

Effect of Administration with the Extract of *Gymnema sylvest* R. Br Leaves on Lipid Metabolism in Rats

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Extract of *Gymnema sylvest* R. Br leaves (GE) was orally administered once a day to rats fed a high fat diet or normal fat diet for 3 weeks to investigate its influence on lipid metabolism. As a result, GE did not influence body weight gain or feed intake in both diet groups during the experimental period. The apparent fat digestibility was significantly decreased by GE in both diet groups for the last 2 weeks of the experimental period, though not the apparent protein digestibility. In addition, the excretion of neutral sterols and acid steroids into feces was increased by GE in both diet groups. Furthermore, GE decreased the total cholesterol and triglyceride levels in serum.

On the other hand, blood lecithin-cholesterol acyltransferase (LCAT) activity was increased by GE. Moreover, it was suggested that GE influenced cecal fermentation and that propionic acid and acetic acid contents in cecum were significantly increased by GE. Consequently, it was suggested that GE improved serum cholesterol and triglyceride levels through influence over a wide range of lipid metabolism in rats.

Key words *Gymnema sylvest*; lipid metabolism; propionic acid; cholesterol; triglyceride

Gymnema sylvest R. Br belonging to Asclepidaceae is a native plant in the south west of India, Australia and tropical Africa. From ancient times, *G. sylvest* has been used in Indian traditional medicine (“Ayurvedic medicine”) and is considered to be effective in improving urination, stomach stimulation, and diabetes.^{1–4} The life style habit diseases prevailing recently are closely associated with obesity.⁵ In addition, the metabolism of carbohydrates and lipids play a considerable part in obesity. From this point of view, the influence of *G. sylvest* a substance known to be effective in improving carbohydrate metabolism, on improvement of lipid metabolism under a high fat diet, is very interesting. Reports dealing with the influence of *G. sylvest* on lipid metabolism are fewer than those on effects on carbohydrate metabolism. In our previous study, the extract of *G. sylvest* leaves lowered liver lipids and improved obesity histopathologically in rats fed on a high fat diet, similar to the case of chitosan (Shigematsu N., *et al.*, unpublished results). From these results, *G. sylvest* may influence a wide range of lipid metabolism. In order to elucidate this possibility, we investigated the influence of *G. sylvest* on lipid excretion to feces, blood lipid and cecal fermentation.

This experiment was done according to “the Experimental Animal Regulation” (IEXAS).

MATERIALS AND METHODS

Materials Male Jcl; Wistar rats (5 weeks old) were used and were provided by Nihon Crea Co., Ltd. CE-2 feed (product of Nihon Crea Co., Ltd.) was used for normal fat diet (NF), and in the case of the high fat diet (HF), beef tallow was added to CE-2 at 20% by weight. The nutritive constituents of NF and HF were as follows; water content (NF: 8.8%, HF: 7.0%), protein (NF: 25.2%, HF: 20.7%), fat (NF: 4.4%, HF: 23.5%), fiber (NF: 4.4%, HF: 3.5%), ash (NF: 7.0%, HF: 5.5%), nitrogen free extract (NF: 50.2%, HF: 39.8%), total calories (NF: 341 Kcal/100 g, HF: 460 kcal/

100 g).

The leaves of *G. sylvest* were dried and extracted with 50% hydrous alcohol at 60 °C for 3 h. The resulting extract was then dried before being used as gymnema extract (GE) (total gymnemic acid content⁶): 2.4%).

Rearing Condition Rats were individually put in a cage made of poly-carbonate (CL-0106, product of Nihon Crea Co., Ltd.) with a size of 345×403×177 mm and maintained at room temperature (23±2 °C) and humidity (55±5%) on a light/dark cycle of 14 h/10 h (lighted up between 6:00 a.m. and 8:00 p.m.). All rats were provided with NF for two weeks after the initiation of this preliminary rearing, and thereafter they were divided into two groups of NF-fed and HF-fed group. NF-fed and HF-fed groups were provided with NF and HF respectively until the end of the experiment. Four weeks after division into the NF-fed and HF-fed groups, each of the two groups (11-week-old rats) was divided into the following two groups, a control group (cont-NF, cont-HF; *n*=10, respectively) and the GE orally treated groups (G-NF, G-HF; *n*=10, respectively). The treated groups (G-NF, G-HF) were orally administered with GE once a day (between 10:00 a.m.—12:00 at noon) for 3 weeks. The oral dose of GE was 33 mg/kg and was given through a stomach tube after suspension in 2 ml of distilled water. The control groups (cont-NF, cont-HF) were dosed with 2ml of distilled water in the same way. From the start of rearing to the beginning of GE administration, the body weight and the feed intake of all rats were measured twice a week. After the beginning of GE administration, they were measured every day. Moreover, rats were allowed *ad libitum* to access the feed stuff and tap water during the experimental period. Furthermore, feces were collected from each group for the last 2 weeks of the experimental period, weighed, freeze-dried, and subjected to analysis. Feces collected in the last 3 d of the experimental period were subjected to analysis for fecal neutral sterol and acidic steroids.

At the end of the experiment, the rats were fasted for 18 h

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and blood samples were collected from the jugular vein for analysis. The liver, mesentery, epididymal fat and cecum were extracted under anesthesia using sodium pentobarbital and weighed.

Apparent Digestibility and Determination of Lipid in the Feces After drying the feces and determining the moisture, nitrogen values and feed intake were determined by the micro Kjeldahl method. Apparent protein digestibility was calculated using the following equation;

$$\left[\frac{(\text{N of the intake feed} - \text{N of the feces})}{(\text{N of the intake feed} - \text{N of GE})} \right] \times 100 (\%)$$

Lipids extracted from feces and intake feed were determined by Soxhlet extraction, and the apparent fat digestibility was calculated using the following equation;

$$\left[\frac{(\text{lipid of the intake feed} - \text{lipid of the feces})}{(\text{lipid of the intake feed} - \text{lipid of GE})} \right] \times 100 (\%)$$

Furthermore, the acidic steroids and neutral sterol in feces were determined by the method of Sheltway and Courchain *et al.*^{7,8)}

Determination of Blood Lipids After separating serum, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels were determined using a commercial assay kit (Spotchem SP-4410, Kyoto Daiichi Kagaku Co.). The phospholipid level was also determined using a commercial assay kit (Determiner PL, Kyowa Medics Co.).

LCAT Activity LCAT activity was determined according to the self-substrate method⁹⁾ which directly quantifies free cholesterol by the following procedure. Lecithin suspension (0.25%) and 150 mM sodium iodo-acetic acid solution was prepared using 50 mM phosphate buffer (pH 7.4). The subject serum was prepared by adding 20 μ l of 0.25% lecithin suspension and 20 μ l of 50 mM phosphate buffer (pH 7.4) to 40 μ l of serum, meanwhile, the control serum was prepared by adding 20 μ l of 0.25% lecithin suspension and 20 μ l of 150 mM sodium iodo-acetic acid solution to 40 μ l of the serum. After incubation of subject serum and control serum for 40 min at 37 °C, 20 μ l of 150 mM sodium iodo-acetic acid solution was added to the subject serum and 20 μ l of 50 mM phosphate buffer (pH 7.4) was added to the control serum, and the free cholesterol in both was determined using a commercial assay kit (Free cholesterol E test Wako, Wako Pure Chemical Ind., Ltd.). LCAT activity was calculated as the decreased level (μ g) of free cholesterol in 1 ml of serum per 1 h by subtraction of the free cholesterol level of subject serum from that of control serum.

Determination of Organic Acid Contents in Cecum The content was drained off from the extracted cecum and weighed. The content was mixed with the same volume of ethanol and the mixture centrifuged (3000 rpm for 5 min). The supernatant was filtered through a membrane filter. Organic acids in the filtrate were determined by HPLC (separation column: Lompac KC-811, KC-810p, Showa Denko Co., Ltd., column temperature; 45 °C, detector; UV 445 nm, eluate; 2 mM sodium perchlorate solution, elution speed; 1 ml/min).

Statistical Analysis The significant differences between the control and GE administered groups were tested by Student's *t*-test. When $p < 0.05$, it was considered to be significant.

Table 1. Apparent Fat and Protein Digestibility, Fecal Lipid Excretion in Rats

	NF group		HF group	
	Cont-NF	G-NF	Cont-HF	G-HF
Apparent digestibility				
Fat (%)	70.5 \pm 3.2	65.3 \pm 2.8**	88.8 \pm 1.4	87.3 \pm 1.7 [#]
Protein (%)	72.8 \pm 3.8	72.3 \pm 3.3	69.5 \pm 3.9	66.3 \pm 4.3
Fecal weight (g/rat/last 3 d)				
Wet	4.8 \pm 1.6	4.9 \pm 1.4	3.4 \pm 0.8	3.5 \pm 1.1
Dry	3.5 \pm 1.0	3.6 \pm 0.9	2.6 \pm 0.6	2.7 \pm 0.7
Fecal moisture (%)	27.0 \pm 6.8	26.8 \pm 6.3	23.6 \pm 6.1	23.7 \pm 8.3
Fecal lipid (mg/rat/last 3 d)				
Neutral sterol	25.8 \pm 17.8	58.4 \pm 24.0*	32.0 \pm 11.2	45.4 \pm 13.1 [#]
Acid steroid	18.9 \pm 3.8	23.3 \pm 3.9*	16.6 \pm 2.4	19.2 \pm 2.8 [#]

Mean \pm S.D. ($n=10$). Statistical difference: * $p < 0.05$, ** $p < 0.01$ compared with cont-NF. [#] $p < 0.05$ compared with cont-HF.

RESULTS

Feed Intake and Change in Body Weight There was no influence of experimental diet or GE dosage on feed intake on any of the groups during the experimental period. In the HF-fed group, the body weight in cont-HF (initial body weight; 118.0 \pm 5.2 g, final body weight; 395.5 \pm 21.6 g) displayed an increasing tendency compared to G-HF (initial body weight; 118.0 \pm 5.2 g, final body weight; 379.5 \pm 20.0 g), although it was not statistically significant. In the NF-fed group, the body weight in cont-NF (initial body weight; 118.0 \pm 5.2 g, final body weight; 374.7 \pm 20.7 g) was not influenced by GE, compared with G-NF (initial body weight; 118.0 \pm 5.2 g, final body weight; 372.5 \pm 17.8 g). In addition, the feed efficiency was not significantly influenced by GE in both diet groups (data not shown).

Apparent Fat and Protein Digestibility, Fecal Lipid Excretion Apparent fat, protein digestibility, and fecal lipid excretion levels of feces are shown in Table 1. Effects of GE dosage on apparent protein digestibility in rats were not observed in both NF- and HF-fed groups (cont-NF vs. G-NF, cont-HF vs. G-HF). However, GE dosage significantly decreased the apparent fat digestibility in both NF- and HF-fed groups compared with each control group. Effects of GE dosage on the weight and water content of feces were not found in both NF- and HF-fed groups, compared with each control group. Meanwhile, GE dosage in both NF- and HF-fed groups was found to significantly promote the excretion of neutral sterol and acidic steroids to feces, compared with each control group (cont-NF vs. G-NF, cont-HF vs. G-HF).

Blood Lipid The results of blood lipid measurement are shown in Fig. 1. In the NF-fed group, the serum total cholesterol level in G-NF was significantly decreased ($p < 0.05$) when compared to cont-NF. Moreover, in the HF-fed group, the serum total cholesterol level in G-HF was significantly decreased ($p < 0.05$) when compared to cont-HF with the same result as the NF-fed group. Concerning serum HDL-cholesterol levels, there was no influence of GE oral dosage in both the NF- and HF-fed groups (cont-NF vs. G-NF, cont-HF vs. G-HF). In NF-fed groups, the serum triglyceride level in the G-NF group was significantly decreased ($p < 0.05$) when compared to cont-NF. Moreover, in HF-fed groups, the

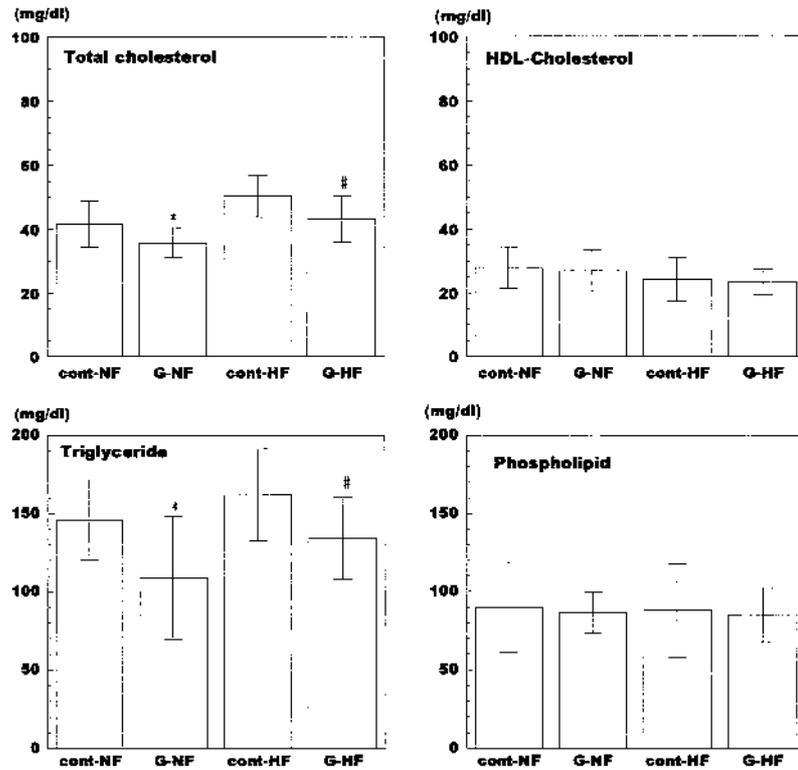


Fig. 1. Serum Lipid in Rats at the End of the Experiment

Vertical bars represent S.D. ($n=10$). Statistical difference: * $p<0.05$ compared with cont-NF, # $p<0.05$ compared with cont-HF.

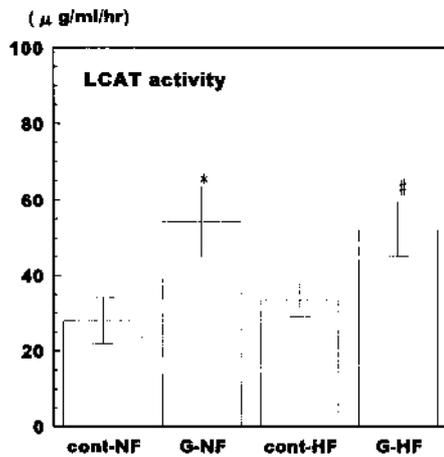


Fig. 2. Effect of GE on Serum LCAT Activity in Rats

Vertical bars represent S.E. ($n=10$). Statistical difference: * $p<0.05$ compared with cont-NF, # $p<0.05$ compared with cont-HF.

serum triglyceride level in G-HF was significantly decreased ($p<0.05$) compared to cont-HF with the same results as the NF-fed group.

Regarding serum phospholipid levels, there was no influence of GE oral dosage in the NF- and HF-fed groups (cont-NF vs. G-NF, cont-HF vs. G-HF).

LCAT Activity The results of LCAT activity measurement are shown in Fig. 2. In NF-fed groups, the value in G-NF was significantly activated ($p<0.05$) compared to cont-NF. In the HF-fed group, GE dosage significantly activated LCAT with similar results to the NF-fed group. Namely, its value in G-HF was significantly activated ($p<0.05$) compared to cont-HF.

Table 2. Organ and Epididymal Fat Weight in Rats at the End of the Experiment

	NF group		HF group	
	Cont-NF	G-NF	Cont-HF	G-HF
Liver (g/rat)	13.9±3.5	13.3±1.8	12.4±2.1	12.6±1.5
Mensentery (g/rat)	3.9±1.0	3.5±0.6	5.0±0.8	4.8±1.3
Epididymal fat (g/rat)	4.8±1.1	5.0±0.6	6.8±1.6	7.0±1.9
Cecum (g/rat)	3.1±1.1	3.1±0.7	2.3±0.4	2.6±0.5

Mean±S.D. ($n=10$).

Table 3. Cecal Content of Organic Acids

	NF group		HF group	
	Cont-NF	G-NF	Cont-HF	G-HF
Cecal content (g)	3.0±0.3	3.1±0.2	2.3±0.1	2.5±0.2
Acetic acid (%)	0.26±0.01	0.46±0.01**	0.22±0.01	0.25±0.01##
Propionic acid (%)	0.06±0.00	0.11±0.00**	0.06±0.00	0.08±0.00##
Succinic acid (%)	0.01±0.00	0.01±0.00	0.01±0.00	0.02±0.00
<i>n</i> -Valeric acid (%)	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
iso-Valeric acid (%)	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00

Mean±S.E. ($n=10$). Statistical difference: ** $p<0.01$ compared with cont-NF, ## $p<0.01$ compared with cont-HF.

Organ Weight In both NF- and HF-fed groups, the effects of GE dosage on organ weights (liver, mensentery, epididymal fat and cecum) were not found (Table 2).

Organic Acid Contents in Cecum Organic acid contents in cecum are shown in Table 3. GE dosage did not significantly influence cecal content volume in both NF-and

HF-fed groups. However, in both the NF- and HF-fed groups, acetic acid and propionic acid in cecal content were significantly increased by GE dosage, when compared to control. Additionally, small amounts of succinic acid, *n*-valeric acid and iso-valeric acid contents were detected in both diet groups.

DISCUSSION

In the present study, the extract of *G. sylvestre* leaves (GE) was orally administrated to rats fed on a high fat or normal fat diet, and the effects on lipid metabolism in both diet groups were investigated.

GE decreased the apparent fat digestibility and promoted the excretion of neutral sterol and acidic steroids to feces in both NF-fed and HF-fed groups (Table 1). In addition, the serum total cholesterol and triglyceride levels in both diet groups were decreased by GE administration (Fig. 1). In addition, the serum HDL-cholesterol and phospholipid levels were not affected by GE. Therefore, the decrease in serum total cholesterol level without a change in the HDL-cholesterol level can be ascribed to the reduction of LDL-cholesterol and VLDL-cholesterol. On the other hand, we have already found that the accumulation of liver lipids in rats fed a high fat diet was safely suppressed by long-term oral administration of GE (Shigematsu, *et al.* unpublished results). VLDL is biosynthesized in liver and secreted into blood, and transformed into LDL *via* IDL by lipoprotein lipase. In general, low VLDL levels in blood are possibly induced by a) repression of hepatic synthesis of VLDL, b) elevation of fatty acid oxidation, or c) inhibition of VLDL secretion from the liver.^{10,11} Although we can not determine whether administration of GE is associated with these mechanism, the excretion of neutral sterols and acidic steroids to feces by GE may also influence hepatic biosynthesis of cholesterol and VLDL. Excretion of neutral sterol and acidic steroids to feces is often observed with dietary fibers and saponins,^{12–14} which can decrease blood cholesterol and triglyceride levels. As for gymnemic acid, the possibility was reported by Nakamura, *et al.*¹⁵ that gymnemic acid influenced the excretion of lipid to feces when administrated to rats in a large amount (0.5–1.0 g/kg of the extract containing 58% of gymnemic acid). Total gymnemic acid content of GE was only 2.4% and the dose of GE was 33 mg/kg in the present study. Therefore, it may not be a suitable explanation that gymnemic acids solely stimulated fecal excretion of neutral sterols and acidic steroids although the diet used in the present study differed from the one used in the study by Nakamura, *et al.*¹⁵ In addition, when rats fed on cholesterol-free casein diet were administered with cholestyramine which stimulate excretion of acid steroid to feces, the plasma cholesterol level was not decreased.¹⁶ Moreover, although plasma cholesterol level in rats were decreased by the intraperitoneal injection of propionic acid, excretion of bile acid and neutral sterol to feces was not stimulated.¹⁷ Consequently, a simple conclusion cannot be drawn regarding the decrease in serum lipids, or that it is accomplished only by the stimulating effect of GE on the excretion of neutral sterol and acidic steroids to feces.

In general, LCAT activity is influenced by apoprotein A-I, C-II, its substrates and properties of HDL such as HDL3 or HDL2 on which LCAT mainly reacts.¹⁸ Therefore, the LCAT

activity obtained in this study is cholesterol-esterifying activity in whole serum, rather than a definite enzymatic activity of LCAT. In this study, LCAT was activated by administration of GE (Fig. 2). It is reported that LCAT is activated by edible fat, and also that LCAT shows a different specificity according to fatty acid as substrate.¹⁹ In this study, a promoting effect of GE on LCAT activity was observed in both NF- and HF-fed groups. Therefore, no influence was shown on LCAT activity by addition of beef tallow. Although the effects of GE on apoprotein A-I and C-II, or cholesterol ester-transfer protein have not been investigated, there are possibilities that administration of GE stimulates esterification of cholesterol, and/or that GE participates in the counter transport system of cholesterol.

As for organic acids in cecum, propionic acid and acetic acid contents were increased by administration of GE in both NF-fed and HF-fed groups (Table 3). There have been many reports concerning the relationships between organic acid fermentation in the cecum and blood lipid concentration.^{20,21} The co-fermentation products of cecal contents and beet fiber decreased plasma cholesterol in rats due to organic acids such as propionic, acetic or butyric acid.²² Moreover, the intraperitoneal injection of propionic acid to rats reduced blood cholesterol.¹⁷ Therefore, the reduction of serum cholesterol level observed in this study may be related to propionic acid content. Therefore, it is assumed that GE may influence the metabolism of intestinal bacteria and organic acid fermentation in cecum and consequently, improve serum lipids. However, this is a subject for further investigation.

As a result of these studies, the possibility was obtained that GE influences lipid metabolism in a wide range of living systems. Remaining problems for investigation are; how do the components of GE undergo transition in blood? and what are the active components of GE?

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