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Research article

Ya That Somdun improves hepatic steatosis in hyperlipidemic rats

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ABSTRACT

Ya That Somdun (YTS) is a traditional Thai medicine composed of six herbs used as a strengthening tonic. Some of the herbs constituting YTS have antihyperlipidemic and anti-obesity activities. The objective of this study was to elucidate the antihyperlipidemic properties of YTS extract in rats with cholesterol suspension-induced hyperlipidemia. Male Sprague-Dawley rats were subdivided into four groups: normal control (NC), hyperlipidemic control (HC), and those who were administered 100 (YTS100) and 200 mg (YTS200) of YTS/kg body weight (BW). Hyperlipidemic rats were orally administered YTS extract for four consecutive weeks from the fifth week of cholesterol suspension. Serum lipid profiles, body weights, liver and renal functional markers, gene expression involved in lipid metabolism, and liver histopathology were examined. The HC and YTS groups showed a significant increase in body weights compared with the NC group. The YTS100 and YTS200 groups showed no significant difference in serum triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels compared with the NC and HC groups. YTS treatment (100 mg/kg BW) downregulated sterol regulatory element binding protein 1c (*SREBP-1c*) mRNA expression and alleviated hepatic steatosis. In conclusion, the YTS extract reduced hepatic lipid accumulation in hyperlipidemic rats by reducing SREBP-1c expression levels.

1. Introduction

Cardiovascular diseases (CVDs) are the main causes of mortality worldwide, accounting for 17.9 million deaths each year [1]. Several factors increase the risk of CVDs, including diabetes, hypertension, dyslipidemia, and obesity [2]. Dyslipidemia is a significant modifiable risk factor for CVDs and is defined by high levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, or low levels of high-density lipoprotein cholesterol (HDL-C) [3]. Thus, maintaining blood lipid levels within the normal range is an effective strategy for preventing and treating CVD progression. The current treatment for dyslipidemia involves lipid-lowering medications like statins and fibrates [4]. Although statins and fibrates effectively lower lipid levels, long-term treatment has several side effects [4]. Statins elevate the levels of liver enzymes called transaminases, which increase the risk of developing diabetes mellitus and muscle-related diseases [5]. Fibrates are also associated with myopathy, cholelithiasis, and venous thrombosis [6]. The combination of fibrates and statins has been reported to cause myopathy [7]. Therefore, research is being conducted on developing novel hypolipidemic medicines with high efficacy and low side effects for treating dyslipidemia.

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Traditional Thai herbal medicine has a long history of use as an alternative treatment and offers therapeutic options for dyslipidemia [8,9]. Ya That Somdun (YTS) is a traditional Thai medicine containing the aerial parts of *Acanthus ilicifolius* L., fruit of *Aegle marmelos* (L.) Corrêa, rhizome and root of *Boesenbergia rotunda* (L.) Mansf., corm of *Cyperus rotundus* L., fruit of *Piper nigrum* L., and stem of *Tinospora crispa* (L.) Hook. F. & Thomson, in equal proportions [10]. YTS has traditionally been used as a strengthening tonic [10]. The six herbal components of YTS are similar to the constituents of the adaptogen and anti-obesity medicine described in the book on traditional Thai medicine named Petch Narm Ake and a traditional Thai antihypertensive herbal (TTAH) recipe [11]. The active compounds in the recipe are piperine, adenosine, pinocembrin, and pinostrobin [11]. Three herbs present in YTS have hypolipidemic and anti-obesity activities. Panduratin A, a compound isolated from *B. rotunda* (L.) Mansf., decreased weight gain and fat mass, healed fatty liver, and improved serum lipid profiles in obese mice [12]. *C. rotundus* extract reduced weight gain and serum lipid levels in preclinical and clinical studies [13]. *P. nigrum* L. extract decreased body and fat weights and improved serum lipid profiles in high-fat rodent models [14,15]. Piperine, an alkaloid constituent of *P. nigrum*, inhibited the mRNA expression of SREBP-1c, a transcription factor that regulates genes involved in the synthesis of fatty acids and triglycerides, such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), [16,17]. While the individual components of the YTS recipe have been studied separately, data on the efficacy of the Ya That Somdun recipe in hyperlipidemic rats remain lacking. Therefore, this study aimed to evaluate the anti-hyperlipidemic properties of YTS in rats with experimentally induced hyperlipidemia.

2. Materials and methods

2.1. Chemicals and reagents

This study used the following reagents: ethanol absolute anhydrous (Carlo Erba Reagents, Germany); acetic acid (Merck, Germany); acetonitrile (RCI Labscan, Thailand); methanol (RCI Labscan); sodium carbonate (Na_2CO_3; Ajax Finechem, Australia); Folin–Ciocalteu's reagent (Loba Chemie, India); gallic acid (Glentham Life Sciences, UK); aluminium chloride ($AlCl_3$; Loba Chemie); rutin hydrate (Glentham Life Sciences); coconut oil (Pipek, Thailand); cholesterol (Glentham Life Sciences); GENEzol® reagent (Geneaid Biotech Ltd., Taiwan); iScript cDNA synthesis kit (Bio-Rad, CA, USA); EvaGreen® qPCR Mix Plus (ROX; Solis BioDyne, Estonia).

2.2. Plant material and extraction

The ingredients of YTS (Table 1) were bought from Pharmacy Ltd. (Bangkok, Thailand). Each plant was cleaned and dried in an oven (UEF700; Memmert, Germany) at 45 °C for three days. The dried plants were ground to a coarse powder using an electric grinder (SM 200; Retsch, Germany). Nine hundred grams of YTS were prepared by mixing equal amounts of each of the six plants and macerating three times each for three days using 9 L of 95 % ethanol. After filtration, the extract was evaporated using a rotary evaporator (R-210; Buchi, Switzerland). The YTS extract was weighed and stored at −20 °C until use.

2.3. Determination of total phenolic content

The total phenolic content was identified using the Folin-Ciocalteu method [18]. In brief, 200 μL of YTS extract (2 mg/mL) were mixed with 2 mL of 2 % Na_2CO_3 solution and 100 μL of 50 % Folin–Ciocalteu's reagent. After 30 min, the absorbance was measured at 750 nm using a spectrophotometer (UV-1800; Shimadzu Scientific Instruments, Japan). A standard curve for total phenolic content was constructed using a gallic acid standard. The total phenolic content is expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g of extract).

2.4. Determination of total flavonoid content

The total flavonoid content was determined using the aluminum chloride colorimetric method [19]. One milligram of YTS extract (0.4 mg/mL) was added to 1 mL of 2 % $AlCl_3$–methanol solution. After 15 min, absorbance was measured at 430 nm using a spectrophotometer (UV-1800). Rutin was used as the standard for the calibration curve. The total flavonoid content is expressed as milligrams of rutin equivalent per gram of extract (mg rutin/g of extract).

Table 1
Ingredients of Ya That Somdun recipe.

Number	Scientific name	Part used
1	*Acanthus ilicifolius* L.	Aerial part
2	*Aegle marmelos* (L.) Corrêa	Fruit
3	*Boesenbergia rotunda* (L.) Mansf.	Rhizome and root
4	*Cyperus rotundus* L.	Corm
5	*Piper nigrum* L.	Fruit
6	*Tinospora crispa* (L.) Hook. f. & Thomson	Stem

2.5. High-performance liquid chromatography-ultraviolet (HPLC-UV) analysis of the extract

HPLC analysis of the YTS extract was conducted using a Thermo Scientific Dionex UltiMate 3000. For analysis, the YTS extract was subjected to filtration through a 0.22-μm membrane filter. The analyte was resolved using a reversed-phase analytical column (VertiSep™ USP C18 HPLC column; 4.6 × 250 mm). The mobile phase consisted of acetonitrile as solvent A and 1 % (v/v) acetic acid as solvent B at a flow rate of 1 mL/min. The gradient flow was incrementally adjusted from 10 % to 75 % of solvent A in solvent B over 38 min. For the detection of piperine in the samples, absorbance was measured at 341 nm. The retention times and spectra in the chromatogram obtained from the analysis of the YTS extract were compared with those of piperine to identify the piperine peaks in the chromatogram. The sensitivity of the analytical method was assessed by establishing the limit of detection (LOD) and limit of quantification (LOQ). The precision was assessed by injecting various concentrations of piperine in triplicates.

2.6. Experimental animals

Twenty-four male Sprague–Dawley rats (150–180 g) were purchased from Nomura Siam International Co., Ltd. (Thailand). The rats were kept in a standard laboratory setting with a 12-h light/dark cycle, a temperature of 23 ± 1 °C, and a relative humidity of 50–70 %. The rats were fed a normal diet and water *ad libitum*. All experiments were reviewed and approved by the Animal Ethics Committee of Walailak University (approval number: WU-ACUC-65023).

The rats were randomly divided into four groups, each containing six rats. Group 1, the normal control (NC) group, was orally administered a solution containing 7 % Tween 80 and 3 % ethanol daily for eight weeks. Experimental hyperlipidemia was induced by oral gavage of a suspension of 6 % cholesterol and 0.5 % cholic acid in coconut oil (5 mL/kg body weight [BW]) for eight weeks. Four weeks after hyperlipidemia induction, Group 2 served as the hyperlipidemic control (HC) group. Groups 3 (YTS100) and 4 (YTS200) were orally administered the YTS extract at doses of 100 and 200 mg/kg BW, respectively, for four weeks. The dosages of YTS extract used in this experiment were based on those of a traditional Thai antihypertensive herbal recipe (TTAH), which contains the same constituents as in a previous study [20]. Oral administration of TTAH at dosages of 40, 200, and 1000 mg/kg resulted in blood parameters for all treated groups remaining within the normal range for rats, with no histological changes observed in the livers and kidneys [20]. Therefore, we used 100 and 200 mg/kg for the YTS extract in this study. The YTS extract was dissolved in a solution containing 7 % Tween 80 and 3 % ethanol. After treatment, the rats were anesthetized by intraperitoneal injection of Zoletil™ (25 mg/kg BW), and the anesthetized condition was confirmed by checking the toe pinch.

After opening the thorax, blood was collected via cardiac puncture, and the rats were immediately euthanized by cutting the hearts. The livers were quickly removed, rinsed with physiological saline solution, and weighed. The left lobe of the liver was preserved with 10 % neutral buffered formalin, and the remaining liver tissue was stored at −80 °C until use.

2.7. Biochemical analysis of serum

The sera were separated from blood samples by centrifugation at 2000×*g* for 10 min and used to determine triglyceride, total cholesterol, LDL-C, HDL-C, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine levels. All parameters were measured using enzymatic methods in a fully automated biochemistry analyzer (ABX Pentra 400; Horiba, France).

2.8. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from hepatic tissues using GENEzol™ reagent following the provided instructions. One milligram of the liver tissue was homogenized in GENEzol™ reagent and incubated for 5 min at room temperature. Two hundred microliters of chloroform were added and shaken for 10 s. After centrifugation, the aqueous phase was transferred into a new tube; for RNA precipitation, isopropanol was added to this solution. Subsequently, the RNA pellet was cleaned with 70 % ethanol, dried, and dissolved in RNase-free water. One microgram of total RNA was converted to cDNA using the iScript cDNA synthesis kit.

The genes of interest were analyzed using quantitative reverse transcription polymerase chain reaction (qRT-PCR) using a QuantStudio 3 Real-Time PCR System (Applied Biosystems, USA). The qRT-PCR reaction volume was 20 μL; the reaction mix contained cDNA, EvaGreen® qPCR Mix Plus, forward and reverse primers, and PCR-grade H_2O. The samples were examined in triplicate. The qRT-PCR was programmed as follows: initial denaturation at 95 °C for 12 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s, and extension at 72 °C for 20 s. The relative mRNA expression level of each gene was normalized to that of β-actin using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are listed in Table 2.

Table 2
Primer sequences used for quantitative real-time polymerase chain reaction (qRT-PCR).

Gene	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
ACACA	TGAGGAGGACCGCATTTATC	GCATGGAATGGCAGTAAGGT
SREBP-1c	CCCTGCGAAGTGCTCACAA	GCGTTTCTACCACTTCAGGTTTCA
β-Actin	GACCTCTATGCCAACACAGT	GGTGTAAAACGCAGCTCAGTA

ACACA, acetyl-CoA carboxylase alpha; SREBP-1c, sterol regulatory element binding protein 1c.

2.9. Histological analysis

The liver tissues were fixed in 10 % neutral buffered formalin at room temperature, embedded in paraffin, and cut into 5-μm-thick sections. The sections were stained with hematoxylin and eosin (H&E) for histological examination. The histological changes were analyzed in a blinded manner using a light microscope. The histopathological changes were assessed based on steatosis, inflammation, and degenerative and necrotic changes [21].

2.10. Statistical analysis

The software GNU PSPP (version 1.4.1-g79ad47) was used for data analysis, and the data are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to assess differences in outcomes among experimental groups, followed by Tukey's post hoc test. The Kruskal–Wallis test was conducted if the data did not follow a normal distribution. Statistical significance was set at $P < 0.05$.

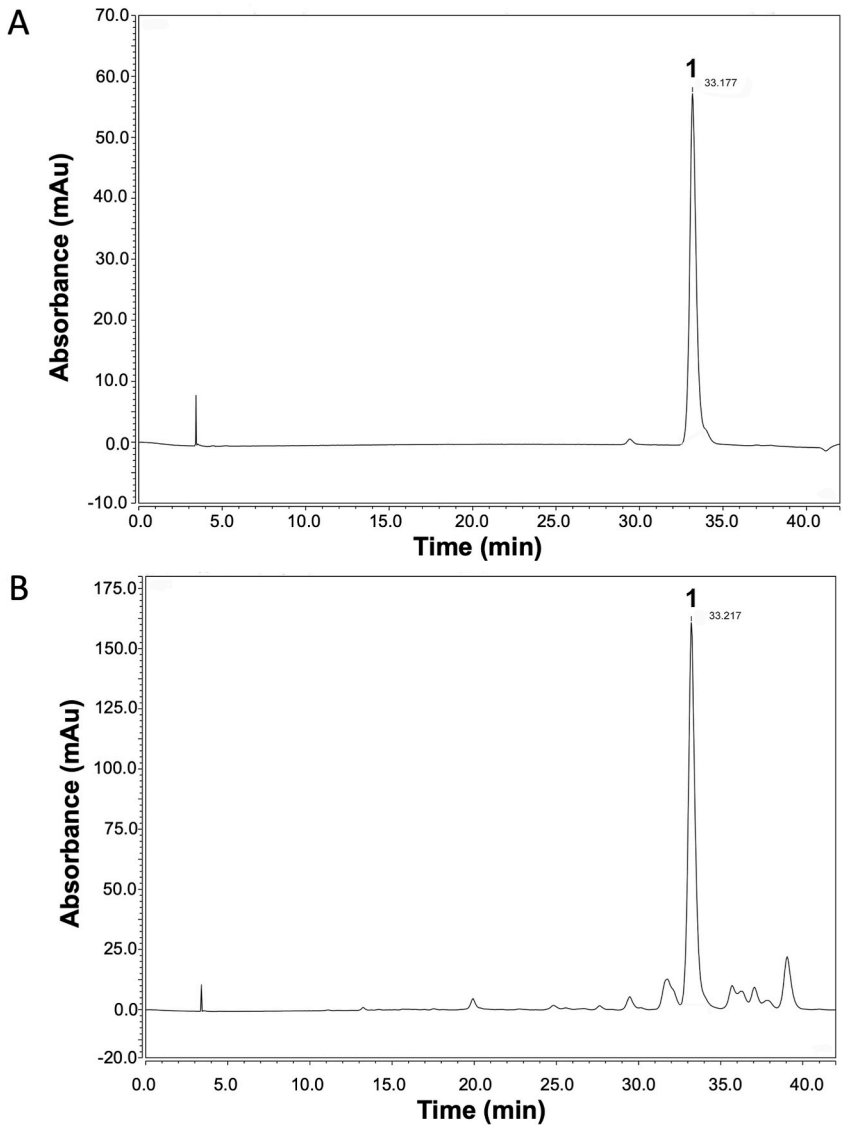

Fig. 1. HPLC chromatograms of piperine (**1**) standard solution (25 μg/mL, A) and the Ya That Somdun extract (1 mg/mL, B) detected at 341 nm.

3. Results

3.1. Total phenolic and flavonoid contents of Ya That Somdun extract

The calibration curves for gallic acid and rutin showed linearity in the range of 10–100 μg/mL; the correlation coefficients (R^2) of the graphical curves for gallic acid and rutin were 0.9997 and 0.9943, respectively. The total phenolic and flavonoid contents in the extract were 57.12 ± 0.97 mg GAE/g of extract and 37.5 ± 0.97 mg rutin/g of extract, respectively.

3.2. Quantification of piperine in Ya That Somdun extract

The HPLC-UV conditions for piperine were established using a gradient mobile phase system, resulting in a retention time of approximately 33 min for the compound. The limits of detection (LOD) and quantification (LOQ) for piperine were determined to be 0.661 and 2.004 μg/mL, respectively. A calibration curve for piperine was prepared by comparing the sample concentrations with those of an external standard, whose concentrations ranged from 3.13 to 100 μg/mL; the calibration curve for piperine had an R^2 value of 0.9996. The repeatability of the method was assessed by conducting multiple analyses using a standard piperine solution. The resulting coefficients of variation (%CVs) ranged from 0.23 to 1.92 %, indicating good repeatability of the method.

The extraction yield of YTS was 6.72 % of the dry weight. The HPLC chromatograms for both the piperine standard (Fig. 1A) and YTS extract (Fig. 1B) are presented in Fig. 1. Using the calibration curve, the piperine content of the YTS extract was determined to be 66 ± 0.79 μg/mg.

3.3. Effect of Ya That Somdun extract on body and liver weights in rats

Table 3 shows no significant differences in initial body weights among the experimental groups. At the end of eight weeks, the body weights and liver index (the ratios of liver weights to body weights) of the HC, YTS100, and YTS200 groups were significantly higher than those of the NC group ($P < 0.05$). The body weights and liver index of the YTS100 and YTS200 groups did not show any significant difference compared to the HC group. ($P > 0.05$).

3.4. Effect of Ya That Somdun extract on lipid levels

Serum triglyceride levels were significantly higher in the HC group than those in the NC group ($P < 0.05$) (Fig. 2A). The groups treated with YTS (100 and 200 mg/kg) showed slightly decreased serum triglyceride levels, but these differences were not statistically significant ($P > 0.05$). There were no significant changes in serum total cholesterol, LDL-C, and HDL-C levels among the experimental groups ($P > 0.05$) (Fig. 2B, C, and 2D).

3.5. Effect of Ya That Somdun extract on liver and kidney functions

The liver and kidney function parameters are shown in Table 4. Serum levels of ALT, AST, BUN, and creatinine did not significantly differ among the experimental groups ($P > 0.05$).

3.6. Effect of Ya That Somdun extract on acetyl-CoA carboxylase alpha (ACACA) and sterol regulatory element binding protein 1c (SREBP-1c) mRNA expression

To evaluate the effects of YTS extract on *ACACA* and *SREBP-1c* mRNA expression in the liver, qRT-PCR was performed. Fig. 3 shows no significant differences in *ACACA* mRNA levels among the experimental groups ($P > 0.05$) (Fig. 3A). *SREBP-1c* mRNA expression was significantly downregulated in the YTS100 group compared with that in the HC group ($P < 0.05$); however, *SREBP-1c* mRNA expression was significantly ($P < 0.05$) upregulated in the YTS200 group compared to that in the HC and YTS100 groups (Fig. 3B).

Table 3
Effect of Ya That Somdun extract on body and liver weights.

Group	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Liver index (%)
NC	159.25 ± 3.99	542.72 ± 8.11	383.48 ± 11.03	2.97 ± 0.35
HC	159.99 ± 10.32	577.68 ± 12.35[a]	417.70 ± 13.98	3.67 ± 0.54[a]
YTS100	162.16 ± 6.53	563.26 ± 23.87[a]	401.10 ± 25.51	3.51 ± 0.24[a]
YTS200	158.57 ± 7.77	570.15 ± 38.17[a]	411.58 ± 36.47	3.63 ± 0.31[a]

Data are expressed as mean ± standard deviation (SD; n = 6).

[a] $P < 0.05$ compared with the normal control (NC). HC, hyperlipidemic control; YTS100, group administered 100 mg of Ya That Somdun/kg body weight (BW); YTS200, group administered 200 mg of Ya That Somdun/kg BW.

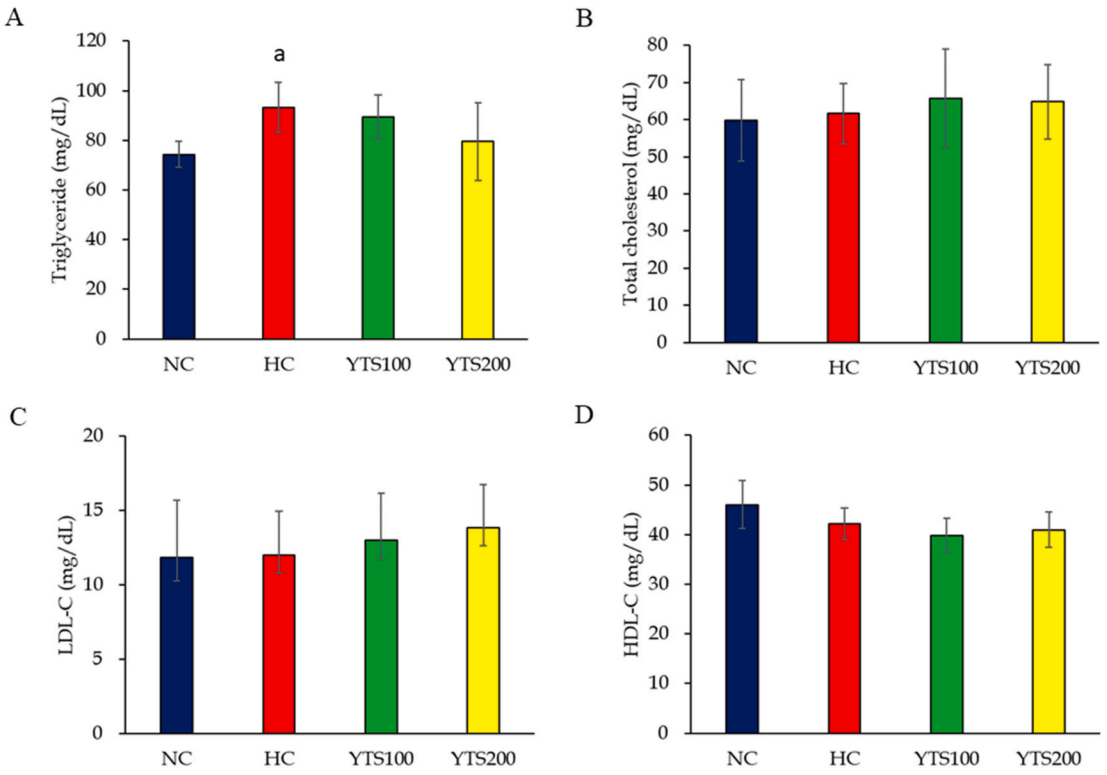

Fig. 2. Effect of Ya That Somdun extract on serum levels of triacylglycerol (A), total cholesterol (B), low-density lipoprotein cholesterol (LDL-C) (C), and high-density lipoprotein cholesterol (HDL-C) (D) in different experimental groups of rats. NC, normal control; HC, hyperlipidemic control; YTS100, group administered 100 mg of Ya That Somdun/kg body weight (BW); YTS200, group administered 200 mg of Ya That Somdun/kg BW. [a] $P < 0.05$ compared with NC. Data are expressed as mean ± standard deviation (SD; n = 6).

Table 4
Effect of Ya That Somdun extract on liver and kidney functions.

Group	Liver function		Kidney function	
	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Creatinine (mg/dL)
NC	21.67 ± 4.89	32.17 ± 10.23	12.83 ± 0.75	0.61 ± 0.04
HC	19.50 ± 6.28	30.83 ± 4.12	11.67 ± 1.03	0.56 ± 0.05
YTS100	19.17 ± 3.54	29.17 ± 4.17	11.83 ± 0.75	0.56 ± 0.05
YTS200	22.50 ± 9.18	34.00 ± 4.90	12.17 ± 1.83	0.57 ± 0.08

Data are expressed as mean ± SD (n = 6). NC, normal control; HC, hyperlipidemic control; YTS100, group administered 100 mg of Ya That Somdun/kg BW; YTS200, group administered 200 mg of Ya That Somdun/kg BW.

3.7. Effect of Ya That Somdun extract on liver histopathology

Histopathological evaluation of the liver samples is shown in Fig. 4. Analysis of liver tissue morphology of the NC group revealed normal hepatocytes with red-pink cytoplasm and normal features in the hepatic sinusoids (Fig. 4A). H&E staining of the liver tissue did not show sinusoidal vasodilation or inflammatory infiltration. In the HC group, most hepatocytes showed variable degrees of cytoplasmic vacuolation, while some contained multiple small or large vacuoles (Fig. 4B). Necrosis was observed in some areas. In the YTS100 group, improvement in histopathological conditions was observed (Fig. 4C) compared with that in the HC and YTS200 groups (Fig. 4D).

4. Discussion

Dietary intake of saturated fats increases serum LDL-C levels and, consequently, the risk of CVDs [22]. High-fat diet is commonly used as a model for hyperlipidemia [23–25]. Common fat sources used in high-fat diets are lard, corn oil, and coconut oil [23–25]. Hyperlipidemia was induced in rats by feeding them cholesterol suspended in coconut oil for eight weeks, which elevated serum triglyceride levels in the HC group. This indicates that feeding of cholesterol and coconut oil-induced dyslipidemia. Studies on animals

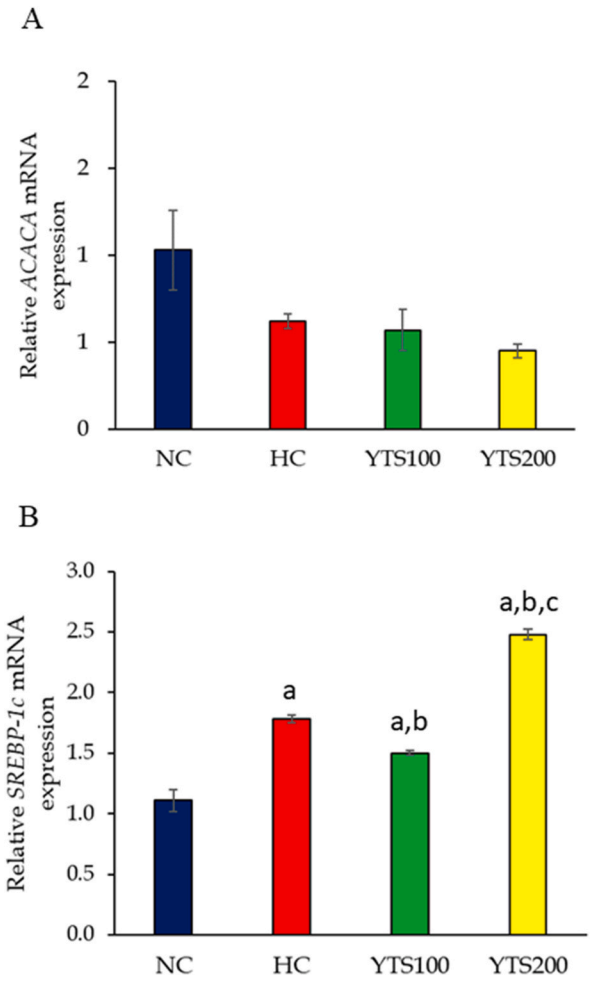

Fig. 3. Relative acetyl-CoA carboxylase alpha (*ACACA*) and sterol regulatory element binding protein 1c (*SREBP-1c*) mRNA levels in different experimental groups of rats. NC, normal control; HC, hyperlipidemic control; YTS100, group administered 100 mg of Ya That Somdun/kg BW; YTS200, group administered 200 mg of Ya That Somdun/kg BW. [a] $P < 0.05$ compared with NC; [b] $P < 0.05$ compared with HC; [c] $P < 0.05$ compared with YTS100. Data are expressed as mean ± SD (n = 6).

have revealed that a high-fat diet consisting of coconut oil and cholesterol increases the levels of total cholesterol and triglycerides [23,25]. However, in this study, we did not observe an increase in the total cholesterol levels in the HC group. A possible explanation for this discrepancy may be the different amounts of cholesterol used in these studies. High levels of dietary cholesterol result in a significant increase in serum LDL-C concentrations [26].

SREBP-1c, a transcription factor, controls the expression of genes involved in the synthesis of fatty acid and triglyceride, including fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and fatty acid synthase [16]. The results of this study showed that *SREBP-1c* mRNA expression was upregulated in the HC group, consistent with the hepatic lipid accumulation observed in this group. This finding is similar to that of a previous study on rats with high-fat diet-induced hyperlipidemia, which supports the importance of SREBP-1c in lipogenesis [27,28]. The rate-limiting step in synthesizing fatty acids is the carboxylation of acetyl-CoA to malonyl-CoA, catalyzed by ACC [29]. The *ACACA* gene encodes ACC, which regulates lipid accumulation in the liver [29,30]. In the present study, no changes were observed in *ACACA* mRNA levels in the HC group. The possible reason for this observation could be that *ACACA* mRNA expression is regulated by multiple factors.

The recipe for preparation of YTS was obtained from a book on royal Thai traditional medicine [10]. This recipe uses the same ingredients as those used in a traditional Thai antihypertensive herbal (TTAH) recipe [11]. In this study, piperine was identified in the YTS extract as YTS is similar to TTAH, and piperine is a major constituent of TTAH [11] and exhibits hypolipidemic activity [14,16]. Previous studies have demonstrated that piperine reduces the hepatic mRNA levels of *SREBP-1c* in mice fed a high-fat diet [16]. This study provided for the first that treatment of rats with 100 mg YTS/kg BW downregulated *SREBP-1c* mRNA expression and ameliorated hepatic steatosis. This suggests that YTS extract reduces hepatic lipid accumulation by suppressing *SREBP-1c* expression. According to previous literature, the three herbal components of YTS exhibit hypolipidemic activity. Panduratin A, an active constituent found in *B. rotunda* (L.) Mansf., activates peroxisome proliferator-activator receptors alpha/delta (PPARα/δ) and decreases high-fat

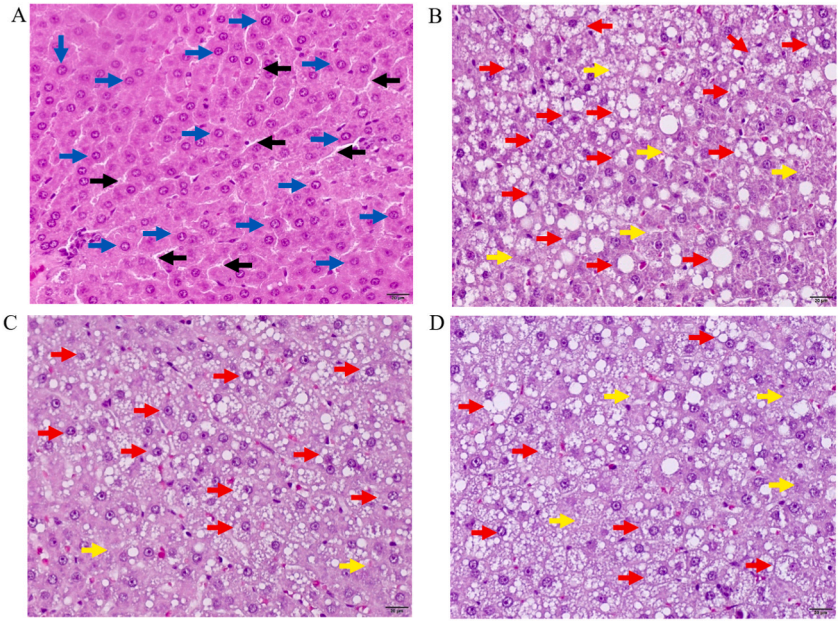

Fig. 4. Histopathological changes in liver tissues of the normal control group (A), hyperlipidemic control group (B), group treated with 100 mg of Ya That Somdun extract/kg BW (C), and group treated with 200 mg of Ya That Somdun extract/kg BW (D). All images were acquired at 400 × magnification. Blue arrows, normal hepatocytes; Black arrows, hepatic sinusoids; Red arrows, cytoplasmic vacuolation in hepatocytes; Yellow arrows, Necrosis area.

diet-induced obesity and lipid metabolism dysregulation [11]. The *C. rotundus* L. extract comprises piceatannol, scirpusin A, and scirpusin B, which improve the serum lipid profiles and reduce the body weights of overweight individuals [12]. Dietary piperine reduces the mRNA levels of the liver X receptor (LXR) α and its lipogenic target gene (*SREBP-1c*) [16]. Based on the information obtained in our study, the active compounds present in YTS should be identified in future studies.

Concerning toxicity, the present study, 200 mg YTS/kg BW increased SREBP-1c mRNA expression and might have exacerbated hepatic steatosis. These findings differ from those of a previous toxicity test [20] in which the authors observed no effect on blood chemistry and histological changes in rats. This could be due to the difference in the forms of herbs used. The present study used the ethanoic extract of YTS, which may have different phytochemicals than powder [20]. Studies have suggested that *T. crispa* can induce hepatotoxicity and nephrotoxicity in rats [31,32]. Furthermore, several substances isolated from herbs have been shown to modulate the activity of liver X receptors (LXRs) [33]. Natural compounds may provide therapeutic benefits while minimizing certain side effects [33]. Cyanidin, a natural flavonoid, activated LXRs responsive genes, including *SREBP-1c* [34]. The YTS recipe comprises six herbal plants, each containing several bioactive compounds. Therefore, the dose 200 mg YTS/kg BW can increase *SREBP-1c* mRNA expression. The prolonged high doses use of YTS should prompt concerns regarding hepatotoxicity.

The present study has a limitation. Feeding cholesterol suspended in coconut oil was not sufficient to increase the serum LDL-C and total cholesterol levels. Thus, a high-fat diet may be more suitable for development of a hyperlipidemia model in future studies [23, 24].

5. Conclusions

The findings of this study revealed that Ya That Somdun, a traditional Thai medicine, reduced hepatic lipid accumulation in hyperlipidemic rats by inhibiting *SREBP-1c* expression. The active compounds present in YTS should be analyzed in future studies.

CRediT authorship contribution statement

Suchittra Samuhasaneeto: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Gorawit Yusakul:** Writing – original draft, Methodology, Investigation. **Chuchard Punsawad:** Writing – original draft, Investigation. **Kingkan Bunluepuech:** Resources.

Ethical approval statement

All experiments were reviewed and approved by the Animal Ethics Committee of Walailak University (approval number: WU-ACUC-65023).

Data availability statement

The data presented in this study are available on request from the corresponding author.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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