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STUDIES ON *GANODERMA LUCIDUM*

II. The effects of *G. lucidum* on lipid metabolim in rats[1,2]

MING-SHI SHIAO[3], TSUNG-CHE TSENG, YUN-YUN HAO and YUH-SHYAN SHIEH

Institute of Botany, Academia Sinica, Taipei, Taiwan 11529, Republic of China

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Abstract

Ganoderma lucidum, a Polyporaece used in traditional Chinese medicine, was fed to rats at two distinct physiological states representing low cholesterogenesis and high lipogenesis. Its effects on serum cholesterol and triacylglycerol contents were examined. Animals were treated with 2% cholesterol diet to suppress sterol biosynthesis. Supplement of *G. lucidum* (Strain No. TP-1) to diet (4.7%, w/w) increased serum cholesterol and triacylglycerol levels by 43% and 22%, respectively. Fat-free high carbohydrate diet was applied to induce lipogenesis in rats. If *G. lucidum* was added to diet, cholesterol level of animal serum was higher than that of control value by 38%. Triacylglycerol content in serum was not significntly affected. However, the amount of triacylglycerols and total lipids in liver were both reduced.

Key words: *Ganoderma lucidum*; animal test; lipid metabolism; cholesterol; triacylglycerols.

Introduction

Ganoderma lucidum, a famous Polyporaece used in traditional Chinese folk medicine, was cultured in liquid media and for fruit body formation in our laboratory (Tseng *et al.*, 1984). Recently, six cytotoxic oxygenated lanostane acids have been isolated from mycelium in liquid culture of *G. lucidum* (Toth *et al.*, 1983). Several oxygenated triterpenes have heen demonstrated to be responsible for the bitter flavor of this medicinal fungus (Nishitoba *et al.*, 1985). Among those triter-

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[3] Present address: Department of Medical Research, Veterans General Hospital, Shih-pai, Taipei, Republic of China.

penes, a few compounds have structures analogous to the known oxygenated sterols with hypocholesterolemic activity (Schroepfer, 1982). To elucidate the effect of *G. lucidum* on mammalian lipid metabolism we decided to carry out study based on animal test in rats at two distinct physiological states representing high lipogenesis and low cholesterogenesis respectively (Dietschy *et al.*, 1978). Test animals were fed with fat-free diet to induce lipogenesis. Physiological state of low cholesterol biosynthesis was generated by feeding rats with 2% cholesterol diet. This report is the preliminary results of the animal test.

Materials and Methods

Animals

Male Wistar rats, average body weight of 80±10 g, were obtained from the Animal Center, University Hospital, National Taiwan University. Animals were maintained in the animal house at 12 hour light-dark cycle. Metabolic test diet was provided *ad lib* and the body weight was followed daily at 3 to 5 p.m. Animals were rejected if the body weight difference was greater than 10% of mean value. Routinely, six to nine rats were used for each independent test.

Metabolic Diets

Complete rat chow supplemented with 2% cholesterol and fat-free diet were purchased from ICN Nutritional Biochemicals Co. (Cleveland, USA). The fat-free diet is consisted of vitamin free casein 21.1%, alphacel 16.45, sucrose 58.45%, salt mixture USP XIV 4.0%, ICN vitamin diet fortified mix, and choline chloride at

Table 1. *Chemical compositions of mycelium of* ***Ganoderma lucidum***

Ganoderma lucidum was cultured in potato-dextrose broth at 28± 2°C for two weeks.

Composition	Percentage of dry weight
Carbohydrates	75
Reducing sugars	29.8
Non-reducing sugars	45.3
Lipids	4.6
Saponified	1.4
Non-saponified	2.9
Soluble Proteins	8.3
Ashes	3.6

272.59 g/100 lb. Control rat chow was manufactured by Ralston Purina Co. (St. Louis, USA). *G. lucidum* was cultured in liqnid potato-dextrose borth for 14 days and supplemented to the diet on 4.7% dry weight basis (Table 1) (Tseng *et al.*, 1984). In preparation of test diet, pre-heated water agar (2%, w/w) was cooled down to 60°C before *G. lucidum* was added to avoid possible heat destruction.

Clinical Test Kits

Kits for total serum cholesterol determination were obtained from E. Merck Co. (Darmstadt, W. Germany). Acetic anhydride and $CHCl_3$ used in Libermann-Burchard test were distilled. Cholesterol and its oleate were obtained from Aldrich Chemical Co. (Milwaukee, USA) and were recrystallized. Fatty acid methyl esters were purchased from Sigma Chemical Co. (St. Louis USA). Kits in triacylglycerol determination were manufactured by Nissui Yaku Co. (Tokyo, Japan).

Sera and Liver Samples

Animals were sacrificed and blood was collected by cardiac puncture. Serum was obtained by allowing blood to coagulate at 30°C for 2 hours and stored at 4°C overnight. Liver was excised, weighed and immediately frozen.

Pretreatment of animals

Rats were fed with normal diet for a few days before test. Test started after two-day fasting.

Determination of Lipids

Crude total lipids were determined by direct gravimetric method (Willams, 1984). Sera were extracted 5 times (5×1 v) with a mixture of $CHCl_3$: MeOH (2:1, v/v). Organic layers were pooled and solvent removed by rotary evaporation until the difference between two consecutive weighing was less than 2 mg. Total lipids were also measured by the method using vanillin-phosphoric acid-conc. sulfuric acid colorimetrically.

Cholesterol was measured by Libermann-Burchard method and HPLC (Column, μ-Bondapak C-18; Solvent. MeOH, isocratic elution, RI detection). Total cholesterol was determined by a coupled enzymatic assay which esterified cholesterol was reacted subsequently with lipase, oxidase and the total amount was followed by quantity of iodide reduced at 365 nm (Richmond and Fu, 1974).

Triacylglycerol was also monitored by a coupled enzymatic assay (Jacobs *et al.*, 1960). Compositions of fatty acids in the lipid subclasses were determined by gas chromatography as their methyl esters.

Results and Discussion

The effect of feeding *Ganoderma lucidum* on lipid metabolism in rats was studied in this report. Accordingly, animal test was carried out at two distinct physiological states. Treatment of rats with 2% cholesterol diet for 10 days was chosen to represent the state that feed-back regulation of cholesterol biosynthesis is highly functioning (Mahley and Holcombe, 1977). Conditions of animals on 2% cholesterol diet and *G. lucidum* are summaried in Table 2. Rats in Control II group were fed with 2% cholesterol diet supplemented with sterilized potato-dextrose broth to eliminate the possible ambiguity due to growth media. No significant effect was observed due to broth itself. In the test group, *G. lucidum* was added on 4.7% dry weight basis for 10 days. Supplement of *G. lucidum* at this ratio was not expected to change the calorific uptake of test animals. The lipid contents in *G. lucidum* accounted for only about 0.2% of diet on dry weight basis. Animals were carefully examined and no fatality or obvious illness was found.

Table 2. *Condition and treatment of animals*

Group (No. of rats)	Average body weight (g)	Period of fasting (day)	Period of refeeding (day/diet*)	Period of test (day/diet*)	Average final weight liver/body (g/g)	Average body weight increment (g/day)
Control I (6)	97	2	7/N	10/N	6.7/153	3.3
Control II (7)	99	2	7/C	10/B	7.0/162	3.7
Test (7)	103	2	7/C	10/A	8.7/173	4.1

* Diets: N=normal diet.
A=2% cholesterol diet+*Ganoderma lucidum* (4.7%).
B=2% cholesterol diet+sterilized broth.
C=2% cholesterol diet.

Table 3. *Routine biochemical analysis of rat serum*

Component or determination	Unit	Control (8)*	CJ-3 (8)	AT-4 (8)	AT-5 (8)
Glucose	mg/dl	66	69	58	68
Creatine	mg/dl	0.5	0.5	0.5	0.4
Cl^-	meq/l	98	96	97	97
Na^+	meq/l	138	134	134	138
ALP	U/l	599	711	827	782
ALT	U/l	41	46	41	29
AST	U/l	196	175	174	158

ALP: Alkaline phosphatase.
ALT: Alanine aminotransferase.
AST: Aspartate aminotransferase.

* No. of rats. Rats were fed with normal diet supplemented with *Ganoderma lucidum* (2.5%, w/w) for 17 days.

Blood samples were also subjected to routine biochemical test and values were normal (Table 3). Slightly higher ratio of liver to body weight was found in the test group (Table 2). The serum total cholesterol in the test group was 43% higher than those of control I and II groups (112 mg/dl vs. 78 and 79 mg/dl, $p<0.001$). Triacylglycerol level was also increased by 22% ($p<0.01$). Difference in free cholesterol between test and control II group was not statistically significant. However, both values were found higher than that in control I group (Table 4).

Table 4. *Levels of serum cholesterol and triacylglycerols in rats on 2% cholesterol diet supplemented with* ***Ganoderma lucidum***

Group (No. of rats)	Level of serum cholesterol		Relative levels of triacylglycerols to that of control I (%)
	Free (mg/dl)	Total (mg/dl)	
Control I (6)	28±3.4	78±9.3	100±20
Control II (7)	41±8.2	79±7.1	145±15
Test (7)	40±2.8	112±12.3	177±22

Rats fed with fat-free high carbohydrate diet would stimulate the conversion of carbohydrates into lipids (Nepokroeff *et al.*, 1974). The activities of lipogenic enzymes are greatly induced and degradation of lipids is suppressed in this physiological state. Conditions of treated animals in the test period are summarized in Table 5. Daily body weight increment and ratios of liver to body weight in the final were found uniform in all experimental groups. Serum total cholesterol level in the test group was significantly higher by 38% in comparison with those of control I and II groups (94 mg/dl vs. 73 and 68 mg/dl, $p<0.005$) (Table 6). Free cholesterol was only marginally increased comparing with that in control II group (36 mg/dl vs. 34 mg/dl). Levels of of serum triacylglycerols in test and control II groups were all increased, an indication that lipogenic enzymes were induced,

Table 5. *Condition and treatment of animals*

Group (No. of rats)	Average body weight (g)	Period of fasting (day)	Period of feeding (day/diet*)	Average final weight liver/body (g)	Average body weight increment (g/day)
Control I (8)	73	2	10/N	6.8/136	6.3
Control II (8)	78	2	10/C	7.1/140	6.2
Test (8)	70	2	10/A	7.0/134	6.4

* Diets: N=normal diet.
A=fat-free diet+*Ganoderma lucidum* (4.7%).
C=fat-free diet+sterilized broth.

but difference between them was not significant (Table 6). However, total lipids in the liver of test group were lower than that of control II group ($p<0.001$). Triacylglycerol content in liver of test group was also lower in comparison with that of control II group (by 46%, $p<0.005$) (Table 7).

Table 6. *Levels of serum cholesterol and triacylglycerols in rats on fat-free diet supplenemted with* ***Ganoderma lucidum***

Group (No. of rats)	Levels of serum cholesterol		Relative levels of triacylglycerols to that of control I (%)
	Free (mg/dl)	Total (mg/dl)	
Control I (8)	29±2.3	68±3.4	100±12
Control II (8)	34±4.3	73±8.0	140±10
Test (8)	36±4.0	94±4.7	132±20

Table 7. *Amounts of liver triacylglycerols and total lipids in rats on fat-free diet supplemented with* ***Ganoderma lucidum***

Group	Triacylglycerides		Total lipids (Relative %)
	A_{585} in enzymatic assay	Relative %	
Control I	0.134±0.001	100	100
Control II	0.265±0.002	197	187
Test	0.203±0.018	151	96

This animal study concluded that feeding of *G. lucidum* to rats at physiological states of low cholesterogenesis or high lipogenesis had no hypocholesterolemic effect. In fact, serum total cholesterol levels were significantly elevated. In conjunction with this observation, our previous study also showed that feeding of mycelia of *G. lucidum* to rats on normal diet (2.5%, w/w) did not reduce serum cholesterol level to any significant extent (Fig. 1). In present cases levels of triacylglycerols were found not affected or even slightly decreased by supplement of *G. lucidum* to diet.

Although several oxygenated triterpenes identified in *G. lucidum* have structures analogous to those known cholesterol-lowering sterols and triterpenes, hypocholesterolemic activity was not observed in this animal test. We speculate that their contents were too low to be effective in mycelia supplemented to the diet. More detailed study on purified individual components of these oxygenated triterpenes and determinetion of lipid profiles in the lipoprotein subclasses of tested animals is essential.

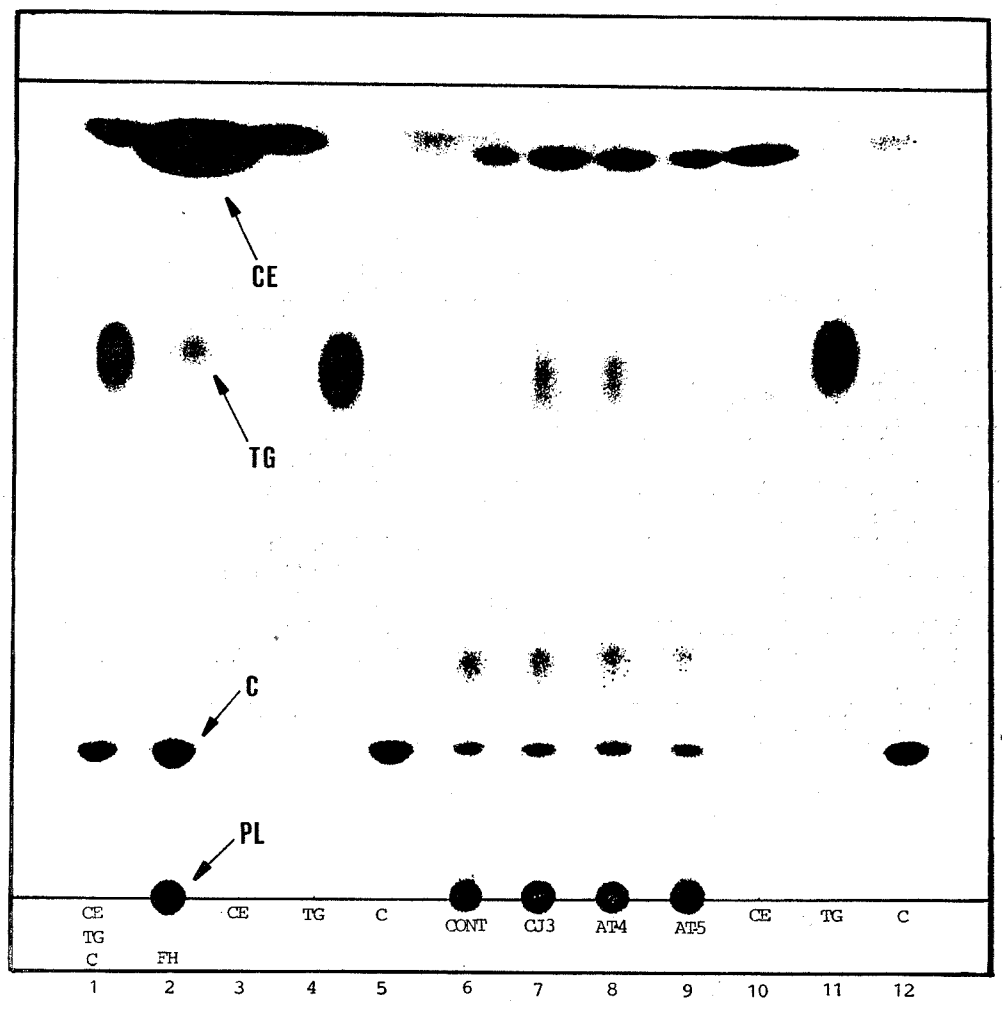

Fig. 1. Thin-layer chromatogram (silica gel, 20×20 cm, 0.25 mm of gel thickness; solvents, Hexane: Et_2O: HOAc=85:15:1, v/v; plate visualized with I_2 vapor) of serum lipid profiles and lipid standards. Column 7-9 were serum lipids of rats on normal diet supplemented with 2.5% (w/w) *Ganoderma lucidum* (Strains CJ3, AT-4, and AT-5) for 17 days. Column 6 was control rat serum lipids. Column 2 was the serum lipids of a human subject with familial hypercholesterolemia (FH) for comparison. Abbreviations of authentic compounds are; C, cholesterol; CE, cholesterol esters; TG, triacylglycerols; PL, phospholipids. All three test groups contained higher cholesterol, particularly cholesterol esters, than that of control.

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靈 芝 之 研 究

(二) 對大白鼠脂質代謝的作用

蕭明熙 曾聰徹 郝箐箐 謝毓賢

中央研究院植物研究所

本研究係探討靈芝對大白鼠血清膽固醇及三羧酸甘油酯含量的影響，將大白鼠飼以含2%膽固醇飼料，造成高攝取以誘導固醇類生物合成受抑制的生理狀態，若投以液相培養靈芝，其血清中總膽固醇和三羧酸甘油脂含量各增加43%與22%。如果利用高醣缺脂者進食，使大白鼠處於趨脂酵素誘生狀態，再投以靈芝，則血清膽固醇增高38%，但三羧酸甘油酯無變化，而肝中的三羧酸甘油酯及總脂量則均下降。