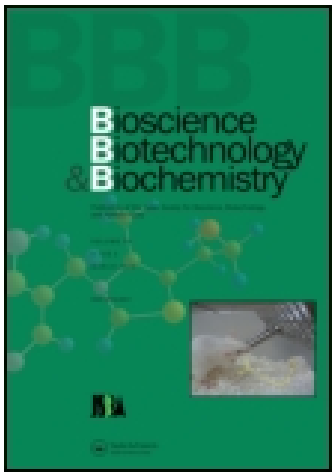

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Effect of Astilbin in Tea Processed from Leaves of Engelhardtia chrysolepis., on the Serum and Liver Lipid Concentrations and on the Erythrocyte and Liver Antioxidative Enzyme Activities of Rats

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Note

Effect of Astilbin in Tea Processed from Leaves of *Engelhardtia chrysolepis* on the Serum and Liver Lipid Concentrations and on the Erythrocyte and Liver Antioxidative Enzyme Activities of Rats

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The effects of astilbin in Kohki tea, which is produced from the leaves of *Engelhardtia chrysolepis* HANCE (Chinese name, huang-qui), and of an aglycone of astilbin, taxifolin, on the serum and liver lipid concentrations, and on the erythrocyte and liver antioxidative enzyme activities were determined with rats fed on a cholesterol-free diet. The total liver cholesterol concentration tended to be decreased by feeding with astilbin, and significantly decreased by feeding with taxifolin. The liver phospholipid concentration was decreased by feeding with both astilbin and taxifolin. In addition, astilbin and taxifolin lowered the serum and liver TBARS concentrations, but did not influence the serum and liver antioxidative enzyme activities, suggesting the possibility that these compounds acted to lower the TBARS concentration by their direct antioxidative action *in vivo*, almost without influencing the antioxidative enzyme activities.

Key words: kohki-tea; astilbin; thiobarbituric acid-reactive substances; antioxidative enzyme; lipid level

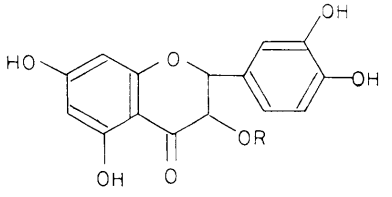
Green tea is drunk as a daily beverage in Asian countries such as Japan and China. Its physiological functions have recently been receiving strong attention, and there are many reports dealing with antioxidative activity,[1-4] angiotensin I-converting enzyme inhibitory activity,[5] antimutagenic and anticancer effects,[4,6] platelet aggregation inhibitory activity,[7] and cholesterol-lowering activity.[8,9] In many cases, it has been reported that these physiological functions were closely concerned with the catechins (catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, *etc.*) contained in green tea. In addition to green, oolong and black teas, many other kinds of tea are manufactured and consumed daily. Leaves of *Engelhardtia chrysolepis* HANCE (Chinese name, huang-qi), a sweet chinese tea and one of the most traditional, has started to be consumed as a beverage (Japanese name, Kohki tea) in Japan. Kohki tea contains astilbin (3-*O*-rhamnosyl taxifolin) as the major flavonoid (about 5% content on a dry matter basis),[10] although the physiological functions of astilbin have not yet been fully examined.

In this report, the effects of astilbin and of its aglycone, taxifolin, on the concentrations of serum and liver lipids and of serum and liver thiobarbituric acid reactive substances (TBARS), and on the erythrocyte and liver antioxidative enzyme activities in rats, which may be related to the serum and liver TBARS contents, were examined.

Astilbin was prepared from leaves of *Engelhardtia chrysolepsis* according to the method of Kasai *et al.*[10] Taxifolin was prepared by hydrolyzing astilbin with 2 N HCl at 60°C for 5 h and by purifying with silica gel column chromatography ($CHCl_3$–MeOH = 10–1, v/v as the eluent), and was crystallized from ethyl alcohol–H_2O.

Five-week-old male weanling Wistar-strain rats (Japan SLC, Hamamatsu, Japan), each weighing about 70 g, were randomly divided into three groups of 5–6 rats each. The rats were individually housed in stainless-steel cages with screen bottoms, and kept under controlled conditions with a 12-h light-dark cycle (06:00–18:00 light), a temperature range of 22–24°C, and relative humidity of about 55%. The basal diet (diet 20C) contained (by weight) 20% casein, 5% corn oil, 1% Harper's vitamin mixure[11] (Oriental yeast Co., Tokyo, Japan), 5% Harper's mineral mixture[11] (Oriental Yeast Co.), 0.1% choline chloride, and 68.9% α-cornstarch. The astilbin (A)- or taxifolin (T)-added diet (diet 20C + A or 20C + T) was made up by adding 0.074% astilbin or 0.05% taxifolin (equal to 0.074% astilbin on a molar basis) to the basal diet at the expense of α-cornstarch. The 0.074% astilbin and 0.05% taxifolin levels were used in this experiment because 0.05% taxifolin has lowered the atherogenic index and tended to lower serum and liver total cholesterol in rats fed with a cholesterol-enriched diet as previously reported.[12] Food and water were provided *ad libitum* for 10 days. At the end of the feeding period, the rats were anesthetized with Nembutal (Dainippon Pharmaceutical Co., Osaka, Japan) after 12 h of starvation and bled by heart puncture. The liver of each rat was immediately removed, weighed and stored at −20°C until subjected to the lipid analysis. To measure the serum TBARS concentration and prepare erythrocytes, a 0.1-ml aliquot of the blood was mixed with 1.9 ml of physiological saline by gently shaking, before the mixture was centrifuged at $1000 \times g$ for 10 min. The supernatant (0.5 ml) was analyzed for its TBARS content, which was determined according to the method of Yagi,[13] the concentration of serum TBARS being expressed as nmol of malondialdehyde per ml of blood. The serum for measuring lipids was separated by centrifuging the blood at $1000 \times g$ for 15 min. Liver lipids were extracted and purified by the method of Folch *et al.*[14]

Total cholesterol, HDL-cholesterol, and triacylglycerol in the serum were enzymatically measured by using commercial kits (cholesterol E-test, HDL-cholesterol test, and triglyceride E-test, respectively, from Wako Pure Chemical Industries, Osaka, Japan). Serum phospholipid was enzymatically measured with the commercial phospholipid B-test kit (Wako Pure Chemical Ind.). Total cholesterol in the liver extract, which had been dissolved in isopropyl alcohol, was enzymatically determined by a cholesterol mono-test kit (Boeringer Manheim Yamanouchi Co., Tokyo,



Astilbin: R = rhamnose
Taxifolin: R = H

Fig. Chemical Structures of Astilbin and Taxifolin.

Japan). To determine liver TBARS, 1 g of liver was homogenized with 9 ml of 1.15% KCl, and 0.5 ml of the resulting homogenate was then analyzed for its TRARS content by using the method of Uchiyama *et al.*[15]

The activities of erythrocyte superoxide dismutase (SOD),[16,17] catalase,[18] and glutathione peroxidase (GSH-Px)[19] were measured by using a hemolysate prepared by adding H_2O to the erythrocytes.[20] One unit of SOD and catalase activity is defined as the amount of enzyme requied to inhibit the rate of diformazan formation from nitro-blue tetrazolium (NBT) by 50% per mg of hemoglobin, and the amount required to decompose 1 μmol of H_2O_2 in 1 min per mg of hemoglobin, respectively. One unit of GSH-Px activity, which was measured by reacting residual GSH with 5,5'-dithiobis-2-nitrobenzoic acid after incubating a reaction mixture composed of H_2O_2, GSH and the enzyme solution, is defined as the amount of enzyme required for a decrease of 0.001 in absorbance at 412 nm in 1 min per mg of hemoglobin.[19] To determine the liver SOD activity, 1 g of frozen liver was homogenized with 5 ml of 0.2% Triton X-100 (v/v) at 4°C by using a glass-glass homogenizer, and then centrifuged at $10,000 \times g$ for 30 min at 4°C. An aliquot of the supernatant was then treated with a 0.25 volume of ethyl alcohol and 0.15 volume of chloroform, before being centrifuged at $5000 \times g$ for 20 min at 4°C. The supernatant obtained was used for an assay of the Cu-Zn SOD activity,[21] one unit being defined as already described. In addition, all the liver enzyme activity is expressed as that per mg of protein. The enzyme solution for determining the liver catalase activity was prepared according to the method of Rao *et al.*,[22] frozen liver tissue (0.6 g) being homogenized in 4 ml of a buffer containing 0.32 mol sucrose and 1 mmol EDTA per liter of 10 mM Tris–HCl (pH 7.8) in a Teflon-glass homogenizer. The supernatant obtained by centrifuging the homogenate at $13,600 \times g$ for 30 min at 4°C was used for measuring the enzyme activities. The activity of liver catalase was measured by using the same method as that used for measuring the erythrocyte catalase activity. The liver GSH-Px activity was measured according to the method of Hafeman *et al.*,[19] using the supernatant obtained by centrifuging the liver homogenate at $105,000 \times g$ for 60 min at 4°C,[23,24] in which the homogenate was prepared immediately after excising the liver from the rats. One unit of GSH-Px activity is defined as already mentioned. The activity of glutathione reductase (GSSG-R) was measured by the method of He *et al.*,[25] using the supernatant applied for measuring the liver GSH-Px activity, one unit of activity being defined as the amount of enzyme required to oxidize 1 μmol of NADPH in 1 min per mg of protein. The hemoglobin content in the hemolysate was determined by using a commercial Hemoglobin-test kit (Wako Pure Chemical Ind.), and the protein content in the enzyme solution was determined as described by Lowry *et al.*[26]

The data for each of the three groups were statistically analyzed by Duncan's multiple-range test after an analysis of variance (ANOVA). The data from the control group and from the group fed with astilbin or taxifolin were also compared, and significant differences between the two groups were determined by Student's *t*-test.

As shown in Table I, the ingestion of astilbin and taxifolin did not stastically affect the daily food intake, body weight gain, or the concentrations of serum total cholesterol, HDL-cholesterol, triacylglycerol, and phospholipid, although the serum TBARS concentrations in the rats fed with astilbin and taxifolin showed a significant decrease, or tendency to decrease, respectively, when analyzed by Student's *t*-test. The concentrations of total liver cholesterol in the rats fed with astilbin and taxifolin tended to decrease and significantly decrease, respectively, when analyzed by Student's *t*-test. The concentrations of liver phospholipid and TBARS were significantly decreased by feeding with astilbin and taxifolin, indicating that astilbin and taxifolin had activity to lower liver TBARS and phospholipid, and may act antioxidatively *in vivo*. Since it has been previously found that dietary antioxidative flavonoids such as isorhamnetin, rhamnetin, and quercetin caused a decrease in the contents of both liver total cholesterol and TBARS in the rats fed on a cholesterol-free diet,[20] it is noteworthy that astilbin and taxifolin, which are flavonoids, tended to decrease liver total cholesterol and significantly decreased the liver TBARS content. However, further studies are necessary to clarify any correlation between the cholesterol-lowering and antioxidative activities of astilbin and taxifolin.

In order to determine the cause of lowering the serum and liver TBARS concentrations by feeding with astilbin and taxifolin, the effects of these compounds on the erythrocyte and liver antioxidative enzyme activities were also determined. The activities

Table I. Effects of Astilbin and Taxifolin on the Growth, Food Intake, Body Weight Gain, and Serum and Liver Lipid Concentrations in Rats Fed on a Cholesterol-free Diet

	20C	20C + A	20C + T
Initial body weight (g)	69 ± 1[a]	69 ± 2[a]	69 ± 2[a]
Food intake (g/10 days)	110 ± 1[a]	111 ± 2[a]	111 ± 2[a]
Body weight gain (g/10 days)	38 ± 1[a]	36 ± 3[a]	36 ± 2[a]
Liver weight (% of body weight)	3.4 ± 0.1[a]	3.5 ± 0.1[a]	3.7 ± 0.1[a]
Serum lipids			
Total cholesterol (mg/dl)	82.0 ± 2.5[a]	78.5 ± 3.7[a]	81.4 ± 2.5[a]
HDL-cholesterol (mg/dl)	57.0 ± 1.3[a]	56.3 ± 3.2[a]	58.2 ± 2.2[a]
Triacylglycerol (mg/dl)	28.1 ± 2.2[a]	27.2 ± 2.1[a]	30.3 ± 2.9[a]
Phospholipid (mg/dl)	120 ± 3[a]	118 ± 5[a]	126 ± 3.7[a]
TBARS[1] (nmol/ml of blood)	2.16 ± 0.16[a]	1.77 ± 0.02[a]*	1.66 ± 0.25[a]
Liver lipids			
Total cholesterol (mg/g of liver)	2.58 ± 0.10[a]	2.20 ± 0.20[a]	2.22 ± 0.08[a]*
Triacylglycerol (mg/g of liver)	8.06 ± 0.38[a]	7.77 ± 0.76[a]	7.68 ± 0.48[a]
Phospholipid (mg/g of liver)	12.6 ± 0.3[a]	9.92 ± 0.72[b]	10.6 ± 0.3[b]
TBARS[1] (nmol/g of liver)	72.5 ± 5.0[a]	53.4 ± 8.1[a]*	54.3 ± 6.7[a]*

20C, 20%-casein diet; 20C + A, 20%-casein + 0.074%-astilbin diet; 20C + T, 20%-casein + 0.05%-taxifolin diet.

[1] Thiobarbituric acid-reactive substances (TBARS) are expressed as the amounts of malondialdehyde (nmol).

Values are means ± SEM of 5 to 6 rats per group. Differences between three groups were analyzed by Duncan's multiple-range test: values within the same row and not sharing a common superscript letter are significantly different at $p < 0.05$. * Significantly different from the 20C group at $p < 0.05$ when analyzed by Student's *t*-test.

Table II. Antioxidative Enzyme Activities in the Erythrocytes and Liver of Rats Fed with Astilbin and Taxifolin

	20C	20C + A	20C + T
Erythrocytes			
SOD (U/mg of Hb)	2.77 ± 0.13[a]	2.78 ± 0.11[a]	2.85 ± 0.14[a]
Catalase (U/mg of Hb)	45.5 ± 2.5[a]	50.9 ± 2.1[a]	54.0 ± 4.0[a]
GSH-Px (U/mg of Hb)	8.06 ± 0.99[a]	7.45 ± 1.31[a]	9.55 ± 2.74[a]
Liver			
SOD (U/mg of protein)	3.36 ± 0.07[a]	3.31 ± 0.11[a]	3.60 ± 0.17[a]
Catalase (U/mg of protein)	276 ± 20[a]	277 ± 13[a]	256 ± 16[a]
GSH-Px (U/mg of protein)	9.70 ± 0.60[a]	9.80 ± 0.90[a]	10.4 ± 0.9[a]
GSSG-R (U/mg of protein)	8.15 ± 0.40[a]	9.63 ± 0.66[a]	9.32 ± 0.80[a]

SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GSSG-R, glutathione reductase.

Values are means ± SEM of 5 to 6 rats per group. Values within the same row and not sharing a common superscirpt letter are significantly different at $p < 0.05$.

of erythrocytes SOD and GSH-Px in the rats fed with astilbin and taxifolin did not differ from those of the control rats, although the catalase activity tended to be increased by feeding with these compounds. The activities of liver SOD, catalase, GSH-Px, and GSSG-R were not influenced by feeding with astilbin and taxifolin (Table II). On the other hand, astilbin and taxifolin showed antioxidative activity *in vitro* when the antioxidative activity of these compounds was measured by using a β-carotene-lipoxygenase-linoleic acid system[20] (inhibition of carotene bleaching: astilbin, 12%; taxifolin, 14%). These results suggest that the serum and liver antioxidative enzyme activities were scarcely influenced by the dietary astilbin and taxifolin, and that the lower TBARS concentration that resulted by fedding with these compounds was mainly brought about by their direct antioxidative activities *in vivo*. However, since it has already been reported that the gastrointestinal absorption of taxifolin was minimal,[27] the amounts of astilbin and taxifolin absorbed from the gastrointestinal tract may be small. No difference in activity between astilbin and taxifolin for lowering liver total cholesterol and phospholipid, and serum and liver TBARS concentrations may suggest that astilbin and taxifolin had almost the same activities after being absorbed by the gastrointestinal tract, and/or that astilbin was hydrolyzed to taxifolin or its related compounds *in vivo* to demonstrate the same activity as that of taxifolin. In the previous paper, since it was found that the fecal excretion of cholesterol and bile acid was hardly influenced by feeding taxifolin to the rats fed on a cholesterol-free diet,[12] taxifolin may exert cholesterol-lowering activity through its influence on the endogenous cholesterol metabolism. This study indicates that astilbin, the major flavonoid in Kohki tea, had activities to decrease the concentrations of serum and liver TBARS and of liver phospholipid in the rats.

References

1) T. L. Lunder, in "Phenolic Compounds in Food and Their Effects on Health II," ed. by M-T. Huang, C-T. Ho, and C. Y. Lee, American Chemical Society, Washington, 1992, pp. 114–120.
2) T. Matsuzaki and Y. Hara, *Nippon Nōgeikagaku Kaishi* (in Japanese), **59**, 129–134 (1985).
3) G. C. Yen and H. Y. Chen, *J. Agric. Food Chem.*, **43**, 27–32 (1995).
4) T. Okuda, Y. Kimura, T. Yoshida, T. Hatano, H. Okuda, and S. Arichi, *Chem. Pharm. Bull.*, **31**, 1625–1631 (1983).
5) Y. Hara, T. Matsuzaki, and T. Suzuki, *Nippon Nōgeikagaku Kaishi* (in Japanese), **61**, 803–805 (1987).
6) A. H. Conney, Z. Y. Wang, C-T. Ho, C. S. Yang, and M-T. Huang, in "Phenolic Compounds in Food and Their Effects on Health II," ed. by M-T. Huang, C-T. Ho, and C. Y. Lee, American Chemical Society, Washington, 1992, pp. 284–291 (1992).
7) K. Namiki, M. Yamanaka, C. Tateyama, M. Igarashi, and M. Namiki, *Nippon Shokuhin Kogyo Gakkaishi* (in Japanese), **38**, 189–195 (1991).
8) M. Fukuyo, Y. Hara, and K. Muramatsu, *Nippon Eiyo Shokuryo Gakkaishi* (in Japanese), **39**, 495–500 (1986).
9) K. Muramatsu, M. Fukuyo, and Y. Hara, *J. Nutr. Sci. Vitaminol.*, **32**, 613–622 (1986).
10) R. Kasai, S. Hirono, W-H. Chou, O. Tanaka, and F.-M. Chen, *Chem. Pharm. Bull.*, **36**, 4167–4170 (1988).
11) A. E. Harper, *J. Nutr.*, **68**, 405–419 (1959).
12) S. Itaya and K. Igarashi, *Biosci. Biotech. Biochem.*, **56**, 1492–1494 (1992).
13) K. Yagi, *Biochemical Medicine*, **15**, 212–216 (1976).
14) J. Folch, M. Lees, and H. S. Stanley, *J. Biol. Chem.*, **226**, 497–509 (1957).
15) M. Uchiyama and M. Mihara, *Anal. Biochem.*, **86**, 271–278 (1978).
16) C. Beauchamp and I. Fridovich, *Anal. Biochem.*, **44**, 276–287 (1971).
17) T. Imanari, M. Hirota, M. Miyazaki, and K. Hayakawa, *Igaku no Arumi* (in Japanese), **101**, 496–497 (1977).
18) K. Tomita and M. Sano, in "Kasanka Shishitsu Jikkenhō" (in Japanese), ed. by T. Kaneda and N. Ueda, Ishiyaku Shuppan Co., Tokyo, 1983, pp. 149–150.
19) D. G. Hafeman, R. A. Sunde, and W. G. Hoekstra, *J. Nutr.*, **104**, 580–587 (1974).
20) K. Igarashi and M. Ohnuma, *Biosci. Biotech. Biochem.*, **59**, 595–601 (1995).
21) D. I. Paynter, R. J. Moir, and E. J. Underwood, *J. Nutr.*, **109**, 1570–1576 (1979).
22) G. Rao, E. Xia, M. J. Nadakavukaren, and A. Richardson, *J. Nutr.*, **120**, 602–609 (1990).
23) P. B. Rowe and J. B. Wyngaarden, *J. Biol. Chem.*, **24**, 5571–5576 (1966).
24) F. Stirpe and E. D. Corte: *J. Biol. Chem.*, **244**, 3855–3863 (1969).
25) P. He and K. Yasumoto, *Nippon Eiyo Shokuryo Gakkaishi* (in Japanese), **43**, 121–125 (1990).
26) O. H. Lowry, N. J. Rosebrough, A. J. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265–275 (1951).
27) S. Brown and L. A. Griffiths, *Experimentia*, **39**, 198–200 (1983).