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Studies on the Activities of Tannins and Related Compounds from Medicinal Plants and Drugs. VII.[1] Effects of Extracts of Leaves of *Artemisia* Species, and Caffeic Acid and Chlorogenic Acid on Lipid Metabolic Injury in Rats Fed Peroxidized Oil

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The effects of oral administration of the extracts from the leaves of several *Artemisia* species, and of chlorogenic acid, which is one of their components, and also of caffeic acid (which can be produced by partial hydrolysis of caffeoylquinic acids, which are the main polyphenolic components in these species) on lipid metabolic injury produced in rats by feeding peroxidized oil were investigated. The acetone-water extracts of the leaves of *Artemisia princeps, A. montana* and *A. capillaris* reduced the elevation of lipid peroxide concentration in the serum in peroxidized oil-fed rats, and the above extracts reduced the levels of liver triglyceride. The extracts of *A. montana* and *A. capillaris* inhibited the elevation of serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase. Caffeic acid and chlorogenic acid also inhibited the elevation of serum triglyceride, lipid peroxides, total cholesterol, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase, as well as liver lipid peroxide.

Keywords——*Artemisia princeps*; *A. montana*; *A. capillaris*; tannin; caffeetannin; lipid metabolism; caffeic acid; chlorogenic acid; liver injury

The dried leaves of some *Artemisia* species have been used for the treatment of inflammation, blood diseases caused by disturbance of menses, hematemesis and hemorrhoids in Chinese and Korean traditional medicine. In Japan, *A. princeps* PAMPAN. and *A. montana* PAMPAN. are the main species used for analogous purposes. Furthermore, *A. capillaris* THUNB. is used for the treatment of liver injury in Japanese traditional medicine.

Okuda *et al.*[2] previously reported the isolation of chlorogenic acid, methyl chlorogenate, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid from the leaves of *A. montana*. These compounds have sometimes been called "caffeetannins."[3] Among them, dicaffeoylquinic acids, which are also contained in the leaves of *A. princeps* and *A. capillaris*, were found to be the main components responsible for the tannic activities of these species, as determined[2] by the relative astringency (RA) and relative affinity for methylene blue (RMB) methods.[4] In a previous paper,[5] we reported that dicaffeoylquinic acids strongly inhibited the adenosine 5′-diphosphate (ADP) and ascorbic acid-induced lipid peroxidation in mitochondria and the ADP and reduced nicotinamide adenine dinucleotide phosphate (NADPH)-induced lipid peroxidation in microsomes of liver.

In higher animals, lipid peroxides are known to injure the liver and lipid metabolism.[6]

The present paper describes the *in vivo* effects of extracts of several species of *Artemisia*, and of chlorogenic acid, and also of caffeic acid, which is produced by hydrolysis of caffeoylquinic acids contained in these extracts, on rat lipid metabolic injury induced by oral administration of peroxidized corn oil.

Materials and Methods

Materials——*Artemisia princeps* was collected in the garden of the Faculty of Pharmaceutical Sciences, Okayama University, in October, 1983. *A. montana* and *A. capillaris* were collected in Tobetsu-cho, Hokkaido, and Asahikawa, Okayama, in September and October, 1983, respectively. The plant extracts were obtained as follows. Dried leaves (30 g) of each species of *Artemisia* were homogenized in acetone–water (7 : 3, v/v, 3 × 300 ml) and filtered. The combined filtrate was evaporated under reduced pressure below 40 °C. The yields of extracts were as follows: *A. montana* (8.9 g), *A. princeps* (10.6 g), *A. capillaris* (6.0 g). Chlorogenic acid and caffeic acid were purchased from Wako Chemical Co. Peroxidized corn oil was prepared by bubbling oxygen through corn oil at 150 °C for 1 h. The lipid peroxide value of the corn oil increased from 0.56 to 54.7 nmol/ml during this treatment.

Animals——Male Wistar-King strain rats weighing 150—160 g (6 weeks old) were housed in a room maintained at 25 ± 1 °C with 60% relative humidity and given free access to food and water. The room was illuminated for 12 h a day starting at 7 : 00 a.m.

Measurements of Serum and Liver Lipids, and Serum Transaminases in Rats Fed the Peroxidized Corn Oil——The rats were given the peroxidized corn oil (10 ml/kg body weight × 2 times/d) orally for 1 week. The same rats received the extracts (250 mg/kg × 2 times/d), caffeic acid (25 or 50 mg/kg × 2 times/d) or chlorogenic acid (12.5 or 25 mg/kg × 2 times/d) orally for 1 week together with the peroxidized corn oil. Blood was taken by cardiac puncture 4 h after the administration of the peroxidized corn oil and centrifuged at 1630 × *g* for 10 min to separate the serum. Total cholesterol (TC), triglyceride (TG), free fatty acids (FFA), high density lipoprotein-cholesterol (HDL-ch), lipid peroxide (LPO), glutamic oxaloacetic transaminase (GOT) (EC 2.6.1.1) and glutamic pyruvic transaminase (GPT) (EC 2.6.1.2) in the sera were determined by the methods of Zak,[7] Fletcher,[8] Itaya and Ui,[9] Ash and Hentschel,[10] Ohkawa *et al.*[11] and Reitman and Frankel,[12] respectively. After the liver weight had been estimated, 1 g of liver tissue was homogenized in 9 ml of saline solution. The homogenate (0.2 ml) was extracted with $CHCl_3$–MeOH (2 : 1) (4 ml), and the extract was dried. The residue was analyzed for TC and TG by the methods described above. Liver homogenate was directly used for estimation of LPO.

TABLE I(a). Content of Caffeoylquinic Acids in the Extracts of *Artemisia* Species

Source of extract	Chlorogenic acid (%)	3,5-Di-*O*-caffeoyl-quinic acid (%)	3,4-Di-*O*-caffeoyl-quinic acid (%)	4,5-Di-*O*-caffeoyl-quinic acid (%)
Artemisia princeps	3.7	17.0	3.0	1.6
Artemisia montana	3.4	13.4	3.2	1.4
Artemisia capillaris	3.9	6.8	1.0	1.6

TABLE I(b). Tannin Activity of the Extracts of *Artemisia* Species and Caffeoylquinic Acids

	RMB	RA
Source of extract		
Artemisia princeps	0.10	0.06
Artemisia montana	0.09	0.04
Artemisia capillaris	0.05	0.02
Caffeoylquinic acids		
Chlorogenic acid	0.004	0.05
3,5-Di-*O*-caffeoylquinic acid	0.25	0.18
3,4-Di-*O*-caffeoylquinic acid	0.25	0.09
4,5-Di-*O*-caffeoylquinic acid	0.15	0.11

The RMB and RA values (as tannin) were expressed in units based on tannic acid (JP X) as a standard.

Determination of Caffeoylquinic Acids in the Extracts of *Artemisia* Species by High-Performance Liquid Chromatography (HPLC)——Determination of chlorogenic acid, methyl chlorogenate, 3,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid was performed on a YMC-Pack ODS (A312) column (6 × 150 mm) using ultraviolet (UV) absorption measurement at 254 nm for detection, with 0.1 M H_3PO_4–0.1 M KH_2PO_4–EtOH–EtOAc (17 : 17 : 4 : 2) as the eluent. Estimation of the amount of each caffeoylquinic acid was based on the peak area on the chromatogram relative to that of a standard sample. The results are shown in Table I(a).

Determination of Tannin Activity of the Extracts of *Artemisia* Species and Caffeoylquinic Acids——The activities of the extracts and caffeoylquinic acids (as tannin) were determined by the RMB and RA methods previously reported.[4] The RA and RMB values were expressed in units based on tannic acid (JP X) as a standard. The values determined are shown in Table I(b).

Statistical Analysis——The data were expressed as means ± standard errors. The statistical analysis was done by the use of Student's *t*-test.

Results

Effects of the Extracts of *Artemisia* Species on Body and Liver Weight, Serum and Liver Lipids, and Serum Transaminases

As shown in Tables II, III and IV, administration of the peroxidized corn oil for 1 week caused reduction of body weight, hyperlipemia with the elevation of TC, LPO, FFA and TG, fatty liver with the accumulation of TC, TG and LPO, and liver injury with the elevation of GOT and GPT as compared to the control group. However, body and liver weights were not changed in the rats orally given the extracts of the leaves of *Artemisia* species (500 mg/kg/d) as compared to the peroxidized corn oil-treated rats. Serum LPO levels were reduced in the rats orally given the extracts (500 mg/kg/d) of the leaves of *A. princeps, A. montana* and *A. capillaris* as compared to the peroxidized oil-fed rats. The elevation of serum TG was inhibited by oral administration of the extracts of the leaves of *A. princeps*, and liver LPO levels were reduced by the extracts of *A. princeps* and *A. capillaris* as compared to the peroxidized corn oil-treated group. Liver TC levels were also reduced by the extracts of *A. capillaris*. However, these extracts had no effect on the serum TC and atherogenic index (TC − HDL-ch/TC).

Effects of Caffeic Acid and Chlorogenic Acid on Body and Liver Weights, Serum and Liver Lipids, and Serum Transaminases

As shown in Table V, it was found that the oral administration of caffeic acid and chlorogenic acid (50 mg/kg/d) slightly inhibited the reduction of body weight as compared to the peroxidized oil-fed rats, while the liver weight was not changed. As shown in Tables VI

TABLE II. Effects of the Extracts of *Artemisia* Species on Body Weight and Liver Weight in Rats Fed Peroxidized Oil for 1 Week

	Body weight (g)		Liver weight (g)
	Initial	Final	
Control	152 ± 3	203 ± 2[a]	8.0 ± 0.32 N.S.
Peroxidized oil-treated rats (5)	153 ± 2	164 ± 7	7.9 ± 0.33
Artemisia princeps, extract (500 mg/kg/d) (6)	151 ± 4	171 ± 8 N.S.	8.1 ± 0.54 N.S.
Artemisia montana, extract (500 mg/kg/d) (6)	153 ± 3	171 ± 7 N.S.	8.3 ± 0.50 N.S.
Artemisia capillaris, extract (500 mg/kg/d) (6)	151 ± 5	161 ± 9 N.S.	7.8 ± 0.42 N.S.

Significantly different from the peroxidized oil-treated rat group. *a*) $p < 0.05$. N.S., not significant. The results are expressed as means ± standard errors.

TABLE III. Effects of the Extracts of *Artemisia* Species on Serum Lipids in Rats Fed Peroxidized Corn Oil for 1 Week

	TC (mg/dl)	HDL-ch (mg/dl)	Atherogenic index	LPO (MDA nmol/ml)	FFA (meq/l)	TG (mg/dl)
Control (5)	67.3±6.9[a]	40.7±1.26[a]	0.37±0.07[a]	3.03±0.42[a]	0.125±0.026[a]	61.6± 6.46[a]
Peroxidized oil-treated rats (5)	172.7±27.2	32.8±2.53	0.79±0.037	10.1 ±3.34	1.19 ±0.180	378.8± 86.2
Artemisia princeps, extract (500 mg/kg/d) (6)	124.0±10.6 N.S.	39.7±3.70 N.S.	0.67±0.043 N.S.	4.56±0.64[a]	0.830±0.119 N.S.	117.9± 38.6[a]
Artemisia montana, extract (500 mg/kg/d) (6)	139.4±21.7 N.S.	44.7±1.39[a]	0.65±0.040 N.S.	4.27±0.94[a]	0.786±0.117 N.S.	171.1±104.8 N.S.
Artemisia capillaris, extract (500 mg/kg/d) (6)	153.3±42.8 N.S.	39.1±4.87 N.S.	0.72±0.052 N.S.	4.30±0.62[a]	0.935±0.081 N.S.	210.8± 92.5 N.S.

Significantly different from the peroxidized oil-treated group. *a*) $p<0.01$. N.S., not significant. TC, total cholesterol; HDL-ch, high density lipoprotein-cholesterol; atherogenic index, TC−HDL-ch/TC; LPO, lipid peroxides; FFA, free fatty acids; TG, triglyceride; MDA, malondialdehyde. The results are expressed as means±standard errors.

TABLE IV. Effects of the Extracts of *Artemisia* Species on Serum Transaminases and Liver Lipids in Rats Fed Peroxidized Corn Oil for 1 Week

	Serum		Liver		
	GOT (Karmen units)	GPT (Karmen units)	TC (mg/g)	TG (mg/g)	LPO (MDA, nmol/g)
Control (5)	103.5±5.61[b]	21.0± 1.63[b]	4.40±0.15[b]	11.0±1.57[b]	114.5±11.2[b]
Peroxidized oil-treated rats (5)	405.6±92.3	222.0±41.5	10.1 ±0.84	28.6±3.42	263.7±39.3
Artemisia princeps, extract (500 mg/kg/d) (6)	228.7±20.2 N.S.	144.0±43.9 N.S.	9.03±0.97 N.S.	13.9±3.10[b]	108.8± 7.41[b]
Artemisia montana, extract (500 mg/kg/d) (6)	151.3±20.6[b]	77.3±14.1[b]	8.44±0.72 N.S.	17.9±2.47[a]	172.7±25.0 N.S.
Artemisia capillaris, extract (500 mg/kg/d) (6)	185.3±12.3[a]	80.7±13.9[b]	7.58±0.56[a]	11.6±2.91[b]	114.5± 9.31[b]

Significantly different from the peroxidized oil-treated group. *a*) $p<0.05$. *b*) $p<0.01$. N.S., not significant. GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; TC, total cholesterol; TG, triglycerides; LPO, lipid peroxides; MDA, malondialdehyde. The results are expressed as means±standard errors.

TABLE V. Effects of Caffeic Acid and Chlorogenic Acid on Body Weight and Liver Weight in Rats Fed Peroxidized Corn Oil for 1 Week

	Body weight (g) Initial	Body weight (g) Final	Liver weight (g)
Control (5)	151±3	202±2[b]	7.8±0.10 N.S.
Peroxidized oil-treated rats (6)	153±1	146±8	7.7±0.53
Caffeic acid (50 mg/kg/d) (6)	153±5	165±3[a]	8.4±0.19 N.S.
(100 mg/kg/d) (6)	151±4	151±5 N.S.	7.7±0.37 N.S.
Chlorogenic acid (25 mg/kg/d) (6)	152±3	160±4 N.S.	8.3±0.39 N.S.
(50 mg/kg/d) (6)	153±2	166±3[a]	8.1±0.21 N.S.

Significantly different from the peroxidized group. *a*) $p<0.05$. *b*) $p<0.01$. N.S., not significant. The results are expressed as means±standard errors.

TABLE VI. Effects of Caffeic Acid and Chlorogenic Acid on Serum Lipids in Rats Fed Peroxidized Corn Oil for 1 Week

	TC (mg/dl)	HDL-ch (mg/dl)	Atherogenic index	LPO (MDA, nmol/ml)	FFA (meq/l)	TG (mg/dl)
Control (5)	73.3± 4.65[b]	28.3±0.65[a]	0.61±0.023[b]	3.22±0.32[a]	0.281±0.041[b]	56.5± 5.69[b]
Peroxidized-treated rats (6)	165.0±16.7	22.2±2.60	0.85±0.027	4.89±0.68	1.23 ±0.221	291.2±39.1
Caffeic acid (50 mg/kg/d) (6)	105.4± 7.10[b]	40.7±3.25[b]	0.60±0.044[b]	2.53±0.46[b]	1.04 ±0.291 N.S.	147.8±17.7[b]
(100 mg/kg/d) (6)	140.7±13.5 N.S.	34.6±6.33 N.S.	0.74±0.056 N.S.	4.48±0.79 N.S.	0.875±0.186 N.S.	145.8±30.7[b]
Chlorogenic acid (25 mg/kg/d) (6)	110.4±10.5[b]	40.1±2.99[b]	0.62±0.046[b]	3.13±0.44[a]	0.510±0.020[b]	60.5±12.3[b]
(50 mg/kg/d) (6)	103.5± 6.71[b]	41.8±2.01[b]	0.59±0.024[b]	2.43±0.33[b]	0.628±0.070[b]	81.1± 7.87[b]

Significantly different from the peroxidized oil-treated group. *a*) $p<0.05$. *b*) $p<0.01$. N.S., not significant. TC, total cholesterol; HDL-ch, high density lipoprotein-cholesterol; atherogenic index, TC−HDL-ch/TC; LPO, lipid peroxides; MDA, malondialdehyde; TG, triglycerides. The results are expressed as means±standard errors.

TABLE VII. Effects of Caffeic Acid and Chlorogenic Acid on Serum Transaminases and Liver Lipids in Rats Fed peroxidized Corn Oil for 1 Week

	Serum GOT (Karmen units)	Serum GPT (Karmen units)	Liver TC (mg/g)	Liver TG (mg/g)	Liver LPO (MDA, nmol/g)
Control (5)	67.0± 8.71[b]	20.8± 2.85[b]	4.54±0.45[b]	10.3±0.74[b]	175.8±21.5[b]
Peroxidized oil-treated rats (6)	499.2±106.8	498.3±93.1	7.86±0.70	22.6±4.52	445.1±82.4
Caffeic acid (50 mg/kg/d) (6)	117.5± 15.5[b]	92.5±14.7[b]	7.68±0.32 N.S.	19.3±3.85 N.S.	247.6±32.7[a]
(100 mg/kg/d) (6)	179.2± 18.1[b]	144.3±23.7[b]	8.61±0.84 N.S.	17.0±2.71 N.S.	257.6±55.5 N.S.
Chlorogenic acid (25 mg/kg/d) (6)	87.5± 7.72[b]	89.2±12.1[b]	8.09±0.42 N.S.	16.4±1.93 N.S.	263.9±30.3[a]
(50 mg/kg/d) (6)	92.5± 11.2[b]	79.6± 7.31[b]	7.40±0.70 N.S.	13.1±1.10[a]	263.9±29.7[a]

Significantly different from the peroxidized oil-treated group. *a*) $p<0.05$. *b*) $p<0.01$. N.S., not significant. GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; TC, total cholesterol; TG, triglycerides; LPO, lipid peroxides; MDA, malondialdehyde. The results are expressed as means±standard errors.

and VII, serum TC, LPO and TG levels, and liver LPO were reduced by caffeic acid (50 mg/kg/d) and chlorogenic acid (25 and 50 mg/kg/d) as compared to the peroxidized oil-fed group, while caffeic acid at the dose of 100 mg/kg/d did not change serum TC or LPO. In the peroxidized corn oil-fed rats, the oral administration of caffeic acid (50 and 100 mg/kg/d) and chlorogenic acid (25 and 50 mg/kg/d) inhibited the elevation of serum GOT and GPT (Table VII).

Discussion

The present investigation demonstrated that the extracts from leaves of *Artemisia* species, as well as caffeic acid and chlorogenic acid, affect liver injury and lipid metabolism in peroxidized corn oil-fed rats. A high-fat diet containing peroxidized corn oil is known to induce fatty liver, hyperlipemia and liver injury with the elevation of serum GOT and GPT in rats.[6] In the present experiments, administration of peroxidized corn oil for 1 week induced hyperlipemia with the elevation of TC, TG, LPO and FFA, fatty liver with the accumulation of liver TC, TG and LPO, and liver injury with the elevation of serum GOT and GPT as compared to the control group. It was postulated that functional disorder of the liver of rats fed peroxidized corn oil might be caused by lipid peroxide accumulated in the liver. Administration of the leaf extract of *A. princeps* reduced the levels of serum and liver TG and LPO as compared to the peroxidized oil-fed rats. The administration of the leaf extract of *A. montana* also reduced the serum LPO, GOT and GPT and liver TG. Furthermore, the administration of the leaf extract of *A. capillaris* also reduced the serum LPO, GOT and GPT, and liver TC, TG and LPO in the peroxidized oil-fed group. Caffeic acid and chlorogenic acid, which are contained in the above medicinal plants, or are presumed to be produced by hydrolysis of caffeoylquinic acids, also inhibited the elevation of serum TC, LPO, TG, GOT and GPT, and liver LPO as compared to the peroxidized oil-treated group. The content of dicaffeoylquinic acids (such as 3,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid) increased in the order *A. capillaris* extract < *A. montana* extract < *A. princeps* extract (used in the experiment, Table I). In a previous paper[5] of this series, we reported that in *in vitro* experiments, caffeic acid and caffeetannins inhibited the production of lipid peroxide induced by ADP and NADPH in liver microsomes and by ADP and ascorbic acid in mitochondria. From these findings, two possible mechanisms can be suggested for the protective actions of the extracts containing caffeetannins and related compounds against liver injury. One is that caffeetannins in the extracts of *Artemisia* species inhibit further production of lipid peroxide in rats fed the peroxidized oil. The other is that these caffeetannins containing the extract of *Artemisia* species inhibit the destructive action of lipid peroxide on liver cells.

In these experiments, the extracts from *Artemisia* species inhibited the elevation of serum and liver TG levels in the peroxidized oil-treated rats. Caffeic acid and chlorogenic acid were also found to reduce the serum and liver lipids. From these results, a possible mechanism is that caffeetannins and related compounds contained in *Artemisia* species may modulate lipid metabolism, *e.g.* by inhibition of lipid absorption in the small intestine, inhibition of the biosynthesis of triglyceride in the liver and acceleration of lipid utilization in muscles. Further work is needed to assess this hypothesis. The administration of 50 mg/kg/d of caffeic acid reduced serum TC, LPO and TG, while the administration of 100 mg/kg/d of caffeic acid had no effect on serum TC and LPO. There was no clear dose-response relationship in the case of caffeic acid. It seems unlikely that lipid metabolism can be regulated by the administration of large amounts of caffeic acid, because of the side effects of caffeic acid. Therefore, further work is needed to clarify the effects of *Artemisia* species and caffeetannins on lipid metabolism in non-treated rats.

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