion

Table 1 shows that the inhibitory ratio of 5,7,4′ -trihydroxy-6,8-diprenylisoflavone is 92.42 ± 2.61% at a concentration of 250 μM and increase in a concentration-dependent manner. The inhibitory ratios of genistein as a non-prenylated isoflavone, mannitol as hydroxy radical scavenger, and α-tocopherol as an antioxidant, are 92.66 ± 6.88, 94.66 ± 3.18, and 63.59 ± 5.73 at a concentration of 250 μM (Table 1). IC_{50} of 5,7,4′ -trihydroxy-6,8-diprenylisoflavone, genistein, mannitol and a-tocopherol are 2.19, 4.48, 8.78, and 62.87 μM.

Genistein is 5,7,4′ -trihydroxyisoflavone. 5,7,4′ -trihydroxy-6,8-diprenylisoflavone has 2 hydroxy groups at 6,8-position (Fig. 1). Isoflavonoids have been shown to have the antioxidative effects [6]. Prenylated flavonones, cycloheterophullin, and artonins A and B, isolated from Artocarpus heterophyllus Lam, have powerful antioxidant properties [7].

Genistein has been demonstrated to have antioxidative effects on oxidative stress [2,3]. The present results demonstrat that prenylflavonoids have more antioxidative effects than non-prenylated flavonoids. Our results are consistent with these previous observations.

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