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Comparative Effects of Crude Drugs on Serum Lipids

EIKO ONISHI,[a] KAZUO YAMADA,[a] TOSHIO YAMADA,[a]
KIYOKO KAJI,[b] HAJIME INOUE,[b] YOSHIYUKI SEYAMA,[b]
and SABURO YAMASHITA*,[b]

Keisai Shoyaku Kenkyusho,[a] 2-36-19 Nakatehara, Kohoku-ku, Yokohama 222, Japan, and Department of Clinical Chemistry,[b] Hoshi College of Pharmacy, Shinagawa-ku, Tokyo 142, Japan

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Aqueous extracts of leaves of *Nelumbo nucifera*, GAERTN. (Nymphaeceae), Kayo, leaves and stems of *Lonicera japonica* THUNB. (Caprifoliaceae) Nindo and stems of *Akebia quinta* (THUNB.) DECNE (Lardiazabalaceae) Mokutsu were administered to rats loaded with a high fat diet containing 1.5% cholesterol and 1.0% cholic acid in order to screen the hypocholesterolemic activity of these crude drugs. The animals were kept on the diet for 5 d, and then crude drug extract, equivalent to the human dose (×1) and higher doses (×10, ×25), was orally administered. Changes of total cholesterol (TC), free cholesterol (FC), cholesterol ester (CE), free and ester cholesterol ratio (Ratio), triglycerides (TG), phospholipid (PL) and free fatty acids (NEFA) were examined. Significant decreases in serum TC, FC, and PL were observed in the high fat-loaded groups given these crude drugs. TC in the liver did not show any reduction in the groups receiving Kayo, Nindo and Mokutsu as compared with the high fat-loaded control group. The overall effects in suppressing serum lipids elevation were in the order of Nindo > Mokutsu > Kayo.

Keywords——crude drug; lipid metabolism; hypercholesterolemia; hyperlipidemia; Caprifiliaceae-leaf; Caprifiliaceae-stem; Lardiazabalaceae-stem; Nymphaceae-leaf

This paper is the first of a series of studies on the pharmacological effects of empirically used crude drugs. Crude drugs obtained from plant sources include many physiologically active ingredients, and since their use as traditional drugs is based on empirical trials, objective evaluations of them with modern techniques are lacking in many cases.

The authors have discovered through their clinical trials as oriental druggists that when Nindo (leaves and stem of *Lonicera japonica* THUNB. (Caprifiliaceae)), Mokutsu (stem of *Akebia quinta* (THUNB.) DECNE (Lardiazabalaceae)) and Kayo (leaves of *Nelumbo nucifera* GAERTN. (Nymphaceae)) were administered to patients with hypercholesterolemia, serum cholesterol levels were lowered (Table I). There is no report concerning the hypocholesterolemic effect of these crude drugs, because the crude drugs have been used for other purposes, so that pharmacological verification of the effect was attempted in animal experiments. Dietary hypercholesterolemia was induced in rats, and screening for hypocholesterolemic effect was carried out with reference to the experimental conditions reported by Tensho *et al.*[1)]

Materials and Methods

Experimental Animals and Diets——Wistar strain rats (Sankyo Labo K.K.; 100±10 g body weight, 5 weeks of age) were kept on the following programs: the control group was kept on the basal powdered diet (Oriental Kobo K.K.); experimental hypercholesterolemia was induced in other groups according to the method described by Tensho *et al.*[1)] by keeping the animals on high cholesterol diet (Table II) for 5 d, and then each crude drug (at various doses) was given once daily for 10 d by means of oral intubation, while the high cholesterol diet was maintained. Diet and

TABLE I. Clinical Effects of Crude Drugs

Patients	Cholesterol levels: Changes in total cholesterol levels of serum: Before treatment (mg/100 ml)	After treatment (mg/100 ml)	Period of treatment (d)
A	301	287	30
B	257	237	70
D	183	163	40
E	264	229	365

Selected examples of clinical hypocholesterolemic effect of the crude drugs. Hypocholesterolemic tendency was shown clinically.

TABLE II. Composition of the High Cholesterol Diet for Rats

Composition of cholesterol diet (%)	
Cholesterol	1.50
Cholic acid	1.00
Milk casein	20.00
Sucrose	49.50
Hydrogenated fat	12.00
Cellulose	4.00
Vitamins	0.35
Dried fish powder	7.50
Minerals	4.00
Choline chloride	0.15

water were given *ad libitum*, and rats in each group were kept separately. Numbers of rats in each group are given in the Tables.

Preparation and Determination of Doses of the Samples——Doses of the crude drugs were determined and prepared, based on the dose for human adults of 60 kg body weight, as follows. Kayo, Nindo or Mokutsu (commercially available), 10 g (daily dose in man), was kept in 100—300 ml of 30% EtOH–water for 2 d at room temperature, then the mixture was boiled on a water bath for 10 min to swell the plant tissue, and the solid was filtered off hot through cotton cloth. The filtrate was concentrated, then frozen and dried. The freeze-dried sample was dissolved in 300 ml of purified water to give the standard dose in 1.0 ml volume per 200 g body weight of rat ($\times 1$: a dose equivalent to that for human adult). When the freeze-dried sample was dissolved in 30 ml of water, it gave a 10 times larger dose than the standard dose ($\times 10$), and in 12 ml, twenty-five times larger ($\times 25$).

Blood Specimens——The animals were fasted for 18 h after the 15-d experimental period, then blood was collected by decapitation. Serum was separated by centrifugation at 3000 rpm at 4 °C for 10 min.

Reagent Kits for the Determination of Lipids——All determinations of lipids were performed using reagent kits based on enzymatic methods.

Cholesterol: Total cholesterol; Cholesterol C-Test Wako. Free cholesterol; Free cholesterol C-Test Wako.

Triglycerides: Iatroset TG-E (Iatron).

Phospholipids: Iatroset PL-E (OM) (Iatron).

Free Fatty Acids: mcl NEFA-E (color) (Iatron).

Determination of total cholesterol (TC) in the liver was performed after extracting fats with Folch's[2] solvent from a certain weight (wet) of the organ, and the obtained value was converted to TC in mg per gram (wet) of the liver.

$$\text{cholesterol ester} = \text{TC (mg/100 ml)} - \text{free cholesterol (mg/100 ml)}$$

$$\text{ester ratio (\%)} = \frac{\text{cholesterol ester (mg/100 ml)}}{\text{total cholesterol (mg/100 ml)}} \times 100$$

Results and Discussion

Kayo, Nindo and Mokutsu have been traditionally used as antipyretics, anti-inflammatory agents, sedatives, *etc.*, and no hypolipidemic effect has so far been reported.

Greenberg *et al.*[3] and Tensho *et al.*[1] reported suitable conditions for the induction of dietary hypercholesterolemia in rats for the purpose of screening for hypocholesterol agents. Based on their conditions, we kept male Wistar strain rats on the basal diet for 4 d, and on the cholesterol (1.5%)-containing diet for 15 d. The body weight of the rats was approx. 150 g and the elevated serum cholesterol level was about 300 mg/100 ml.

We previously used this system[4] to evaluate the hypocholesterolic effect of iodine-enriched eggs (prepared and sold by Nippon Nosan Co.), and the serum cholesterol level was significantly lowered by oral administration for 10 d.

Using the same conditions, Nindo ($\times 1$), was administered for 10 d: it was found that the serum cholesterol level was 282.1 ± 96.2 mg/100 ml ($n=7$) in the control, and 386.6 ± 74.3 mg/100 ml ($n=7$) in the Nindo group. Nindo thus did not lower a serum cholesterol level of around 300 mg/100 ml. We therefore tried to induce a higher initial serum cholesterol level.

Tensho *et al.*[1] reported that the degree of induced dietary hypercholesterolemia was influenced by the feed, strain of rats and initial body weight of rats, and in particular, claimed that the induced serum cholesterol level and the initial body weight of rats were inversely correlated. Thus, the same strain of rat was kept on the high cholesterol content diet from a younger age, without preliminary feeding, starting at approx. 100 g of body weight. Variations of the obtained higher cholesterol level were seen, as reported[1] but the serum cholesterol level

TABLE III. Effect of Crude Drugs on Serum Lipids

Group Number of rats	Control 9	Cholesterol 7	Kayo ($\times 10$) 7	Nindo ($\times 10$) 7	Mokutsu ($\times 10$) 7
Total cholesterol (mg/100 ml)	$62.6 \pm 10.6^{a,c}$ (5)[b]	1287.0 ± 232.8 (100)	809.7 ± 284.0^{c} (63)	757.4 ± 273.1^{c} (59)	994.9 ± 154.7^{d} (77)
Free cholesterol (mg/100 ml)	15.7 ± 2.2^{c} (5)	333.7 ± 69.6 (100)	210.4 ± 67.1^{c} (63)	210.8 ± 82.4^{c} (63)	245.9 ± 20.0^{c} (74)
Ester ratio (%)	74.6 ± 4.2 (101)	74.1 ± 1.8 (100)	72.8 ± 5.6 (98)	72.0 ± 6.7 (97)	75.0 ± 2.3 (101)
Phospholipids (mg/100 ml)	126.6 ± 21.4^{c} (27)	439.4 ± 94.1 (100)	306.5 ± 97.7^{d} (66)	327.6 ± 67.7^{d} (70)	349.5 ± 52.0^{d} (75)
Triglycerides (mg/100 ml)	106.2 ± 12.9^{c} (52)	205.8 ± 28.1 (100)	176.6 ± 41.7 (86)	169.4 ± 35.4 (82)	182.9 ± 27.1 (90)
Free fatty acids (μEq/l)	842.9 ± 227.4 (83)	991.3 ± 202.0 (100)	833.5 ± 257.8 (86)	903.1 ± 224.8 (89)	920.0 ± 135.2 (91)
Total cholesterol / Liver wet weight (mg/g)	5.1 ± 1.2 (15)	34.8 ± 5.4 (100)	35.8 ± 5.2 (105)	34.6 ± 5.4 (102)	32.6 ± 5.4 (96)
Body weight (g) Initial	99.9 ± 6.6 (97)	102.8 ± 8.8 (100)	103.3 ± 7.4 (100)	108.2 ± 6.0 (105)	108.5 ± 6.1 (106)
Body weight (g) Final	190.4 ± 15.1^{c} (128)	152.0 ± 24.4 (100)	137.6 ± 21.6 (93)	154.6 ± 22.2 (104)	148.0 ± 18.8 (99)

Effects of Kayo, Nindo and Mokutsu on the serum levels of lipids and the total cholesterol level in the liver of the experimental rats. Serum total cholesterol level was experimentally elevated with high cholesterol diet to around 1000 mg/100 ml. Doses of crude drugs were 10 times larger than those equivalent to the usual dose for human adults.

a) Mean ± S.D.

b) Percent of the value for the cholesterol group.

Statistically significant difference from the cholesterol group; *c*) $p<0.01$, *d*) $p<0.05$.

TABLE IV. Effect of Nindo on Serum Lipids

Group	Cholesterol	Nindo (×1)	Nindo (×10)	Nindo (×25)
Number of rats	5	5	5	6
Total cholesterol (mg/100 ml)	761.0±84.6[a] (100)[b]	521.4±59.2[c] (69)	679.1±180.0 (89)	730.2±189.3 (96)
Free cholesterol (mg/100 ml)	137.2±13.4 (100)	98.9±17.1[c] (72)	118.8±33.2 (87)	130.3±27.8 (95)
Ester ratio (%)	81.8±1.8 (100)	80.9±3.4 (99)	82.5±0.9 (101)	81.9±1.6 (100)
Phospholipids (mg/100 ml)	299.8±26.4 (100)	117.1±92.7[c] (39)	213.4±38.5[c] (71)	240.8±29.7[c] (80)
Triglycerides (mg/100 ml)	196.4±23.1 (100)	115.1±31.5[c] (59)	136.1±32.7[c] (69)	201.7±49.4 (103)
Free fatty acids (μEq/l)	1617.7±376.0 (100)	1313.2±281.7 (81)	1481.0±154.1 (92)	1339.1±312.4 (83)
Body weight (g) Initial	98.2±3.4 (100)	98.6±3.4 (100)	103.2±4.8 (105)	99.3±7.4 (101)
Body weight (g) Final	137.0±2.1 (100)	138.0±6.7 (101)	150.2±8.5 (110)	147.0±7.7 (107)

Effect of various doses of Nindo extract on the serum lipids and body weight of the experimental rats.
a) Mean±S.D.
b) Percent of the value for the cholesterol group.
Statistically significant difference from the cholesterol group; *c*) $p<0.01$, *d*) $p<0.05$.

TABLE V. Effect of Mokutsu on Serum Lipids

Group	Cholesterol	Mokutsu (×1)	Mokutsu (×10)	Mokutsu (×25)
Number of rats	5	4	5	5
Total cholesterol (mg/100 ml)	761.0±84.6[a] (100)[b]	590.0±91.4[c] (78)	750.4±93.4 (99)	536.8±80.4[d] (71)
Free cholesterol (mg/100 ml)	137.2±13.4 (100)	121.7±21.3 (89)	124.5±21.4 (91)	95.6±26.2[d] (70)
Ester ratio (%)	81.9±1.8 (100)	79.4±0.8[d] (97)	83.5±0.6 (102)	82.4±1.0 (101)
Phospholipids (mg/100 ml)	299.8±26.2 (100)	232.8±36.6[d] (78)	239.7±23.1[d] (67)	140.1±50.3[c] (40)
Triglycerides (mg/100 ml)	196.4±23.1 (100)	99.6±25.4[c] (51)	126.9±30.0[c] (65)	131.1±17.5[c] (67)
Free fatty acids (μEq/l)	1617.2±376.0 (100)	1264.7±111.8 (78)	1504.9±111.1 (93)	1227.5±195.4 (76)
Body weight (g) Initial	98.2±3.4 (100)	98.0±1.4 (100)	104.6±5.4 (107)	103.6±2.6 (105)
Body weight (g) Final	145.8±2.2 (100)	145.0±6.8 (99)	151.8±12.4 (104)	150.2±7.4 (103)

Effect of various doses of Kayo extract on the serum lipids and body weight of the experimental rats.
a) Mean±S.D.
b) Percent of the value for the cholesterol group.
Statistically significant difference from the cholesterol group; *c*) $p<0.01$, *d*) $p<0.05$.

was significantly elevated in the range of 700—1000 mg/100 ml as compared with the result of the previous preliminary test. Nindo, Mokutsu and Kayo (×10) were administered to such hypercholesterolemic rats. The results are summarized in Tables III—VI

High cholesterol-containing diet induced significantly higher total and free cholesterol, phospholipids and triglyceride levels as compared with the control group. Administration of

TABLE VI. Effect of Kayo on Serum Lipids

Group Number of rats		Cholesterol 5	Kayo (×1) 5	Kayo (×10) 5	Kayo (×25) 6
Total cholesterol (mg/100 ml)		761.0±84.6[a] (100)[b]	567.9±74.4[c] (75)	588.9±135.6[d] (77)	604.7±170.4 (80)
Free cholesterol (mg/100 ml)		137.2±13.4 (100)	108.5±19.8[c] (76)	114.0±25.7 (83)	111.4±20.9[d] (81)
Ester ratio (%)		81.9±1.8 (100)	80.8±3.0 (99)	80.5±3.1 (98)	81.0±3.0 (99)
Phospholipids (mg/100 ml)		299.8±26.2 (100)	261.8±16.3 (87)	247.8±32.0[d] (83)	235.6±36.5[c] (79)
Triglycerides (mg/100 ml)		196.4±23.1 (100)	185.1±21.4 (94)	189.4±39.1 (97)	147.4±14.1[c] (75)
Free fatty acids (μEq/l)		1617.7±376.0 (100)	1557.6±88.0 (96)	1345.2±336.9 (83)	1741.9±370.2 (108)
Body weight (g)	Initial	98.2±3.4 (100)	99.0±4.1 (101)	102.2±7.0 (104)	101.8±3.1 (104)
	Final	145.8±2.2 (100)	142.6±9.3 (98)	153.6±8.4 (105)	138.3±12.9 (95)

Effect of various doses of Mokutsu extract on the serum lipids and body weight of the experimental rats.
a) Mean±S.D.
b) Percent of the value for the cholesterol group.
Statistically significant difference from the cholesterol group; *c*) $p<0.01$, *d*) $p<0.05$.

these 3 crude drugs (×10) to these animals lowered the serum total and free cholesterol and phospholipid levels significantly. However, the ester ratio and free fatty acid levels, which were not greatly elevated in the cholesterol group, were not lowered by these crude drugs. The cholesterol level in the liver was not reduced by these crude drugs.

Thus, hepatic cholesterol level was not lowered by Nindo, Mokutsu and Kayo, but serum total and free cholesterol and phospholipid levels were reduced by these crude drugs.

The effects of Nindo, Mokutsu and Kayo, at doses of ×1, ×10 and ×25, on the serum cholesterol and other serum lipids were examined: Nindo, at a dose of ×1, lowered the serum total and free cholesterol levels, as well as phospholipids and triglyceride levels, and Nindo appeared to have a stronger effect on cholesterol and phospholipid levels than did the other two crude drugs. Suppression of serum lipid increase was not reduced by increasing the dose of Nindo (×10→×25), but rather weakened. Mokutsu, ×1, suppressed total cholesterol as well as phospholipid and triglyceride levels, but free cholesterol and free fatty acid levels were not lowered. Contrary to the case of Nindo, larger doses (×10, ×25) of Mokutsu suppressed total and free cholesterol and phospholipid levels, and the suppression of phospholipid level by ×25 Mokutsu was significantly greater than that by ×1 ($p<0.01$). Kayo, ×1, suppressed total and free cholesterol levels, but triglyceride and phospholipid levels were not suppressed unless the dose was increased to ×10 or ×25.

In conclusion: (1) elevated cholesterol level was lowered by these three crude drugs, and phospholipid and triglyceride levels were somewhat reduced; (2) the suppression of serum lipid elevation was in the order of Nindo>Mokutsu>Kayo.

References

1) A. Tensho, I. Shimizu, T. Takenawa, H. Kikuchi, and T. Rokujo, *Yakugaku Zasshi*, **92**, 879 (1972).
2) J. Folch, M. Lees, and G. H. S. Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
3) C. M. Greenberg, C. A. Bocher, J. F. Kerwin, S. M. Greenberg, and T. H. Lin, *Am. J. Physiol.*, **202**, 732 (1961).
4) K. Kaji, Y. Seyama, S. Yamashita, Y. Mamiya, and T. Ishikawa, *Rinsho To Kenkyu*, **60**, 140 (1983).