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Studies on the Activities of Tannins and Related Compounds from Medicinal Plants and Drugs. IV.[1] Effects of Various Extracts of Geranii Herba and Geraniin on Liver Injury and Lipid Metabolism in Rats Fed Peroxidized Oil

YOSHIYUKI KIMURA,*[,a] HIROMICHI OKUDA,[a] KAZUKO MORI,[b] TAKUO OKUDA,[b] and SHIGERU ARICHI[c]

2nd Department of Medical Biochemistry, School of Medicine, Ehime University,[a] Shigenobu-cho, Onsen-gun, Ehime 791-02, Japan, Faculty of Pharmaceutical Sciences, Okayama University,[b] Tsushima, Okayama 700, Japan, and The Research Institute of Oriental Medicine, Kinki University,[c] Sayama-cho, Minamikawachi-gun, Osaka 589, Japan

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The effects of several kinds of extracts from the herb *Geranium thunbergii* and of geraniin, the main components of tannin of this herb, on liver injury produced in rats by feeding peroxidized oil were investigated. The acetone–water and water extracts, and also geraniin, were found to reduce lipid peroxide concentrations in the serum and liver of the animals with liver injury. These extracts and geraniin were also found to reduce the levels of serum cholesterol, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in the peroxidized oil-treated rats.

Keywords——*Geranium thunbergii*; tannin; lipid metabolism; lipid peroxide; liver injury; glutamic oxaloacetic transaminase; glutamic pyruvic transaminase; geraniin

Geranii Herba ("Gen-no-shoko" in Japanese), the herb of *Geranium thunbergii* SIEB. *et* ZUCC. has been used in Japanese traditional medicine as a remedy for diarrhea induced by inflammation of the small intestine. In a previous paper,[2] we reported that geraniin[3] and corilagin[3] isolated from Geranii Herba strongly inhibited the adenosine 5′-diphosphate (ADP) and ascorbic acid-induced lipid peroxidation in mitochondria and the ADP and nicotinamide adenine dinucleotide phosphate (NADPH)-induced lipid peroxidation in microsomes. Furthermore, geraniin and corilagin inhibited the adrenaline-induced lipolysis in isolated fat cells and enhanced the ACTH-induced lipolysis in fat cells.[4]

In higher animals, lipid peroxides are known to injure the liver, kidney and blood vessels.[5,6] The present paper describes the *in vivo* effects of various extracts from Geranii Herba, and of geraniin, the main component of tannin of this herb, on rat liver injury induced by oral administration of peroxidized corn oil, and on lipid metabolism.

Materials and Methods

Materials——The preparation of the various extracts of Geranii Herba is shown in Chart 1. Geraniin was isolated by the method described by Okuda *et al.*[3a] Peroxidized corn oil was prepared by bubbling oxygen through corn oil at 180 °C for 1 h. The lipid peroxide value of corn oil increases from 1.9 nmol to 126.9 nmol/ml during this treatment.

Animals——Male Wistar–King strain rats weighing 200—240 g (8 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.

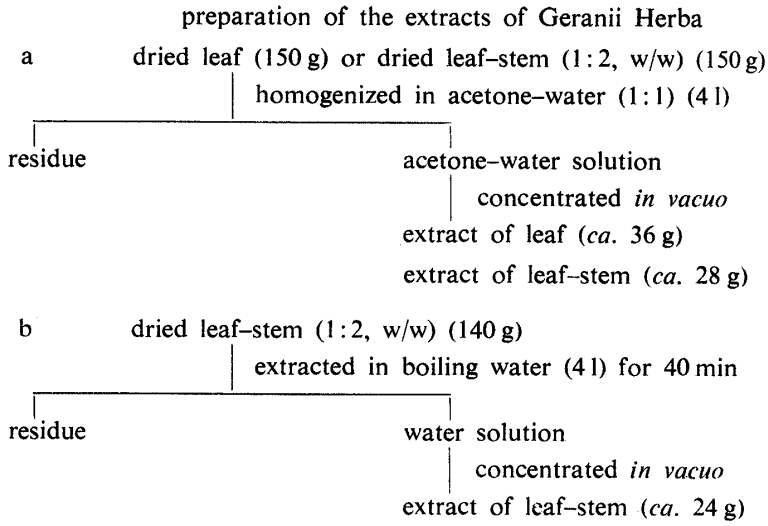

Chart 1

TABLE I. Contents of Tannins and Flavonoids in the Extracts of Geranii Herba

Extraction method	Homogenized in acetone–water (1:1)		Extracted in boiling water
Compounds (%)	Leaf	Leaf–stem (1:2, w/w)	Leaf–stem (1:2, w/w)
Geraniin	18.40	11.70	0
Corilagin	2.95	3.80	7.57
Ellagic acid	0.92	0.75	1.38
Kaempferitrin	2.76	1.48	1.88
Quercetin	<0.1	<0.1	<0.1
Tannin content (determined by RA and RMB method)	RA=0.31 RMB=0.39	RA=0.28 RMB=0.30	RA=0.15 RMB=0.15

A mixture of dried leaf and stem (1:2, w/w). Contents of compounds in the residue after the evaporation of solvents were determined by HPLC.

Measurements of Serum and Liver Lipids, and Serum Transaminases (GOT and GPT) 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 5 d. The same rats received the various extracts (150 mg/kg × 2 times/d) or geraniin (25 mg/kg × 2 times/d or 50 mg/kg × 2 times/d) orally for 5 d together with the peroxidized corn oil. Blood taken by cardiac puncture 5 h after the administration of the peroxidized corn oil was 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 peroxides (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] Yagi *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 and concentrated. The residue was analyzed for TC and TG by the methods described above. Liver homogenate was directly subjected to estimation of LPO.

Measurements of Hydrolyzable Tannins Such as Geraniin and Corilagin, Flavonoids Such as Quercetin and Kaempferitrin, and Ellagic Acid in the Various Extracts of Geranii Herba——Geraniin, corilagin, quercetin, kaempferitrin and ellagic acid in the various extracts were determined by high-performance liquid chromatography (HPLC) based on the methods of Okuda *et al.*[3c,d] This was performed on a YMC-Pack ODS column (6 × 150 mm)

using ultraviolet (UV) abosrption measurement at 280 nm for detection. The analysis of tannins was carried out by developing with mixture of 0.1 M KH_2PO_4 and 0.1 M H_3PO_4, ethanol and ethyl acetate (100 : 10 : 5, v/v) at a flow rate of 2.1 ml/min under a pressure of 85—90 kg/cm^2 at 40 °C. Flavonoids and their glycosides were analyzed by developing with a mixture of 0.1 M KH_2PO_4 and 0.1 M H_3PO_4, and acetonitrile (82 : 18, v/v) at a flow rate of 2.4 ml/min under a pressure of 110—115 kg/cm^2 at 40 °C.

Measurements of Tannin Content——Tannin contents in the various extracts of Geranii Herba were also determined as relative astrigency (RA) and relative activity on methylene blue (RMB) by the methods of Okuda *et al.*[13] The fundamental activities of tannins determined by the RA and RMB measurements are shown in Table I.

Results

Effects of the Various Extracts on Serum and Liver Lipids, and Serum Transaminases (GOT and GPT)

As shown in Table II, the administration of the peroxidized corn oil for 5 d caused hyperlipemia with the elevation of TC, LPO, FFA and TG as compared to the control values. Serum TC levels were found to be reduced in the rats orally given the acetone–water (1 : 1) extract (300 mg/kg/d) of the leaf and the leaf–stem (1 : 2), and the water extract (300 mg/kg/d) of the leaf–stem (1 : 2) as compared with the peroxidized corn oil-treated rats. The oral administration of the above extracts had no effect on the serum HDL-ch, while the atherogenic index (TC–HDL-ch/HDL-ch) was reduced in the rats orally given the above extracts (300 mg/kg/d) as compared with the peroxidized corn oil-treated group. Oral administration of the various extracts to peroxidized corn oil-treated rats slightly inhibited the elevations of serum LPO and FFA.

As shown in Table III, the administration of the peroxidized corn oil for 5 d induced fatty liver and liver injury with the elevation of GOT and GPT as compared with the control group. In the peroxidized corn oil-treated rats, oral administration of the above various extracts slightly inhibited the elevations of serum GOT and GPT levels. Liver LPO was also reduced in the rats orally given the acetone–water (1 : 1) extract (300 mg/kg/d) of the leaf as compared with the peroxidized corn oil-fed group.

Table II. Effects of the Various Extracts of Geranii Herba on Serum Lipids (Total Cholesterol, High Density Lipoprotein-Cholesterol, Lipid Peroxides, Free Fatty Acids and Triglycerides) in Rats Fed Peroxidized Corn Oil for 5 d

	Serum					
	TC (mg/dl)	HDL-ch (mg/dl)	Atherogenic index	LPO (MDA, nmol/ml)	FFA (meq/l)	TG (mg/dl)
Control (5)	86.5±3.73[b]	48.3±5.39 N.S.	0.85±0.13[b]	3.39±0.29[d]	0.164±0.033[e]	131.2±10.9[b]
Peroxidized oil-treated rats (5)	106.4±7.71	46.8±2.24	1.27±0.10	4.92±0.28	1.139±0.144	211.0±26.4
Leaf (acetone–H_2O) extract (300 mg/kg) (7)	73.9±5.88[d]	42.9±2.86 N.S.	0.78±0.19[a]	4.44±0.30 N.S.	0.838±0.081[a]	218.6±17.6 N.S.
Leaf, stem (acetone–H_2O) extract (300 mg/kg) (7)	58.2±4.72[e]	43.9±1.73 N.S.	0.34±0.13[e]	3.60±0.29[c]	0.647±0.032[e]	228.0±28.8 N.S.
Leaf, stem (hot H_2O) extract (300 mg/kg) (6)	58.2±4.33[e]	42.7±2.90 N.S.	0.37±0.08[e]	3.65±0.25[d]	0.686±0.108[b]	260.8±57.0 N.S.

Significantly different from peroxidized oil-treated group; *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.005$, *e*) $p<0.001$; N.S., not significant.

TC, total cholesterol; HDL-ch, high density lipoprotein-cholesterol; atherogenic index, TC–HDL-ch/HDL-ch; LPO, lipid peroxide; FFA, free fatty acids; TG, triglyceride; MDA, malondialdehyde.

The results are expressed as means±standard errors.

TABLE III. Effects of the Various Extracts of Geranii Herba on Serum Transaminases (GOT and GPT) and Liver Lipids (Total Cholesterol, Triglycerides and Lipid Peroxides) in Rats Fed Peroxidized Corn Oil for 5 d

	Serum		Liver		
	GOT (Karmen Unit)	GPT (Karmen Unit)	TC (mg/g)	TG (mg/g)	LPO (MDA, nmol/g)
Control (5)	72.8±5.62[d]	22.4±2.56[d]	4.45±0.37[d]	7.63±1.21[d]	265.4±10.8[d]
Peroxidized oil-treated rats (5)	380.4±25.7	298.8±34.5	10.7±0.31	30.1±2.05	453.8±11.7
Leaf (acetone–H_2O) extract (300 mg/kg) (7)	276.6±18.0[c]	174.9±26.4[b]	10.4±0.54 N.S.	31.7±2.14 N.S.	354.8±19.3[d]
Leaf, stem (acetone–H_2O) extract (300 mg/kg) (7)	264.6±37.4[a]	152.9±28.5[c]	11.4±0.64 N.S.	35.7±2.90 N.S.	469.8±21.8 N.S.
Leaf, stem (hot H_2O) extract (300 mg/kg) (6)	266.3±21.6[c]	167.2±18.9[c]	11.0±0.72 N.S.	32.9±2.77 N.S.	432.7±22.5 N.S.

Significantly different from peroxidized oil-treated group; *a*) $p<0.02$, *b*) $p<0.01$, *c*) $p<0.005$, *d*) $p<0.001$; N.S., not significant.

TC, total cholesterol; TG, triglyceride; LPO, lipid peroxide; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; MDA, malondialdehyde.

The results are expressed as means±standard errors.

Effects of Geraniin on Serum and Liver Lipids, and Serum Transaminases (GOT and GPT)

As shown in Table IV, oral administration of geraniin (50 mg/kg/d or 100 mg/kg/d) inhibited the elevation of serum TC, LPO, FFA, TG and the atherogenic index as compared with the peroxidized corn oil-fed group.

As shown in Table V, liver TC and LPO were significantly lowered by oral administration of geraniin (50 mg/kg/d or 100 mg/kg/d) as compared with the peroxidized corn oil-treated group. In peroxidized corn oil-fed rats, the oral administration of geraniin also inhibited the elevation of serum GOT and GPT levels.

Discussion

The present investigation demonstrated that various extracts from Geranii Herba, as well as isolated geraniin, affect liver injury and lipid metabolism in peroxidized corn oil-fed rats. A high-fat diet containing peroxidized corn oil is known to cause 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 5 d induced hyperlipemia with the elevation of TG, LPO and FFA, fatty liver with the accumulation of liver TC, TG and LPO, and liver injury with elevation of serum GOT and GPT as compared with the control group.

It was postulated that functional disorder of the liver of rats fed peroxidized corn oil might be induced by lipid peroxide accumulated in the liver. Administration of the various extracts of Geranii Herba reduced the levels of serum FFA, TC, LPO, GOT, GPT and liver LPO as compared with the peroxidized oil-treated rats. Geraniin, which is main component of tannin of this herb, also inhibited the elevation of serum TC, TG, FFA, LPO, GOT, GPT and liver LPO in the peroxidized oil-treated rats. The contents of geraniin, corilagin, ellagic acid, kaempferitrin and quercetin in the acetone–water (1 : 1) extract of the leaf were found to be 18.4, 2.95, 0.93, 2.76 and $<0.1\%$, respectively. The contents of geraniin, corilagin, ellagic acid, kaempferitrin and quercetin in the acetone–water (1 : 1) extract of the leaf–stem (1 : 2) were 11.70, 3.80, 0.75, 1.48 and $<0.1\%$, respectively. Geraniin in Geranii Herba is known to be hydrolyzed by treatment with boiling water, to provide several products (including corilagin and ellagic acid), in yields which increase upon prolonged treatment.[4] The activities of

TABLE IV. Effects of Geraniin Isolated from Geranii Herba on Serum Lipids (Total Cholesterol, High Density Lipoprotein-Cholesterol, Lipid Peroxides, Free Fatty Acids and Triglycerides) in Rats Fed Peroxidized Corn Oil for 5 d

	TG (mg/dl)	HDL-ch (mg/dl)	Serum Atherogenic index	LPO (MDA, nmol/ml)	FFA (meq/l)	TG (mg/dl)
Control (5)	89.4±4.61 N.S.	47.4±4.43 N.S.	0.90±0.19[a]	3.40±0.30[c]	0.182±0.031[e]	112.5±12.6[d]
Peroxidized oil-treated rats (5)	111.5±17.8	41.6±2.33	1.63±0.28	6.35±0.91	1.35 ±0.128	351.7±61.8
Geraniin (50 mg/kg) (6)	77.7±9.49 N.S.	44.6±5.59 N.S.	0.80±0.24[b]	4.58±0.41[a]	1.02 ±0.148 N.S.	166.1±29.9[c]
Geraniin (100 mg/kg) (6)	60.3±3.64[c]	42.2±1.14 N.S.	0.44±0.10[e]	4.26±0.74 N.S.	0.806±0.092[d]	130.5±27.3[d]

Significantly different from peroxidized oil-treated group; *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.005$, *e*) $p<0.001$, N.S., not significant.

TC, total cholesterol; HDL-ch, high density lipoprotein-cholesterol; atherogenic index, TC–HDL-ch/HDL-ch; LPO, lipid peroxide; FFA, free fatty acids; TG, triglyceride; MDA, malondialdehyde.

The results are expressed as means ± standard errors.

TABLE V. Effects of Geraniin Isolated from Geranii Herba on Serum Transaminases (GOT and GPT) and Liver Lipids (Total Cholesterol, Triglycerides and Lipid Peroxides) in Rats Fed Peroxidized Corn Oil for 5 d

	Serum		Liver		
	GOT (Karmen Unit)	GPT (Karmen Unit)	TC (mg/g)	TG (mg/g)	LPO (MDA, nmol/g)
Control (5)	83.4±6.17[d]	32.2±2.56[d]	4.02±0.48[d]	6.83±1.41[d]	278.5±11.3[d]
Peroxidized oil treated rats (5)	590.4±123.2	436.8±76.1	12.0±0.84	38.4±2.89	631.2±18.3
Geraniin (50 mg/kg) (6)	316.0±58.4[a]	259.0±70.2 N.S.	9.19±0.41[c]	35.2±2.00 N.S.	523.0±13.9[d]
Geraniin (100 mg/kg) (6)	314.0±39.3[b]	225.0±29.1[b]	12.6±0.45 N.S.	44.7±3.20 N.S.	533.4±15.1[d]

Significantly different from peroxidized oil-treated group; *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.001$; N.S., not significant.

TC, total cholesterol; TG, triglyceride; LPO, lipid peroxide; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; MDA, malondialdehyde.

The results are expressed as means ± standard errors.

tannins, represented by RA and RMB values, in the water extract were also reduced to a certain extent during the above treatment. The data for the boiling water extract showed that any structural change of geraniin during the extraction did not substantially affect the ability to induce liver injury. The degree of inhibitory effect on liver injury in rats fed peroxidized oil was in the order leaf, stem (hot water) extract > leaf, stem (acetone–water) extract > leaf (acetone–water) extract. The contents of corilagin increased in the order leaf, stem (hot water) extract > leaf, stem (acetone–water) extract > leaf (acetone–water) extract. Therefore, it may be suggested that corilagin formed by the hydrolysis of geraniin provides protection against liver injury induced by the administration of peroxidized oil. Flavonols and their glycosides in the extracts are considered not to participate substantially in these activities, since their amounts are small.

In a previous paper[2] of this series, we reported that *in vitro* addition of geraniin, corilagin and ellagic acid inhibited the production of lipid peroxide induced by ADP and NADPH in microsomes, and by ADP and ascorbic acid in mitochondria. From these findings, two possible mechanisms may be suggested for the protective actions of the various extracts containing tannins and related compounds against liver injury. One is that the various extracts containing tannin and isolated geraniin inhibit the production of lipid peroxide in tissues. The other is that the tannins and related compounds inhibit the destructive action of lipid peroxide on liver cells.

Furthermore, we reported in the preceding paper[4] that geraniin and corilagin inhibited the adrenaline-induced lipolysis in isolated fat cells. In the present experiments, the various extracts from Geranii Herba and isolated geraniin inhibited the elevation of serum TC, FFA and TG levels in the peroxidized oil-treated rats. Based on the above *in vitro* experimental results, it is suggested that the *in vivo* anti-hyperlipemic effects of the extracts containing tannins, and geraniin might be partly due to inhibitory actions of the tannins on hormone-induced lipolysis in adipose tissue. However, other mechanisms for this anti-hyperlipemic action, such as inhibition of lipid absorption in the small intestine, inhibition of the biosynthesis of cholesterol in the liver and acceleration of lipid utilization in muscles have not been ruled out. Further work is needed.

References

1) Part III: Y. Kimura, H. Okuda, T. Okuda, T. Yoshida, Y. Hatano, and S. Arichi, *Chem. Pharm. Bull.*, **31**, 2501 (1983).
2) T. Okuda, Y. Kimura, T. Yoshida, T. Hatano, H. Okuda, and S. Arichi, *Chem. Pharm. Bull.*, **31**, 1625 (1983).
3) *a*) T. Okuda, T. Yoshida, and H. Nayeshiro, *Chem. Pharm. Bull.*, **25**, 1682 (1977); *b*) T. Okuda, T. Yoshida, and T. Hatano, *Tetrahedron Lett.*, **21**, 2561 (1980); *c*) T. Okuda, T. Yoshida, and T. Hatano, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 9; *d*) T. Okuda, K. Mori, K. Seno, and T. Hatano, *J. Chromatogr.*, **171**, 313 (1979); *e*) T. Okuda, T. Yoshida, and K. Mori, *Phytochemistry*, **14**, 1877 (1975); *f*) T. Okuda, T. Yoshida, and K. Mori, *Yakugaku Zasshi*, **95**, 1462 (1975).
4) *a*) Y. Kimura, H. Okuda, T. Okuda, T. Yoshida, T. Hatano, and S. Arichi, *Chem. Pharm. Bull.*, **31**, 2497 (1983); *b*) Y. Kimura, H. Okuda, T. Okuda, T. Yoshida, T. Hatano, and S. Arichi, *Chem. Pharm. Bull.*, **31**, 2501 (1983).
5) *a*) H. Izumida, T. Kosugi, T. Shimizu, I. Kobayashi, H. Konno, G. Mizuno, T. Tochohara, S. Tabuchi, and H. Kishi, *J. Jap Atherosclerosis Soc.*, **8**, 295 (1980); *b*) Y. Kimura, M. Kubo, T. Tani, S. Arichi, and H. Okuda, *Chem. Pharm. Bull.*, **30**, 1792 (1982); *c*) Y. Kimura, H. Okuda, T. Tani, and S. Arichi, *Chem. Pharm. Bull.*, **30**, 1792 (1982); *d*) Y. Kimura, H. Okuda, Z. Taira, N. Shoji, T. Takemoto, and S. Arichi, *Planta Medica*, in press.
6) *a*) T. Mizunuma, F. Sato, T. Tezuka, and H. Okuda, *Eiyo To Shokuryo*, **29**, 213 (1976); *b*) H. Okuda, *Igaku To Yakugaku*, **2**, 411 (1979); *c*) T. Mizunuma, Y. Takahashi, Y. Kishino, F. Sato, and H. Okuda, *Lipid Peroxide Res.*, **2**, 88 (1978); *d*) Y. Kimura, H. Ohminami, H. Okuda, K. Baba, M. Kozawa, and S. Arichi, *Planta Medica*, **49**, 51 (1983).
7) B. Zak, *Am. J. Clin. Pathol.*, **27**, 583 (1957).
8) M. J. Fletcher, *Clin. Chim. Acta*, **22**, 393 (1968).
9) K. Itaya and M. Ui, *J. Lipid Res.*, **6**, 16 (1965).
10) K. O. Ash and W. M. Hentschel, *Clin. Chem.*, **24**, 2180 (1978).
11) H. Ohkawa, N. Ohishi, and K. Yagi, *Anal. Biochem.*, **95**, 351 (1979).
12) S. Reitman and S. Frankel, *Am. J. Clin. Pathol.*, **28**, 56 (1957).
13) *a*) T. Okuda, K. Mori, and K. Aoi, *Yakugaku Zasshi*, **97**, 1267 (1977); *b*) T. Okuda, K. Mori, and R. Murakami, *Yakugaku Zasshi*, **97**, 1273 (1977).