

Amelioration effects of traditional Chinese medicine on alcohol-induced fatty liver

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Abstract

AIM: To examine the effects of traditional Chinese medicine (TCM) on alcohol-induced fatty liver in rats. TCM consists of *Astragalus membranaceus*, *Morus alba*, *Crataegus pinnatifida*, *Alisma orientale*, *Salvia miltiorrhiza*, and *Pueraria lobata*.

METHODS: The rats were separated randomly into five groups. One (the CD group) was fed a control diet for 10 wk, another (the ED group) fed an ethanol-containing isocaloric liquid diet for 10 wk, and the last three (the TCM group) were fed an ethanol-containing isocaloric liquid diet for 10 wk and dosed orally with TCM (222 mg/kg·d, TCM222; 667 mg/kg·d, TCM667; and 2 000 mg/kg·d, TCM2000, respectively) weekly during the last 4 wk.

RESULTS: ED group developed fatty liver according to lipid profile and liver histological findings. Compared with the control group, liver/body weight, serum triglyceride (TG) and total cholesterol (TC), liver TG and TC, serum alanine aminotransferase (ALT) and aspartic aminotransferase (AST) significantly increased in the ED group. Whereas, in the rats administered with TCM, liver/body weight, serum TG and TC, liver TG and TC, serum ALT and AST were significantly decreased, and the degree of hepatic lipid droplets was markedly improved compared with those in the ED group.

CONCLUSION: TCM treatment causes significant reduction in alcohol-induced lipid hepatic accumulation, reversing fatty liver and liver damage, and can be used as a remedy for alcoholic fatty liver.

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Key words: Fatty liver; Alcohol; *Astragalus membranaceus*; *Morus alba*; *Crataegus pinnatifida*; *Alisma orientale*; *Salvia miltiorrhiza*; *Pueraria lobata*

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INTRODUCTION

Fatty liver is a common histological finding in human liver biopsies, it has been defined as both, more than 5% of cells containing fat droplets or total lipid exceeding 5% of liver weight^[1,2]. In the majority of cases the accumulating lipid is predominantly TG. Fatty liver can occur in association in the majority of cases due to alcohol excess, and patients with it are susceptible to liver fibrosis and cirrhosis. Fatty liver is also associated with liver cancer^[3-6]. Recovery from fatty liver seems to decrease susceptibility to liver fibrosis and cirrhosis, the more severe liver diseases in terms of liver function and treatment. However, there is as yet no clearly established therapy for reversing fatty liver. We screened a number of liver protective and anti-hepatic steatosis agents from natural products used for the treatment of liver diseases, and selected *Astragalus membranaceus*^[7-9], *Morus alba* (Oh *et al.*, 2002), *Crataegus pinnatifida*^[10,11], *Alisma orientale*^[12,13], *Salvia miltiorrhiza*^[14,15] and *Pueraria lobata*^[16-18]. All of these have been reported to effect liver protection; however, the question of whether a mixture of these continues to be effective is unknown. Thus, the current study investigates the effects of traditional Chinese medicine (TCM) on induced (by administration of alcohol) fatty liver in male Sprague-Dawley rats. Although the active ingredients of TCM is not completely known, any of their synergistic mixtures could play a role in resolving fatty liver, and the potential clinical therapeutic value for subjects with alcoholic fatty liver warrants further attention.

MATERIALS AND METHODS

Preparation of traditional Chinese medicine

TCM is a mixture of traditional medicine, consisting of *Astragalus membranaceus*, *Morus alba*, *Crataegus pinnatifida*, *Alisma orientale*, *Salvia miltiorrhiza*, and *Pueraria lobata* (2:2:2:2:1:1). The materials were obtained from Pharmaceutical Company in Korea, and cut into small pieces. After standing at room temperature for 30 min, the materials were extracted in 10 volumes of distilled water at 95-99 °C, 0.5-0.6 kgf/cm², 3 h. The fluids were then filtered, and thickened under reduced pressure at 70-80 °C. The extracts were spray dried to a dry powder, and maintained in humidity below 5%.

Animals and diets

Male Sprague-Dawley rats (Daehan Biolink, Chungbuk, Korea), weighing about 100 g, were purchased. All animals received humane care in compliance with the institution's guideline criteria for humane care, as outlined in the National Institute of Health Guidelines for the Care and

Use of Laboratory Animals^[19]. The rats were acclimatized for at least 7 d after delivery and were given free access to standard rodent pellet food (Samtaco, Gyeonggi-do, Korea) and water during the adaptation period. The cages were placed in a room with controlled temperature (22 ± 2 °C), relative humidity ($60 \pm 5\%$), and 12 h light/dark cycle (08:00 on–20:00 off). The body weight and general condition of the animals were monitored twice weekly and liquid diet intake was determined daily. The rats were randomly assigned to three groups: one (the CD group) fed a control diet for 10 wk and administered orally daily with saline on each of the last 4 wk, another (the ED group) fed an ethanol-containing isocaloric liquid diet for 10 wk and dosed orally with saline in each of the last 4 wk, and the last three (the TCM groups) were fed an ethanol-containing isocaloric liquid diet for 10 wk and dosed orally with TCM [0.222 g/(kg · d), TCM222; 0.667 g/(kg · d), TCM667; and 2.000 g/(kg · d), TCM2000, respectively] on each of the last 4 wk. Each group included 10 rats. The liquid diet (Table 1) was prepared according to the method described by Lieber-DeCarli^[20] and provided 1 kcal/mL, containing 28% carbohydrate, 20% protein, 15% fat plus 37% ethanol (ED group) or isocaloric maltose dextrin as the control. Animals were started on the diet at a body weight of 125–140 g and ethanol was introduced progressively with 3% for the first 2 d (21% of total energy), 4% for the subsequent 2 d (28% of energy) followed by the final formula containing 5% (36% of energy) thereafter. Ethanol was incorporated into the diet just before the supply of the liquid diet to the rats. The diet was kept refrigerated in the dark and used within 1 wk of preparation.

Table 1 Composition of experimental diets (g/L)

Ingredients	Control	Ethanol
Casein	41.40	41.40
DL-Methionine	0.30	0.30
L-Cystine	0.50	0.50
Cellulose	10.00	10.00
Maltose dextrin	115.20	25.60
Corn oil	8.50	8.50
Olive oil	28.40	28.40
Safflower oil	2.70	2.70
Mineral mix ¹	8.75	8.75
Vitamin mix ²	2.50	2.50
Choline bitartrate	0.53	0.53
Xanthan gum	3.00	3.00
Ethanol	–	45.5

¹AIN-76 mineral mix provided the following g/kg mix: calcium phosphate, dibasic, 500; sodium chloride, 74; potassium citrate · H₂O, 220; potassium sulfate, 52; magnesium oxide, 24; manganous carbonate, 3.5; ferric citrate, 6; zinc carbonate, 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01; chromium K sulfate.12H₂O, 0.55; sucrose, finely powdered, 118.03; Dyets, Bethlehem, PA, USA. ²AIN-76A vitamin mix provided the following g/kg mix: thiamine HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; niacin, 3; calcium pantothenate, 1.6; folic acid, 0.2; biotin, 0.02; vitamin B12 (0.1%), 1; vitamin A palmitate (500 000 IU/g), 0.8; vitamin D3 (400 000 IU/g), 0.25; vitamin E acetate (500 IU/g), 10; menadione sodium bisulfite, 0.08; sucrose, finely powdered, 981.15; Dyets, Bethlehem, PA, USA.

Sampling procedures

On 0, 6, 8, and 10 wk of treatment, animals were killed

and blood and tissue removed for analysis. The rats were anesthetized with ether and killed after 12 h of fasting. Blood samples were collected from hepatic portal vein into vacutainer (Becton Dickinson & Co., Rutherford, NJ, USA) for the separation of serum and centrifuged (3 000 r/min for 15 min at 4 °C). The serum was frozen at -70 °C for biochemical analysis. The liver was removed and then weighed after being cleaned with ice-cold saline, and stored at -70 °C for the lipid analysis.

Biochemical analysis

Biochemical analysis was carried out using commercial kits. The analysis of serum TG, TC, and high-density lipoprotein cholesterol (HDL-C) concentration were done by automatic analyzer techniques (Asan Pharm. Co., Ltd, Korea). The concentrations were measured spectrophotometrically at a wavelength of 550, 500, and 500 nm respectively (Jasco V-530 UV/VIS, Japan). The low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were estimated using the Friedewald formula as follows: LDL-C = TC-(VLDL-C+HDL-C) and VLDL-C = TG/5^[21]. The activities of the serum aspartic aminotransferase (AST) and alanine aminotransferase (ALT) were measured using kits (Asan Pharm. Co., Ltd, Korea). To analyze hepatic lipid content, the lipids were extracted by homogenizing the liver with 2:1 chloroform-methanol (v/v) by the method devised by Folch *et al.*^[22]. Then, TG and TC were measured using commercial kits (Asan Pharm. Co., Ltd, Korea).

Liver histology

A portion of the liver was fixed in 40 g/L formaldehyde and embedded in paraffin wax. Frozen liver sections from fixed tissues were cut into 10-μm pieces and mounted on the slide. The samples were stained for lipid with Oil Red O in propylene glycol with Mayer's hematoxylin as the counter stain, and it calculated the area by densitometer.

Statistical analysis

Statistical evaluation of data was performed by Duncan's multiple range tests to make comparisons among the groups. Values were given as the mean ± SD. Differences of $P < 0.05$ were considered statistically significant.

RESULTS

Effects on body weight and liver weight

Table 2 shows the body weight and liver weight (g/100 g body weight) in all rats during the 10 wk. At 10th wk, the body weight gain was significantly lower in the rats fed alcohol than in the rats fed without alcohol. However, a trend toward increased weights was seen in rats administrated TCM than those in the ED group. The liver weights tended to be higher in alcohol-fed rats than in the CD group. However, after the administration of TCM for 4 wk, the TCM groups showed significantly decreased liver weights above all, and TCM667 group was the lowest of all the TCM groups. It is considered that there is an inhibition of hepatic lipids accumulation in rats treated with TCM.

Table 2 Effect of TCM on body weight and liver weight of rats fed experimental diets for 10 wk and treated with TCM for the last 4 wk

Wk	Group	Body weight (g)	Liver/body weight (g/BW 100 g)
0	-	134.8±5.6	4.03±0.33
6	CD	306.6±14.2	2.69±0.22
	ED	265.4±13.5 ^b	3.14±0.11 ^b
8	CD	309.4±16.3 ^{a,c}	2.74±0.22 ^e
	ED	293.2±17.2 ^c	3.20±0.26 ^a
	TCM2000	315.0±15.2 ^a	2.97±0.14 ^c
	TCM667	299.2±20.9 ^{a,c}	2.90±0.25 ^{c,e}
	TCM222	294.0±15.1 ^c	2.93±0.18 ^{c,e}
10	CD	350.8±19.7 ^a	2.55±0.13 ^e
	ED	308.0±12.7 ^{c,e}	2.99±0.31 ^a
	TCM2000	317.8±15.6 ^c	2.80±0.08 ^{a,c}
	TCM667	319.0±17.8 ^c	2.74±0.21 ^{c,e}
	TCM222	299.4±20.1 ^e	2.84±0.17 ^{a,c}

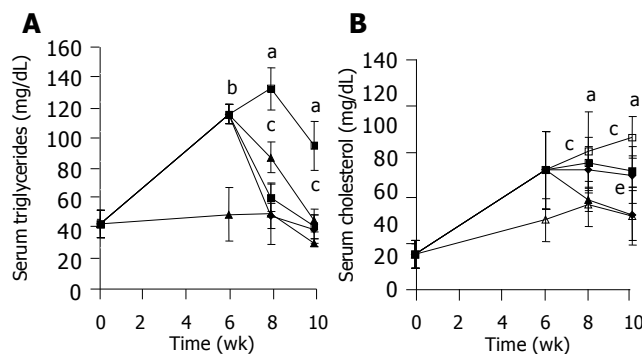
^aP<0.05, ^cP<0.05, ^eP<0.05, ^{vs} others, ^bP<0.001 ^{vs} control.

Effects on serum lipid profiles

Serum concentrations of TG and TC in rats administrated with alcohol increased to 3.8- and 1.5-fold higher levels respectively than those seen in rats given the control diet (Figure 1). Consumption of alcohol for 6 wk caused significant increases in TG and TC. At 10th wk, TG and TC were significantly reduced by consumption of TCM. TG concentration was reduced by 70%, and the TC concentration was significantly decreased to the level of control rats. Especially, a “middle dose” of TCM667 is more effective in decreasing serum TG than a high dose of TCM2000. In the current study, LDL-C and VLDL-C concentration in rats given alcohol significantly increased 5.8- and 3.8-fold higher levels than those seen in control diet-treated rats (Table 3). However, a trend toward decreased LDL and VLDL was seen after TCM administration; especially, TCM667 is more effective in this, than a high dose of TCM2000. On the other hand, HDL-C concentration in rats given alcohol declined significantly. However, the administration of TCM partially returned to normal.

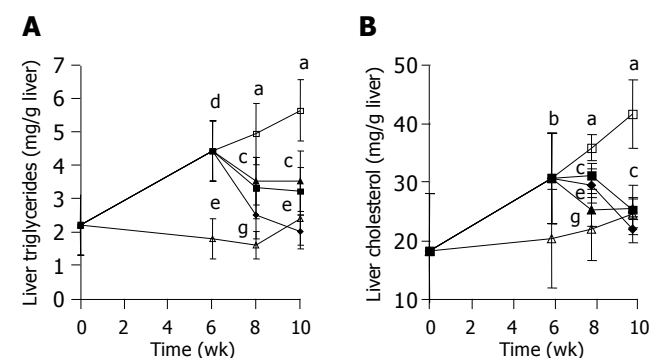
Table 3 Effect of TCM on serum LDL-C, VLDL-C and HDL-C concentration of rats fed experimental diets for 10 wk and treated with TCM for the last 4 wk

Wk	Group	LDL-C	VLDL-C mg/dL	HDL-C
0		16.66±9.58	7.41±1.87	31.20±9.73
6	CD	26.46±13.45	8.62±3.67	35.21±6.98
	ED	41.39±17.36 ^b	22.33±1.33 ^d	28.65±7.68 ^b
8	CD	20.21±9.15 ^c	8.72±4.21 ^e	48.36±4.47 ^a
	ED	49.53±17.02 ^a	25.90±2.88 ^a	24.94±7.93 ^e
	TCM2000	30.15±4.21 ^c	16.43±2.23 ^c	31.70±3.74 ^c
	TCM667	44.78±10.26 ^a	8.47±1.71 ^e	32.94±6.71 ^c
	TCM222	45.55±9.66 ^a	10.94±1.88 ^e	30.67±5.65 ^c
10	CD	12.02±8.78 ^b	4.75±0.60 ^e	55.36±10.48 ^a
	ED	69.94±11.90 ^a	18.05±3.30 ^a	20.42±6.21 ^e
	TCM2000	26.23±9.99 ^e	7.78±1.54 ^c	38.27±9.33 ^c
	TCM667	35.59±8.91 ^c	6.59±1.93 ^c	37.71±6.74 ^c
	TCM222	39.14±8.47 ^c	6.95±1.57 ^c	35.67±3.04 ^c

^aP<0.05, ^cP<0.05, ^eP<0.05, ^{vs} others, ^bP<0.001, ^dP<0.001 ^{vs} control.**Figure 1** Effect of TCM on concentration of serum TGs (A) and TC (B) of rats fed experimental diet for 10 wk and treated with TCM for the last 4 wk. \triangle , CD; \square , ED; \blacktriangle , TCM2000; \bullet , TCM667; \blacksquare , TCM222. ^aP<0.05, ^cP<0.05, ^eP<0.05 ^{vs} others, ^bP<0.0001 ^{vs} control.

Effects on hepatic triglycerides and total cholesterol

In alcohol-fed rats, hepatic lipid content raised significantly compared to rats fed with an isocaloric diet without alcohol (Figure 2). Hepatic triglycerides (TGs) and total cholesterol (TC) concentration showed 2.3- and 2.7-fold increase, respectively, in rats fed an alcohol diet compared to those fed a control diet. Therefore, ingestion of alcohol containing diets for 6 wk causes the onset of fatty liver. At 10th wk, TG and TC were significantly reduced by consumption of TCM. Both the TG and TC concentration was significantly decreased to the level of control rats. Especially, TCM667 is more effective in decreasing serum TG than a high dose of TCM2000.

**Figure 2** Effect of TCM on TGs and TC concentrations in liver of rats fed experimental diet for 10 wk and treated with TCM for the last 4 wk. \triangle , CD; \square , ED; \blacktriangle , TCM2000; \bullet , TCM667; \blacksquare , TCM222. ^aP<0.05, ^cP<0.05, ^eP<0.05, ^gP<0.05 ^{vs} others, ^bP<0.001, ^dP<0.0001 ^{vs} control.

Effects on hepatoprotective activities

The administration of alcohol to the rats significantly raised the activities of AST and ALT (Table 4). Supplementing with TCM reduced the activity of serum AST and ALT in rats with fatty liver. Moreover, TCM exhibited DPPH free radical scavenging effect (data not shown).

Hepatopathological manifestations

Histological examination of liver tissue (Figure 3) showed lipid accumulation greater in rats, which fed the ethanol-containing diets for 10 wk (Figure 3B), compared to control

Table 4 Effect of TCM on serum AST and ALT activities of rats fed experimental diets for 10 wk and treated with TCM for the last 4 wk

Wk	Group	AST (U/mL)	ALT (U/mL)
0		71.79±9.39	32.67±8.52
6	CD	89.72±6.17	28.00±6.63
	ED	133.30±12.78 ^b	68.96±9.22 ^b
8	CD	94.85±8.12 ^{c,e}	38.45±8.97 ^c
	ED	119.52±14.73 ^a	62.60±9.48 ^a
	TCM2000	86.20±9.89 ^e	33.92±9.26 ^{c,e}
	TCM667	99.21±6.41 ^c	29.84±9.44 ^e
	TCM222	103.61±6.92 ^c	59.37±7.07 ^a
10	CD	96.97±9.97 ^{ac}	23.50±3.02 ^e
	ED	105.24±12.06 ^a	56.74±9.76 ^a
	TCM2000	86.13±9.71 ^e	36.23±9.92 ^c
	TCM667	86.54±9.66 ^e	27.73±9.87 ^e
	TCM222	88.34±6.72 ^{c,e}	40.80±9.44 ^c

^a*P*<0.05, ^c*P*<0.05, ^e*P*<0.05, *vs* others, ^b*P*<0.001 *vs* control.

diet-fed rats (Figure 3A). In contrast, in rats administered with TCM for 4 wk, accumulation of lipid droplets was remarkably decreased (Figures 3C-E). Quantification of liver lipids was concordant with the histological findings (Figure 3F). Ethanol feeding increased the liver lipids content by threefold, these results are consistent with the rate of elevation of plasma and hepatic TG in the ethanol-fed rats. In striking contrast, when TCM was administered for the last 4 wk of the ethanol feeding, the lipids content was significantly decreased to the level of control rats.

DISCUSSION

Fatty liver is the most common pathological change induced

by alcohol and is also one of the earliest pathological revelations of alcoholic liver disease. Autopsy studies in previously healthy adults, killed accidentally, have revealed an incidence of 1/3^[1]. Potential pathophysiological mechanisms include: (1) increased uptake of fatty acids, (2) inhibition of fatty acid oxidation, (3) increased endogenous synthesis of fatty acids, and (4) decreased capacity of the liver to export TG in very-low density lipoprotein particles^[23]. Fatty liver can lead to liver fibrosis and cirrhosis; therefore, it is important to treat the condition before it progresses into a more serious form. However, there is no established therapy for the recovery of fatty liver. In this regard, the present study aimed to evaluate the effects of TCM, which depletes levels of serum and hepatic lipid profiles on alcohol-induced fatty liver in rats.

The liquid diet containing alcohol resulted in the accumulation of liver lipids and thus provided a model for alcohol-induced lesions^[20]. Despite strict isocaloric pair feeding, alcohol-fed rats did not gain as much weight as those in their pair fed control groups even though they received diets with the same energy content. The slower weight gain of the animals in this study compared to those reported by some authors may be due to the reduction of intake and absorption of nutrients^[24,25]. The supplement of alcohol for 6 wk to the rats caused a significant increase in liver weight, and this accounted for by TG accumulation. However, body weight and liver weight of TCM groups were close to those of the control groups. Moreover, both serum and liver, lipid concentrations were significantly (*P*<0.0001) increased by alcohol feeding. In the present study, however, TCM administration decreased serum and hepatic lipids concentrations to levels lower than those seen before

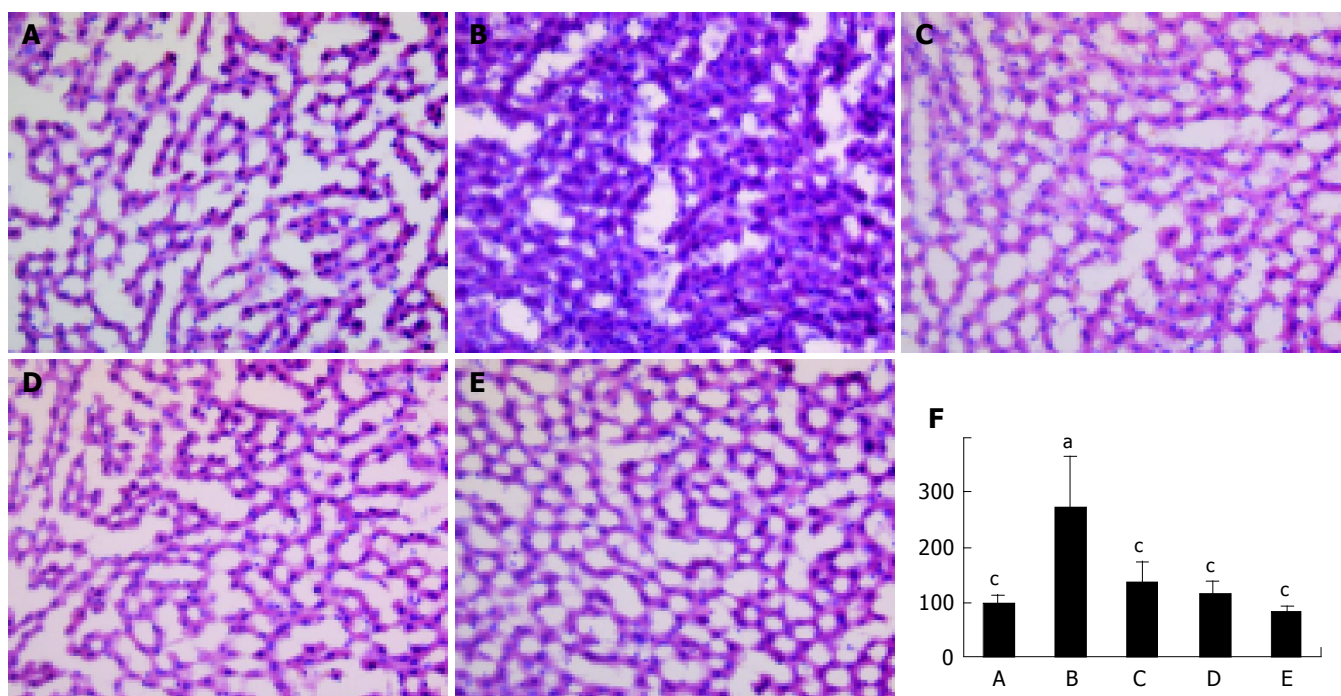


Figure 3 Liver histology in rats treated with alcohol or alcohol plus TCM. Frozen sections of liver (×40) taken from (A) CD+saline, (B) ED+saline, (C) ED+TCM2000, (D) ED+TCM667, (E) ED+TCM222-treated rats on 10 wk, (F) densitometer analysis: the representative experiment A–E were quantified and the integrated area were percentized in control rats (100%). In A–E, frozen sections were stained with Oil Red O and counterstained with Mayer's hematoxylin. Lipid droplets were stained red. ^a*P*<0.05, ^c*P*<0.05 *vs* others.

TCM administration or returned to normal range, even when rats consumed ethanol diets during the period of TCM administration.

Accordingly, we suggest that the treatment of rats with TCM for 4 wk reversed fatty liver to normal values, as appraised biochemically and histologically. In addition, VLDL was made up of TG-rich lipoproteins and transferred TG from the liver to peripheral tissues. Previous studies indicate that the impaired ability of hepatocytes to secrete lipid components as VLDL is likely to be one major mechanism linked to alcoholic fatty liver^[4,26-28]. Accordingly, one important mechanism involved in TCM-induced recovery from an alcoholic fatty liver is decrease in serum LDL and VLDL concentrations. In the current study, the ED group showed increased VLDL and LDL, but the administration of TCM returned to normal. These findings suggest that TCM might enhance the secretion of VLDL from the liver and contribute to the improvement of alcoholic fatty liver. In addition, it seems remarkable that TCM administration increased serum HDL levels. HDL are cholesterol-rich lipoproteins and transferred cholesterol from peripheral tissues to the liver. Therefore, TCM might affect not only TG but also total lipid metabolism, including cholesterol metabolism. Additionally, the current study evaluated whether TCM could protect against liver damage, consequently, TCM takes effects to decrease AST and ALT.

In conclusion, administration of TCM diminishes the accumulation of lipids in the liver. These results suggested that intake of TCM (*Astragalus membranaceus*, *Morus alba*, *Crataegus pinnatifida*, *Alisma orientale*, *Salvia miltiorrhiza*, and *Pueraria lobata*) may be useful in preventing and improving fatty liver induced by alcohol. It is not certain by what mechanism this takes place from the current observation; therefore, follow-up investigations identifying the mechanism related to the reduction of lipids in liver are recommended.

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