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Taiwanofungus camphoratus Activates Peroxisome Proliferator-Activated Receptors and Induces Hypotriglyceride in Hypercholesterolemic Rats

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***Taiwanofungus camphoratus* (*T. camphoratus*), a fungus and a Taiwan-specific, well-known traditional Chinese medicine, has long been used to treat diarrhea, hypertension, itchy skin, and liver cancer. To gain a large amount of *T. camphoratus*, several culture techniques have been developed, including solid-state culture and liquid-state fermentation. Peroxisome proliferator-activated receptor gamma (PPARγ) has been described as a hypoglycemic agent that increases insulin sensitivity in peripheral tissues and results in reduced blood glucose, insulin, and triglyceride levels in insulin-resistant animals and in type-2 (non-insulin-dependent) diabetic patients. In this study, we investigate the possibility that *T. camphoratus* might activate PPARγ *in vitro* and hypolipidemic activity *in vivo*. The results show that an aqueous extract of the wild fruiting bodies of *T. camphoratus* was able to increase the PPARγ activity in cells transfected with the PPARγ expression plasmid and the AOx-TK reporter plasmid. Based on the cell experiment, we examined the hypolipidemic effect of wild fruiting bodies (WFT) and a solid-state culture (SST) of *T. camphoratus* on SD rats fed on a high-cholesterol (HC) diet. The results show that WFT significantly decreased the serum triglyceride level, but could not affect the cholesterol level. SST only slightly decreased the serum triglyceride level. In addition, both WFT and SST significantly decreased the serum alanine transaminase (ALT) level and protected against the liver damage induced by the HC diet from the results of a histological examination. These results suggest that *T. camphoratus* might contain PPARγ ligands and result in a hypotriglyceridemic effect, and that it also exhibits a liver protective activity.**

Key words: *Taiwanofungus camphoratus*; peroxisome proliferator-activated receptor; triglyceride; glucose

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor superfamily and have been initially described as molecular targets for compounds that cause peroxisome proliferation.[1)] To date, three isotypes of PPAR have been described in humans: α; NUC1, also called β or δ; and γ.[2–5)] As with most of the members of the nuclear hormone receptor superfamily, dimerization is essential for the activation of PPARs. They heterodimerize with the 9-cis retinoid X receptor (RXR),[6)]

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; CWE, cold water extract from wild fruiting bodies of *T. camphoratus*; HWE, hot water extract from wild fruiting bodies of *T. camphoratus*; ME, methanol extract from wild fruiting bodies of *T. camphoratus*; WFT, wild fruiting bodies of *T. camphoratus*; SST, solid-state culture of *T. camphoratus*; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HC, high cholesterol; AST, aspartate transaminase; ALT, alanine transaminase; GLU, glucose; BUN, blood urea nitrogen; CRE, creatinine; ALB, albumin

forming a complex that is able to bind to the PPAR response elements (PPRE) located in the promoter of PPAR target genes. The target genes of PPARα are a relatively homogenous group of genes that participate in aspects of lipid catabolism such as fatty acid uptake through membranes, fatty acid binding in cells, fatty acid oxidation, and lipoprotein assembly and transport. Whereas PPARγ influences the storage of fatty acids in the adipose tissue, with the C/EBP transcription factor, PPARγ is part of the adipocyte differentiation program that induces the maturation of pre-adipocytes into fat cells.[7] Several PPs (peroxisome proliferators) have been shown to act as exogenous PPAR activators by cell transactivation assays.[8] These include hypolipidemic drugs such as clofibrate, gemfibrozil, and the experimental drug,[9,10] WY 14,643, leukotriene D4 receptor antagonists,[11,12] industrial compounds, including the phthalate ester metabolite, monoethylhexyl phthalate (MEHP),[13] and trichloroacetic acid, a metabolite of the organic solvent, trichloroethylene.[14] In addition to fibrate, non-steroidal anti-inflammatory drugs like ibuprofen, indomethacin, and fenoprofen have been shown to activate PPARα. In contrast to the previous synthetic PPAR ligands, the insulin-sensitizing anti-diabetic thiazolidinediones (TZD),[15–17] such as rosiglitazone (BRL 49653) show high selectivity for PPARγ. TZD are oral hypoglycemic agents that increase insulin sensitivity in peripheral tissues, which results in reduced blood glucose, insulin, and triglyceride levels in insulin-resistant animals and in type-2 (non-insulin-dependent) diabetic patients. PPARs play an important and central role in lipid homeostasis, which when unbalanced, can lead to obesity, diabetes, and cardiovascular disease. At present, chemicals targeted at PPARs (such as fibrates and thiazolidinediones) have been shown to be effective treatments for hyperlipidemia and insulin resistance. Therefore, many laboratories are interested in developing new and specific ligands for these receptors from natural and synthetic chemicals.

Taiwanofungus camphoratus (aka *Antrodia camphorata*) is a native fungus in Taiwan, grows in a unique host, the endemic perennial tree *Cinnamomun kanehirai* (Bull camphor tree), and is known as a folk medicine in Taiwan. It has been used to treat abdominal pain, diarrhea, drug intoxication, hypertension and skin itching, and to improve the immune system and liver function.[18] Previous studies have demonstrated that extracts of *T. camphoratus* exhibited anti-inflammatory and antioxidative activities, induced vasorelaxation, and decreased the hepatitis B virus. A triterpenoid sesquiterpene lactone, steroid, and polysaccharides are the bioactive ingredients in *T. camphoratus*.[19–22] However, more unknown ingredients remain to be discovered and identified. Two types of artificial cultivation of *T. camphoratus*, a solid-state culture and liquid-state fermentation, were developed to substitute for the rare wild fruiting body. In the present study, we use extracts of the wild fruiting body and solid-state culture of *T. camphoratus* to examine the possible activation of PPAR *in vitro* and the hypolipidemic effects of this on rats fed on a high-cholesterol diet.

Materials and Methods

Materials. Cholesterol was purchased from Sigma Chemical (St. Louis, MO, USA). The dried ground powder of the wild fruiting body and solid-state culture of *T. camphoratus* were provided by Well Shine Biotechnology Development Co. (Taipei, Taiwan).

Preparation of different extracts from T. camphoratus. The wild fruiting body of *T. camphoratus* was sequentially extracted with cold water, methanol, and hot water to give a cold water extract, methanol extract, and hot water extract, respectively, as described previously.[23]

Cell culture and transient transfection. African green monkey kidney COS-1 cells (BCRC 60002, Food Industry Research and Development Institute, Hsinchu, Taiwan) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated bovine serum (Invitrogen Taiwan Ltd., Taipei, Taiwan), 100 units/ml of penicillin, and 100 µg/ml of streptomycin. COS-1 cells were seeded in 24-well plates. After 24 h of incubation, the medium was replaced with the Opi-MEM medium (Invitrogen, Carlsbad, CA, USA) and co-transfected with the PPARγ expression plasmid and AOx-TK reporter plasmid plus the phRL-TK (Promega, Madison, WI, USA) plasmid as an internal control, using Lipofectamine2000™ (Invitrogen). After 48 h of incubation, the cells were treated with various extracts of the wild fruiting body of *T. camphoratus* for 18 h. A total cell lysate was used to determine the luciferase activity with the Dual-Luciferase Reporter Assay kit (Promega) as described previously.[24]

Animals and treatments. Male Sprague-Dawley (SD) rats (6 weeks old) were purchased from the National Laboratory Animal Center (Taipei, Taiwan), housed in stainless steel wire-bottomed cages, and acclimatized under laboratory conditions (19–23 °C, 60% humidity, 12-h light/dark cycle) throughout the experimental period. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Taipei Medical University. The rats were divided into six groups (5 rats/group) and fed with different diets: group 1, a basal diet (ground Purina rat feed, Ralston Purina, St. Louis, MO, USA); group 2, basal diet with 1% WFT; group 3, basal diet with 1% SST; group 4, high-cholesterol diet (basal diet with 1% cholesterol); group 5, high-cholesterol diet with 1% WFT; group 6, high-cholesterol diet with 1% SST. The experiments were carried out for 12 weeks. For the first 6 weeks, food and water were provided *ad libitum*, but these were limited for the last 6 weeks. The rats were

then starved for 16 h, and fasting blood was collected *via* the tail vein or heart puncture under pentobarbital anesthetization.

Determination of plasma chemical and biochemical profiles. Total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride levels were determined with an automated chemistry analyzer (AU-640, Olympus Optical Co., Ltd., Tokyo, Japan). VLDL-cholesterol was calculated as follows: VLDL-cholesterol = total cholesterol − (LDL-cholesterol + HDL-cholesterol). Other biochemical parameters (glucose, BUN, CRE, AST, and ALT) were determined with an automated chemistry analyzer (AU-400, Olympus Optical Co., Ltd., Tokyo, Japan).

Histological examination. Paraffin-embedded rat livers were cut, and the sections were stained with hematoxylin and eosin to allow a pathologist to determine the changes.

Statistical analyse. Animal experimental data were analyzed by two-way ANOVA. When the interaction (HC diet × WFT or HC diet × SST) was significant, Tukey's multiple-comparison test was performed. Cell experimental data were analyzed by one-way ANOVA and followed by Dunnett's multiple-comparison test. The data are expressed as the mean ± S.D., and differences are considered to be significant at $P < 0.05$.

Results

Activation of PPAR by the aqueous extracts of T. camphorates

A series of WFT extracts were prepared: a cold-water extract (CWE), hot water extract (HWE), and methanol extract (ME). These were tested with regard to their activation effects on PPARγ in COS-1 cells. The PPARγ expression plasmid was cotransfected into COS-1 cells with an AOx-TK reporter construct containing three copies of the acyl-CoA oxidase PPAR responsive element (PPRE) upstream of the thymidine kinase (TK) promoter driving luciferase gene expression. When PPARγ are activated by their ligands, they can bind to the PPRE site and drive luciferase gene expression. Among the three *T. camphoratus* extracts, 10 µg/ml of CWE and HWE significantly activated PPARγ by approximately 1.6 and 2.2-fold, respectively (Fig. 1A). ME was unable to activate PPARγ at under 10 µg/ml. A little cytotoxic effect may have occurred in COS-1 cells under 10 µg/ml of ME. When the ME concentration was decreased, ME caused less cell death and no significant PPARγ induction. In contrast, CWE and HWE both induced PPAR activity in a dose-dependent manner, although a higher concentration of CWE or HWE (30 µg/ml) might have had a cytotoxic effect and resulted in lower PPARγ activity (Fig. 1B and C). These results suggest that an aqueous extract of *T. camphoratus* could activate PPARγ and PPAR ligands that might exist in *T. camphoratus*.

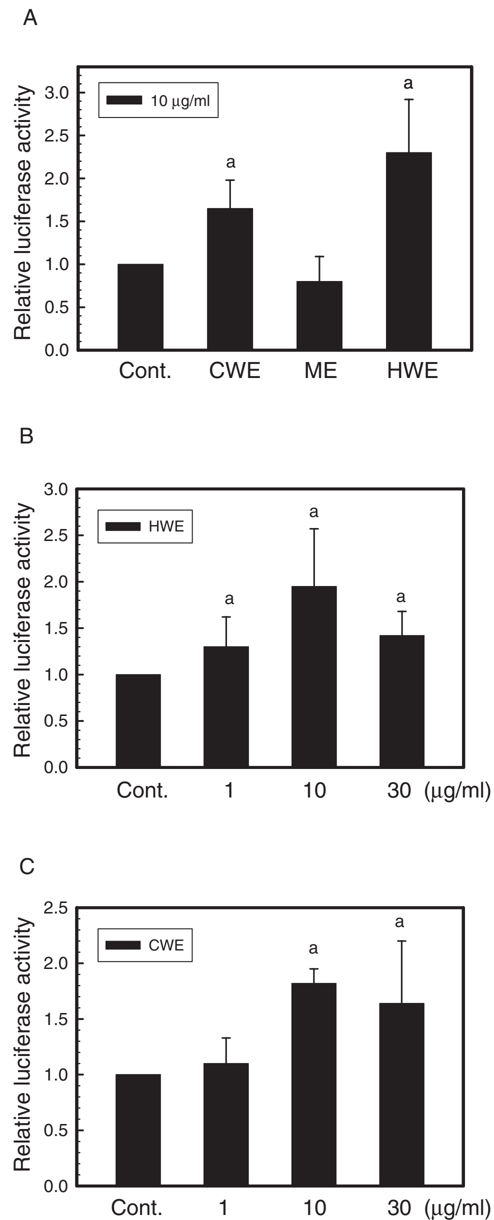

Fig. 1. Effects of *T. camphoratus* on the Activation of PPARγ in Cells.

COS-1 cells were co-transfected with the AOx-TK reporter plasmid and PPARγ expression plasmid, while the phRL-TK plasmid served as an internal control. A, Transfected cells were treated with 10 µg/ml of the cold water extract (CWE), methanol extract (ME), or hot water extract (HWE) of wild fruiting bodies for 18 h. The transfected cells were treated with various concentration of HWE (B), or CWE (C) of wild fruiting bodies for 18 h. Total cell extracts were subsequently assayed for luciferase activity as described in the Materials and Methods section. Each value is presented as the mean ± S.D. of triplicate tests. [a]$P < 0.05$ *versus* control by Dunnett's multiple-comparison test.

Reduction of blood triglyceride and glucose levels by T. camphoratus

Previous studies have demonstrated that PPAR acti-

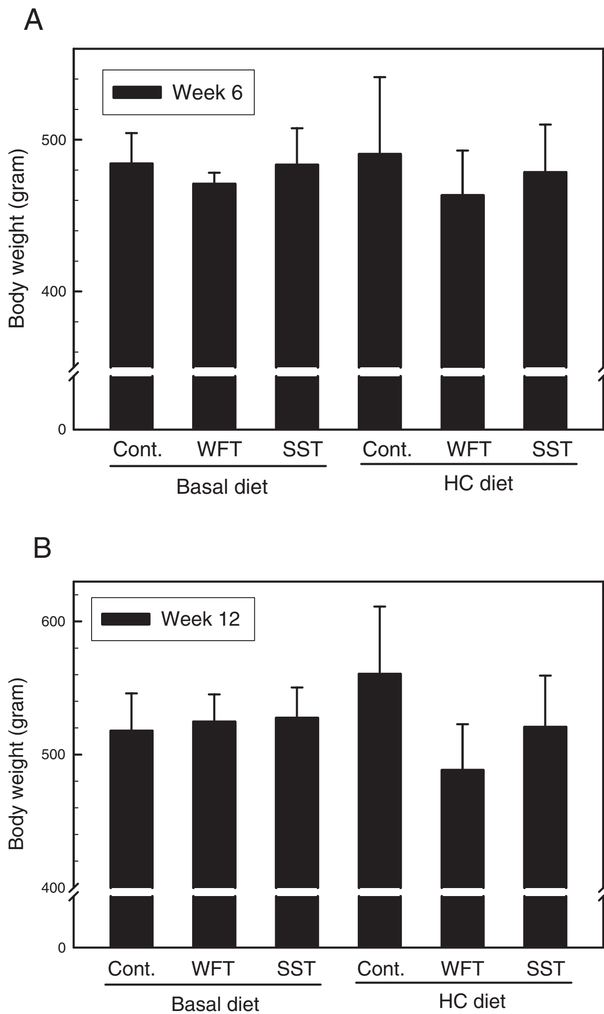

Fig. 2. Effects of *T. camphoratus* on the Body Weight of Rats Fed with the High-Cholesterol Diet for 6 and 12 Weeks.

Cont, control; WFT, 1% wild fruiting body of *T. camphoratus*; SST, 1% solid-state culture of *T. camphoratus*; HC diet, high-cholesterol diet. Data are presented as the mean ± S.D. from five rats per group.

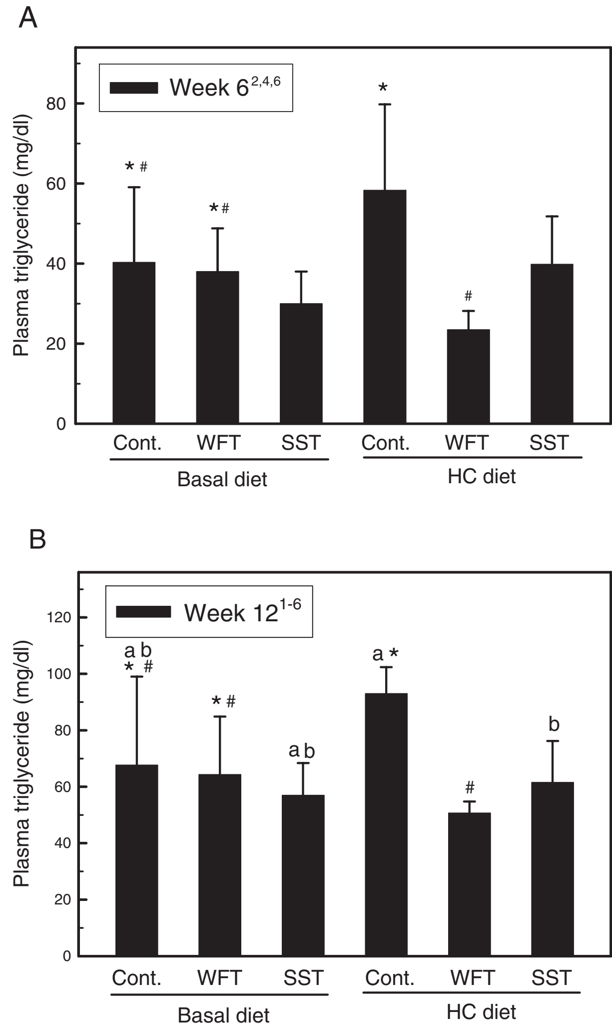

Fig. 3. Effects of *T. camphoratus* on the Serum Triglyceride Levels of Rats Fed with the High-Cholesterol Diet for 6 and 12 Weeks.

Cont, control; WFT, 1% wild fruiting body of *T. camphoratus*; SST, 1% solid-state culture of *T. camphoratus*; HC diet, high-cholesterol diet. Data are presented as the mean ± S.D. from five rats per group. [1]Two-way ANOVA indicated a significant effect of SST ($P < 0.05$). [2]Two-way ANOVA indicated a significant effect of WFT ($P < 0.05$). [3]Two-way ANOVA indicated a significant effect of the HC diet × SST ($P < 0.05$). [4]Two-way ANOVA indicated a significant effect of the HC diet × WFT ($P < 0.05$). [5]Means in a row (HC diet × SST) followed by the same letter are not significantly different by Tukey's *post-hoc* test ($P < 0.05$). [6]Means in a row (HC diet × WFT) followed by the same symbol are not significantly different by Tukey's *post-hoc* test ($P < 0.05$).

vators can be used to treat various diseases, including dyslipidemias and diabetes. Since we found that the aqueous extract of *T. camphoratus* exhibited PPARγ activity, the next test was to investigate whether *T. camphoratus* could alter the lipid metabolism in rats fed on the HC diet. Male SD rats were fed with either the basal diet (group 1), basal diet containing 1% WFT (group 2), basal diet containing 1% SST (group 3), HC diet (basal diet with 1% cholesterol, group 4), HC diet containing 1% WFT (group 5), or HC diet containing 1% SST (group 6) for 12 weeks. In the last 6 weeks, the rats were fed on the same diet as that in the first 6 weeks, although the diet intake was limited. During the feeding period, each group showed no significant difference in the dietary intake (about 23.5 g/rat and 26.2 g/rat in the 6th and 12th week, respectively). During the 6th week, fasting blood was collected *via* the tail vein, and the end of 12th week, the fasting blood was collected *via* a heart puncture, and liver specimens were collected for future examination. As shown in the Fig. 2, the average body weight showed no significant difference among the six groups in the 6th week. However, group 5 seemed to have a lower mean body weight than group 4 at the end of 12th week.

We next found that the serum mean triglyceride level in the HC-fed rats (group 4) was higher than that in the basal diet-fed rats (group 1) but was not significantly different in the 6th week and at the end of the 12th week (Fig. 3). Interestingly, the WFT-fed rats (group 5) on the

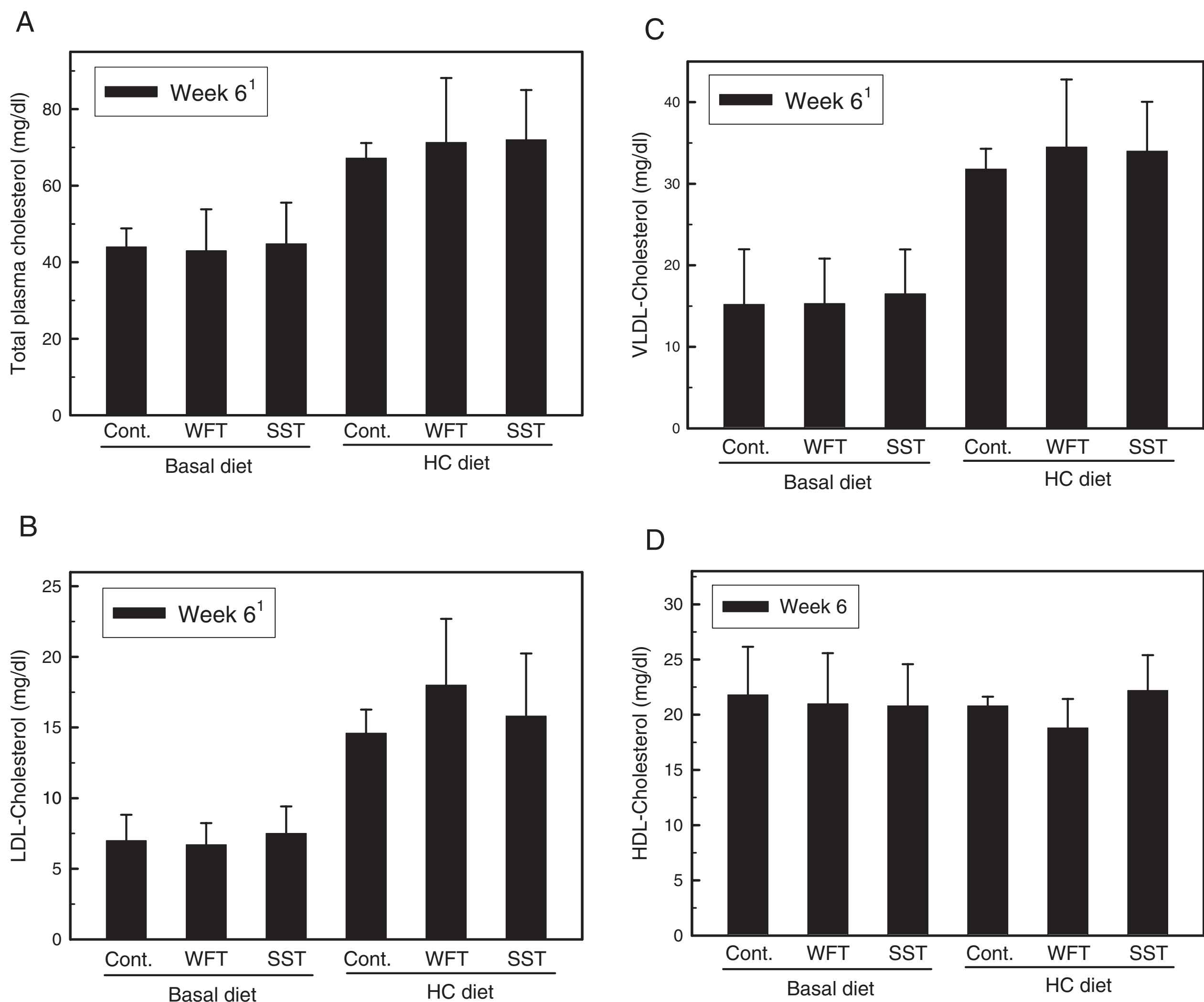

Fig. 4. Effects of *T. camphoratus* on the Total Serum Cholesterol (A), LDL-Cholesterol (B), VLDL-Cholesterol (C), and HDL-Cholesterol (D) Levels of Rats Fed with the High-Cholesterol Diet for the First 6 Weeks.

Cont, control; WFT, 1% wild fruiting body of *T. camphoratus*; SST, 1% solid-state culture of *T. camphoratus*; HC diet, high-cholesterol diet. Data are presented as the mean ± S.D. from five rats per group. [1]Two-way ANOVA indicated a significant effect of the HC diet ($P < 0.05$).

HC diet had a significant lower serum triglyceride level than the HC diet-fed rats (group 4) in both the 6th week and at the end of the 12th week. In addition, the SST-fed rats also had a significant lower level of serum triglyceride than the HC diet-fed rats in the 12th week. These results suggest that WFT and SST might have decreased the serum triglyceride level in the rats on the HC diet. Other lipid parameters were also determined, and the total serum cholesterol, LDL-cholesterol, and VLDL-cholesterol levels were found to be highest in the HC diet rats (group 4) than in the basal diet rats (group 1) in the 6th week (Fig. 4A–C). However, the administration of *T. camphoratus* (groups 5 and 6) did not decrease the total serum cholesterol, LDL-cholesterol, or VLDL-cholesterol level relative to the HC diet-fed rats (group 4). In addition, the HDL-cholesterol level did not differ between the basal diet rats (groups 1–3) and HC diet-fed rats with/without *T. camphoratus* (groups 4–6) in the 6th week (Fig. 4D). During the last 6 weeks, the diet uptake was limited to the same level as that in the first 6 weeks. Under these limited-diet conditions, only the LDL-cholesterol level remained high in the HC diet-fed rats (groups 4–6) compared to the basal diet-fed rats (groups 1–3) (Fig. 5B). The administration of *T. camphoratus* (groups 5 and 6) did not decrease the LDL-cholesterol level compared to the HC diet-fed rats (group 4). These results suggest that *T. camphoratus* could not change the cholesterol level in the HC diet-fed rats and that the limited diet uptake was able to restore the total cholesterol level, even with the diet containing HC. It was not able, however, to restore the triglyceride (Fig. 3B) and LDL-cholesterol levels (Fig. 5B).

On the other hand, the HC diet significantly increased the serum glucose level in the 6th week (Table 1, group 1 *vs.* group 4), and the WFT diet tended to decrease the glucose level (groups 2, 5). At the end of 12th week, no significant difference in glucose level the

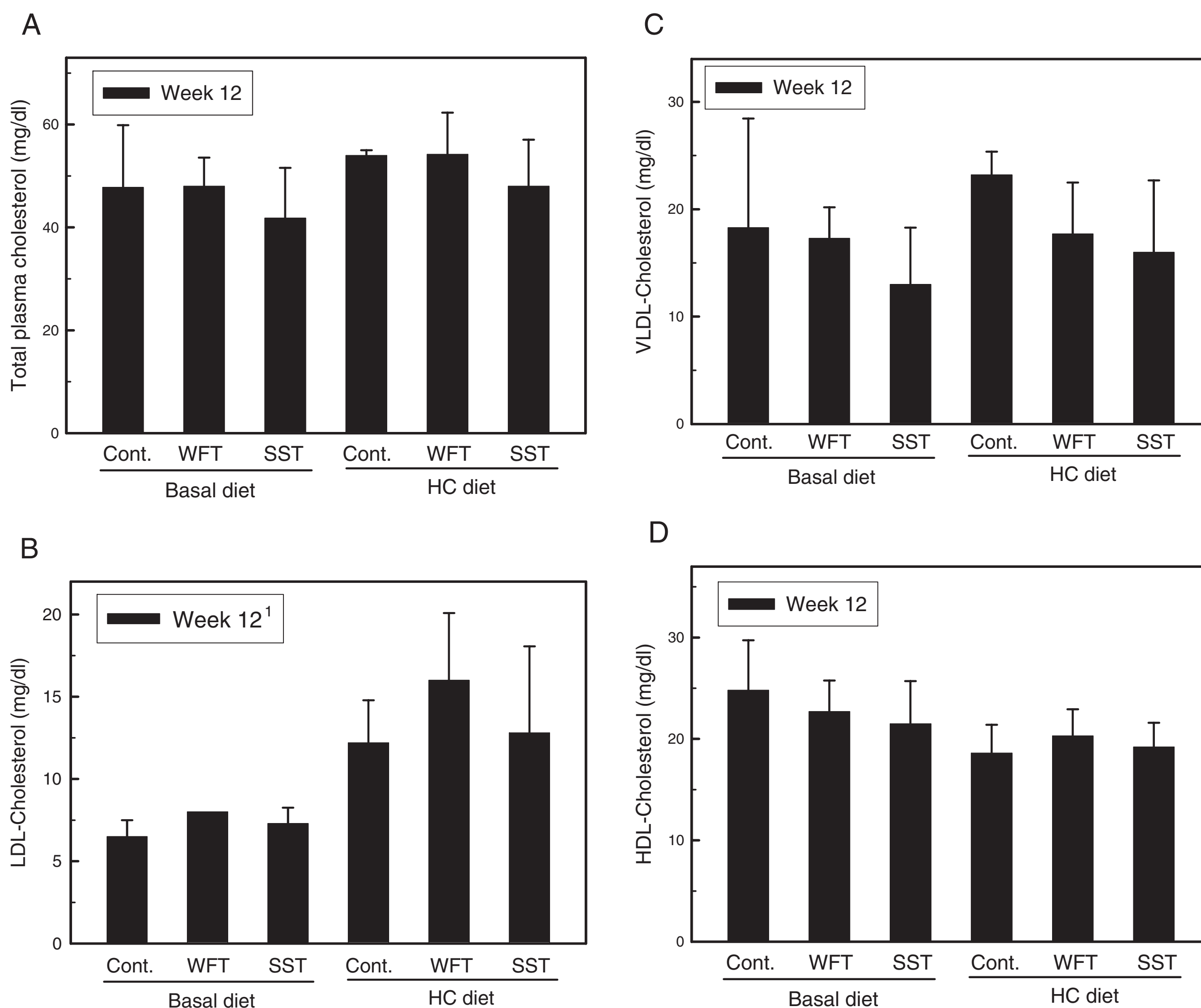

Fig. 5. Effects of *T. camphoratus* on the Total Serum Cholesterol (A), LDL-Cholesterol (B), VLDL-Cholesterol (C), and HDL-Cholesterol (D) Levels of Rats Fed with the High-Cholesterol Diet for 12 Weeks.

Cont, control; WFT, 1% wild fruiting body of *T. camphoratus*; SST, 1% solid-state culture of *T. camphoratus*; HC diet, high-cholesterol diet. Data are presented as the mean ± S.D. from five rats per group. [1]Two-way ANOVA indicated a significant effect of the HC diet ($P < 0.05$).

between the basal diet-fed rats and HC diet-fed rats was apparent (Table 2, group 1 *vs.* group 4), but the WFT diet significantly decreased the glucose level (groups 2 and 5). These results suggest that the wild fruiting body of *T. camphoratus* might improve the serum glucose level under a limited diet uptake condition or normal diet condition.

Liver protective effects of the T. camphoratus *extract on the high-cholesterol diet-fed rats*

After 6 weeks of treatment, the serum levels of AST and ALT were slightly increased in the HC diet-fed rats (group 4) compared to basal diet-fed rats (group 1) (Table 1). The addition of WFT slightly decreased the AST and ALT levels in the HC diet-fed rats (group 5). Although the two-way ANOVA test showed a significant effect of the HC diet × SST, SST did not significantly decrease the ALT level. In the 12th week, the HC diet slightly increased the AST and ALT levels (group 4 *vs.* group 1) (Table 2). The administration of WFT (group 5) and SST (group 6) significantly decreased the ALT level in the HC diet-fed rats (group 4). ALT is a more specific index for liver damage than AST. The ALB level is another index for liver function that was also increased in the rats fed with the basal diet and SST (Table 1, group 1 *vs.* group 3). The histological examination showed that the HC diet-fed rats developed periportal hepatitis, piecemeal necrosis, and inflammation without hepatic steatosis, which was corroborated by a morphological analysis of the abundance of swelling and inflammatory cells around the central vein of the liver (Fig. 6C and D). The histology appeared normal in the rats fed with WFT and the SST (Fig. 6E–H). These results suggest that both WFT and SST might exhibit a liver protective activity against HC-induced liver damage.

In addition, we also examined the serum levels of BUN and CRE that reflect the kidney function. After 6

Table 1. Effects of Wild Fruiting Bodies and the Solid-State Culture of *T. amphoratus* on the Plasma Biochemistry Profiles of SD Rats Fed with a High-Cholesterol Diet for the First 6 Weeks[1]

	Basal diet			HC diet		
	Cont.	WFT	SST	Cont.	WFT	SST
AST (U/l)[2,3,5,7]	71.0 ± 16.7^{a}	69.0 ± 9.9	107 ± 18^{b}	87.0 ± 12.3^{a}	78.0 ± 16.0	71.0 ± 12.2^{a}
ALT (U/l)[2,5,7]	35.0 ± 6.7^{a}	37.0 ± 14.7	38.8 ± 7.3^{a}	46.8 ± 7.7^{a}	41.8 ± 3.5	35.2 ± 4.3^{a}
GLU (mg/dl)[2,3,5,7]	95.1 ± 18.7^{a}	89.6 ± 12.5	101 ± 9^{a}	126 ± 4^{b}	120 ± 8	136 ± 11^{b}
BUN (mg/dl)[2,3,5,7]	17.0 ± 2.9^{a}	21.7 ± 5.7	30.5 ± 9.0^{b}	23.0 ± 2.7^{b}	27.5 ± 8.0	21.6 ± 2.8^{ab}
CRE (mg/dl)[2–8]	$0.45 \pm 0.13^{a*}$	$0.60 \pm 0.17^{\#}$	0.80 ± 0.16^{b}	$0.60 \pm 0.07^{b\#}$	$0.78 \pm 0.15^{\S}$	0.70 ± 0.12^{b}
ALB (g/dl)[2,3,5,7]	2.73 ± 0.75^{a}	3.30 ± 0.61	3.53 ± 0.30^{b}	3.92 ± 0.26^{b}	3.73 ± 0.13	3.98 ± 0.18^{b}

[1]Each value is expressed as the mean ± S.D. (n = 5).
[2]Two-way ANOVA indicated a significant effect of the HC diet ($P < 0.05$).
[3]Two-way ANOVA indicated a significant effect of SST ($P < 0.05$).
[4]Two-way ANOVA indicated a significant effect of WFT ($P < 0.05$).
[5]Two-way ANOVA indicated a significant effect of the HC diet × SST ($P < 0.05$).
[6]Two-way ANOVA indicated a significant effect of the HC diet × WFT ($P < 0.05$).
[7]Means in a row (HC diet × SST) followed by the same letter are not significantly different by Tukey's *post-hoc* test ($P < 0.05$).
[8]Means in a row (HC diet × WFT) followed by the same symbol are not significantly different by Tukey's *post-hoc* test ($P < 0.05$).

Table 2. Effects of Wild Fruiting Bodies and the Solid-State Culture of *T. camphoratus* on the Plasma Bochemistry Profiles of SD Rats Fed with a High-Cholesterol Diet for the Last 6 Weeks[1]

	Basal diet			HC diet		
	Cont.	WFT	SST	Cont.	WFT	SST
AST (U/l)	66.3 ± 11.5	61.0 ± 12.1	71.0 ± 5.5	71.2 ± 16.2	61.8 ± 8.0	66.5 ± 17.3
ALT (U/l)[2–8]	$44.0 \pm 1.4^{ab*\#}$	$38.0 \pm 4.4^{*\#}$	36.3 ± 4.9^{ab}	$48.8 \pm 5.2^{a*}$	$37.0 \pm 5.2^{\#}$	36.8 ± 2.4^{b}
GLU (mg/dl)[2,4,6,8]	$147 \pm 6^{*}$	$130 \pm 10^{\#}$	134 ± 15	$149 \pm 5^{*}$	$130 \pm 11^{\#}$	148 ± 19
BUN (mg/dl)	15.3 ± 1.0	15.7 ± 0.6	17.8 ± 3.9	16.6 ± 2.7	18.8 ± 3.1	16.4 ± 1.5
CRE (mg/dl)	0.55 ± 0.06	0.53 ± 0.06	0.55 ± 0.06	0.54 ± 0.06	0.60 ± 0.08	0.54 ± 0.05
ALB (g/dl)	3.62 ± 0.61	3.83 ± 0.06	3.75 ± 0.26	4.04 ± 0.25	3.95 ± 0.38	3.80 ± 0.29

[1]Each value is expressed as the mean ± S.D. (n = 5).
[2]Two-way ANOVA indicated a significant effect of the HC diet ($P < 0.05$).
[3]Two-way ANOVA indicated a significant effect of SST ($P < 0.05$).
[4]Two-way ANOVA indicated a significant effect of WFT ($P < 0.05$).
[5]Two-way ANOVA indicated a significant effect of the HC diet × SST ($P < 0.05$).
[6]Two-way ANOVA indicated a significant effect of the HC diet × WFT ($P < 0.05$).
[7]Means in a row (HC diet × SST) followed by the same letter are not significantly different by Tukey's *post-hoc* test ($P < 0.05$).
[8]Means in a row (HC diet × WFT) followed by the same symbol are not significantly different by Tukey's *post-hoc* test ($P < 0.05$).

weeks of treatment, the serum levels of BUN and CRE were significantly increased in the HC diet-fed rats (group 4) compared to basal diet-fed rats (group 1) (Table 1). The addition of SST significantly increased the BUN and CRE levels in the basal diet-fed rats, and WFT significantly increased the CRE level in the basal diet-fed rats and HC diet-fed rats. These results suggest that SST and WFT might have an adverse effect on the kidneys.

Discussion

It is well known that PPARα/γ ligands are involved in the negative regulation of leptin and resistin, and participate in lipid metabolism, glucose transport, and insulin sensitivity.[25,26] Activation of PPARγ promotes both lipid storage and lipogenesis, which involves the expression of such genes as aP2, CD36, SCD-1, lipoprotein lipase, and fatty acid transport protein 1. The expression of these genes increases the triglyceride content of adipose tissue and decreases triglyceride and free fatty acids in the circulation, liver and muscles, and finally results in the redistribution of body-wide lipids. It also improves insulin sensitivity as well as lowering glucose level in the blood. High triglyceride and glucose levels in the blood markedly increase the incidence of cardiovascular diseases. In addition, some clinical PPARγ agonist drugs, such as rosiglitazone, have the potential for serious adverse cardiovascular effects for type 2 diabetes patients.[27] Therefore, many academic and pharmaceutical researchers are anxious to discover new and useful PPARγ agonists for the treatment of hyperglycemic and hyperlipidemic patients. Many investigators have sought PPAR agonists in natural products or herbal medicines. Han *et al.* have reported that 20(S)-protopanaxatriol, a ginseng saponin-activated PPARγ, increased PPAR target gene expression, and might improve the insulin resistance associated with diabetes.[28] Our previous study has also demonstrated that several flavonoids were able to activate PPARγ and result in decreased inflammation by a transient transfection assay on mouse macrophages.[24] Several studies

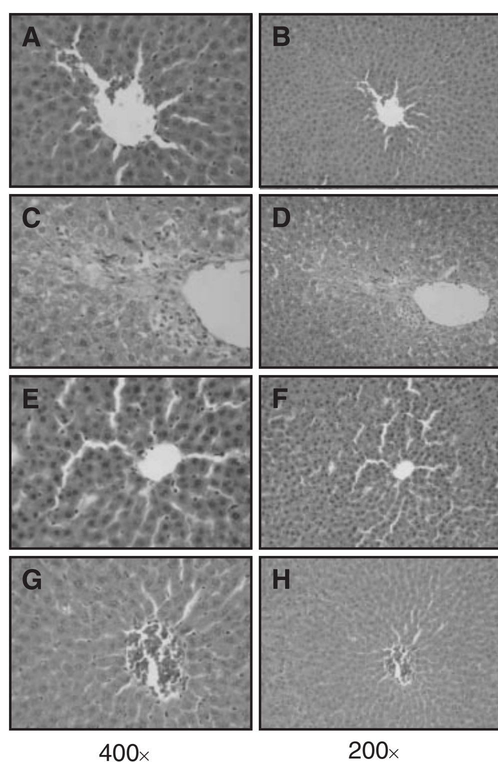

Fig. 6. Effects of *T. camphoratus* on Histopathological Changes to the Liver of Rats Fed with the High-Cholesterol Diet for 12 Weeks.

The liver samples were stained with hematoxylin-eosin and the magnification was shown. (A and B) basal diet; (C and D) high-cholesterol diet; (E and F) high-cholesterol diet with 1% wild fruiting body of *T. camphoratus*; (G and H) high-cholesterol diet with the 1% solid-state culture of *T. camphoratus*.

have demonstrated that a vegetable bitter gourd (*Momordica charantia*) exhibited antidiabetic effects, and that it contained antidiabetic substances such as charantin, vicine, polypeptide-p, 5β,19-epoxy-3β,25-dihydroxycucurbita-6,23(*E*)-diene, and 3β,7β,25-trihydroxycucurbita-5,23(*E*)-dien-19-al.[29,30] Chuang *et al.*[31] have further found that a compound of *Momordica charantia*, 9c, 11*t*, 13*t*-conjugated linolenic acid, was the active compound in wild bitter gourd and acted as a PPARα agonist.

In the first 6 weeks of our study, the rats were provided with food and water *ad libitum*. Under these conditions, we found that the HC diet-fed rats had higher AST and ALT levels than the basal diet-fed rats. On the other hand, a limited diet uptake was able to reverse the rise in AST and ALT levels during the last 6 weeks of feeding (Tables 1, 2). However, liver histological staining data revealed that HC also induced liver damage, even with the limited diet intake. Interestingly, both WFT and SST significantly protected against the liver damage induced by the HC diet (Fig. 6). These results suggest that both WFT and SST might protect directly against liver damage or enable a quick recovery from liver damage, possibly indirectly. Moreover, the HC diet-fed rats had higher serum cholesterol and triglyceride levels than the basal diet-fed rats in the first 6 weeks. Diet limitation allowed a quick return to the normal serum cholesterol level (Figs. 4 and 5), but the triglyceride level remained unchanged (Fig. 3). However, WFT significantly improved the triglyceride level in both the first and last 6-week period, suggesting that WFT might really have hypotriglyceridemic activity *in vivo*.

We have demonstrated in this study that *T. camphoratus* extracts could activate PPARγ in a transient transfection experiment, decrease serum glucose and triglyceride, and protect against liver damage in high-cholesterol diet-fed rats. Although the active compounds wait to be further identified, several terpenoids, such as geraniol, farnesol, and geranylgeraniol, have been confirmed to activate PPARγ and its target gene expression.[32] Since several sesquiterpenes, steroids, triterpenoids, and diterpenoids have been found in abundance in the fruiting body of *T. camphoratus*,[33] it is very likely that the extracts of *T. camphoratus* activated PPARγ mediated by its terpenoid constituents or other steroid constituents. However, we also found that the solid-state culture of *T. camphoratus* had less effect on reducing the serum glucose and triglyceride levels than the wild fruiting bodies of *T. camphoratus*. This have might have resulted from the differing types and quantities of terpenoid constituents present in the two sources of *T. camphoratus*.

In conclusion, the aqueous extract of *T. camphoratus* was able to activate PPARγ according to a transient transfection assay of cells and decrease both the serum glucose and triglyceride levels in HC rats. These results suggest that WFT might have potential as a hypoglycemic and hypotriglyceridemic agent and be useful for the treatment of diabetic patients. Future work will be to identify which components are the PPARγ ligands in *T. camphoratus*.

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