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Original paper

Combined Lowering Effect of Phytosterol Esters and Tea Extracts on Lipid Profiles in SD Rats

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The present study was conducted to determine the effect of oral administration of phytosterol esters (PS, 308 mg/kg·d), tea extracts (TE, 300 mg/kg·d) and their combination on serum, liver and fecal lipids of SD rats induced by high-cholesterol diets. After 12 weeks, animals were sacrificed and followed immediately with the collection of blood and organ samples. Results showed that PS and TE alone retained its own lipid-lowering profiles. The combination of PS and TE decreased both serum cholesterol and triglyceride levels, and produced an additive effect on serum LDL-C level reduction. Most importantly was that PS+TE produced a complementary effect in suppressing body weight and fat mass, promoting fecal lipid excretion and reducing liver weight and liver lipids, suggesting the combination was more effective than either PS or TE alone. These results demonstrated that PS+TE could effectively prevent the formation of obesity, single and mixed hyperlipidemia without the observation of any toxic effect. Therefore, PS+TE could be considered as a potential nutraceutical or functional ingredient to prevent cardiovascular diseases and its related complications.

Keywords: phytosterol esters, tea extract, tea polyphenols, cholesterol-lowering, lipid-lowering

Introduction

Nowadays, cardiovascular disease (CVD) and their complications are considered as the primary causes of morbidity and mortality worldwide (Bahmani *et al*., 2015; Xu *et al*., 2012). Dyslipidemia (or hyperlipidemia) is one of the major risk factors for the development of these related complications (Johnston *et al*., 2017; Hunter and Hegele, 2017). Dyslipidemia is characterized by elevated serum or plasma levels of total cholesterol (TC) or low density lipoprotein cholesterol (LDL-C) and triglycerides (TG), or decreased high density lipoprotein cholesterol (HDL-C) level (Hunter and Hegele, 2017). Apart from dyslipidemia, obesity is another risk factor of cardiovascular disease, which is a complex metabolic disorder caused by an imbalance between fat synthesis and fat breakdown (Suzuki *et al*., 2013; Xu *et al*., 2015). Therefore, it is crucial to prevent the formation of hyperlipidemia and obesity for combating CVD and their complications.

As known to all, drug treatment is highly beneficial to the hyperlipidemic populations by decreasing blood cholesterol or triglycerides. Statins can effectively decrease blood cholesterol level by inhibiting cholesterol biosynthesis as inhibitor of

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3-hydroxy-3-methylglutary coenzyme A reductase (He *et al*., 2011). Ezetimibe, as another effective cholesterol-lowering drug, can inhibit intestinal cholesterol absorption by interaction with Niemann-Pick C1 Like 1 protein (Klop *et al*., 2013). Fibrates, as triglyceride-lowering agent, decreased blood TG level by approximately 30 % and LDL-C level by 8 %, whereas HDL-C level was increased by an average of 9 % (Klop *et al*., 2013). Unfortunately, most drugs possessed undesirable adverse side effects in some individuals, such as myalgia induced by statins, which can hinder medication compliance (Giglio *et al*., 2016; Hunter and Hegele, 2017). As a result, many patients and health-care providers are looking for other ways to reduce blood lipid level. Recently, phytochemicals have gained much attention due to their potential as functional food and dietary supplements to treat or prevent hyperlipidemia, especially for those patients with moderately elevated blood lipids (Liu *et al*., 2015).

At present, plant sterols (phytosterols) have attracted increasing attention because of their remarkable cholesterol-lowering effect (He *et al.*, 2018a; He *et al.*, 2018b). Abundant evidences demonstrated that phytosterols effectively decreased blood cholesterol by inhibiting intestinal cholesterol absorption in animals or humans (Moghadasian *et al*., 2016; Tan and Shahidi, 2012). However, the cholesterol-lowering effect of phytosterols and phytosterol esters is relatively low when compared with cholesterol-lowering drugs (Jia *et al*., 2008). Furthermore, phytosterols can effectively decrease blood cholesterol level, but have little or no effect on blood TG level (Jia *et al*., 2008). Therefore, it's necessary to develop some new formulations to possess higher cholesterol-lowering effect and be able to lower triglyceride level as well. As known to all, tea is the most popular and widely consumed beverage around the world. Green tea or tea extracts (TEs) have been shown to reduce blood triglyceride level (Lee *et al*., 2015; Li *et al*., 2006) and suppress adipose accumulation (Lee *et al*., 2015; Xu *et al*., 2015), thus having the potential to prevent hypertriglyceridemia and obesity.

Both phytosterols and green tea extract are generally considered as important hypocholesterolemic functional candidates. Phytosterols can lower blood cholesterol, but cannot reduce blood triglyceride. Green tea or TEs can be used for the prevention of hypertriglyceridemia. To the best of our knowledge, the interaction of phytosterols and green tea extracts has not been studied. Therefore, the aim of the present study was to investigate whether the combination of phytosterol esters and green tea extracts has the effect of synergistic or additive lipid-lowering or obesity-preventing, thereby providing a theoretical basis for the development of nutraceutical foods.

Materials and Methods

2.1. Materials TC, TG, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), total bile acid (TBA), creatinine (CREA) and uric acid (UA) kits were provided by Sichuan Maccura Biotechnology Co., Ltd. (Chengdu, China). HDL-C and LDL-C kits were provided by Shanghai Ailex Co., Ltd. (Shanghai, China). All other chemicals and solvents used were of analytical grade and provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cholesterol and bile salt were provided by Anhui Tianqi Chemical Engineering Co., Ltd. (Huaibei, China). Egg yolk powder was purchased from Zhengzhou Tianyuan Food Technology Co., Ltd. (Zhengzhou, China). Pork oil was obtained from local markets. Sawdust bedding was provided by the experimental animal center of Jiangsu University.

2.2. Chemical composition of phytosterol esters and tea extracts Phytosterol fatty acid esters were a generous gift from BASF Co., Ltd. (Shanghai, China). Phytosterol fatty acid esters (water dispersible powder, Vegapure® 67 WDP: total phytosterol esters content ≥ 67 %, total phytosterols ≥ 42 %) had the following chemical composition: beta-sitosterol ≥ 46 %, campesterol ≥ 26 %, stigmasterol ≥ 20 %, brassicasterol ≥ 3 %, beta-sitostanol ≥ 1.8 %, campestanol ≥ 1 %. Tea extracts (TE) was purchased from Hangzhou Gosun Biotechnologies Co. Ltd. (Hangzhou, China). Tea extracts (tea polyphenols ≥ 99 %) had the following chemical composition: EGCG ≥ 37.2 %, EGC ≥ 27.8 %, ECG ≥ 27.2 %, EC ≥ 7.0 %.

2.3. Diets Granulated and powdered basic diets with the same formula were purchased from Jiangsu Xietong Organism Co., Ltd. (Nanjing, China). The granulated basic diets were used for feeding normal group rats. The powdered basic diets were used to prepare high-fat-high-cholesterol diets. The high-fat-high-cholesterol diets contained 78.8 % powder basic diet, 10 % egg yolk powder, 5 % sucrose, 5 % pork oil, 1 % cholesterol and 0.2 % bile salts. All high-fat-high-cholesterol diets were prepared weekly and dried naturally. The control diet contained a trace amount of phytosterols and phytosterol esters (<0.01 %), which were insignificant compared with the oral dose of phytsterol esters.

2.4. Animals, Groups and Oral Sample Preparation Fifty male healthy SD rats (Animal Core Facility of Nanjing Medical University, Nanjing, China), weighing 120–140 g, were housed in polypropylene cages (5 per cage) in a room controlled at 25 ± 1 ℃ and 50 ± 10 % humidity with a 12 h light/dark cycle. Animal feeding and experimental operations were approved (permission number: 201602862) and conducted in accordance with the guidelines set by the animal experimental ethical committee, Jiangsu University.

All rats were given basic diets with free access to food and water. After one week of adaptation, the rats were weighed and randomly divided into five groups based on the average body weight (n = 10/group). The rats in normal group (NG) were fed regular granulated rodent chow diet. The rats in high-fat high-cholesterol (HF), phytosterol esters (PS), green tea extract (TE) and the combination of PS and TE (PS+TE) group were given high-fat-high-cholesterol diet. In NG and HF group, the rats

were treated with drinking water. In treatment groups, the rats were orally administered with PS (308 mg/kg·d), TE (300 mg/kg·d) and PS+TE (308 mg/kg·d + 300 mg/kg·d) dissolved in drinking water, respectively, once a day for 12 consecutive weeks.

It has been recommended that daily intake of 2–3 g phytosterols for a subject can effectively reduce blood cholesterol (He *et al*., 2013). In this study, 3.4 g/d phytosterol esters (2.1 g/d of phytosterols) for humans with 70 kg of body weight corresponded to 308 mg/(kg·d) for rats based on the dose translation from rats to humans. The given dose of tea extracts was selected based on the previous literature (Babu and Liu, 2008; Li *et al*., 2013). The maximum oral volume for rats was 20 mL/(kg·d) and the precise volume of gavage was calculated weekly on the basis of animal body weight. The sample concentration was calculated based on the given dose and content of sample. The oral sample was prepared daily with the help of ultrasonic equipment.

2.5. Animal Experiment and Sample Collection During the whole experimental period, the rats were allowed free access to food and drinking water. The fresh water and diets were given to the rats daily, and uneaten food was discarded. Food intake and body weight were measured weekly. Sawdust bedding was changed twice a week. The feces from each group were collected once in four weeks. Blood was obtained from retro-orbital sinus once in four weeks, and the rats were kept fasting overnight before blood sampling. At the end of experimental period, the rats were kept fasting overnight, weighed, measured and sacrificed. Blood was obtained from retro-orbital sinus, centrifuged at 4000 r/min for 10 min, and serum was isolated. Liver was dissected, washed with saline, blotted on filter paper, weighed and divided into two parts. The first liver tissue was fixed in paraformaldehyde solution. The remaining liver tissues were also stored at –80 ℃. Heart, kidney, spleen and lung were also collected and stored at –80 ℃.

2.6. Analysis of Serum Lipids and Biochemical Parameters Serum levels of TC, TG, HDL-C, LDL-C, GLU, AST, ALT, TBA, CREA and UA were measured with the corresponding test kits using an automatic AU2700 biochemical analyser (Beckman Coulter Inc., Brea, CA, USA).

2.7. Analysis of Hepatic Lipids Liver lipids were extracted as described previously (Wang *et al*., 2010). Briefly, liver sample were firstly homogenized in saline. Liver homogenate was extracted with chloroform/ethanol (2/1, v/v) to a final volume of 20 times. Liver cholesterol and triglyceride concentrations were measured using the same method and test kit for serum cholesterol and triglyceride.

2.8. Analysis of Fecal Lipids Fecal lipids were extracted and analyzed as we described previously (He *et al*., 2013). In brief, the feces from each group was collected and fully dried under vacuum at 60 ℃, then ground into fine powder and extracted with chloroform/ethanol (2/1, v/v). The extract was centrifuged at 5000 r/min for 10 min, and the supernatant was collected. A portion of supernatant was taken out, evaporated under a nitrogen stream, saponified at 70 ℃ for 2 h in ethanolic potassium hydrate and evaporated under a nitrogen stream. The samples were re-dissolved in water, neutralized by adding hydrochloric acid and extracted with chloroform. After centrifugation, the upper water layer was removed and the chloroform layer was collected. Fecal cholesterols were analyzed by high performance liquid chromatograph using a symmetry-C_{18} column (5 μm, 4.6 × 150 mm, Waters, USA) eluted with methanol/formic acid (1000/1, v/v) at 0.8 mL/min, the eluate was monitored using a ZAM 4000 evaporative light scattering detector from Schambeck (Bad Honnef, Germany) at 60 ℃ and nitrogen as carrier gas at pressure of 500 hPa. The concentrations of fecal cholesterol were calculated according to a standard curve constructed with cholesterol standards. Fecal triglyceride and bile acid concentration was determined with the corresponding test kit.

2.9. Statistical Analysis The statistical analyses were performed using SPSS 16.0. One-way ANOVA was used to analyze the overall treatment effects. When a statistically significant effect was obtained, the Student–Newman–Keuls test was performed to determine the differences between treatment and control groups. Significance level was set at $p < 0.05$. All data were presented as means ± SEM. Values with different superscript letters were significantly different ($p < 0.05$). Values with the same superscript did not differ significantly ($p > 0.05$).

Results

3.1. Food Intake and Body Weight No significant differences in food intake were observed among the five groups. The average body weights for every group during the whole feeding period (week 0–12) are shown in Fig. 1. No significant difference between HF and PS was observed in the average body weight during the whole feeding period.

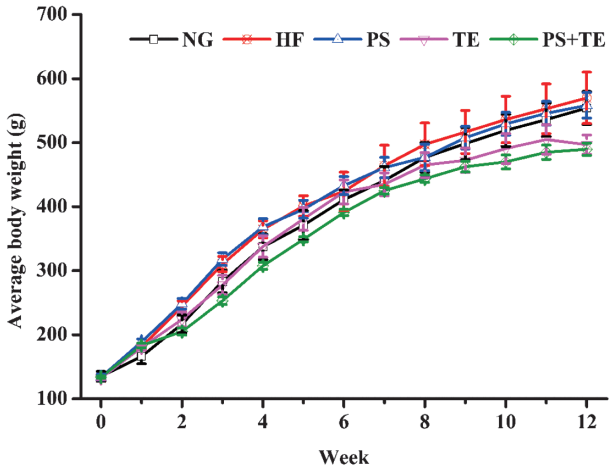


Fig. 1. The average body weight of every group during the whole experiment period. Results are means ± S.E.M. ($n = 10$).

Table 1. Body and relative organ weights in rats of every group

	Group				
	NG	HF	PS	TE	PS+TE
Body weight (g)					
Initial	135.1±7.8	132.5±3.2	134.1±4.3	132.6±2.6	134.0±2.0
Final	554.3±25.7ab	570±40.2a	558.3±19.9a	496.9±14.9b	489.8±9.7b
Relative organ weight (% body weight)					
Heart	0.30±0.01	0.30±0.01	0.32±0.01	0.32±0.01	0.33±0.01
Liver	2.88±0.05c	3.59±0.22a	3.32±0.10ab	3.07±0.12bc	3.11±0.07bc
Spleen	0.15±0.00	0.16±0.01	0.15±0.01	0.16±0.01	0.17±0.01
Lung	0.34±0.03	0.35±0.04	0.34±0.02	0.36±0.01	0.34±0.01
Kidney	0.63±0.03	0.60 ±0.01	0.65±0.02	0.65±0.04	0.64±0.04
Epididymal +Perirenal fat	2.98±0.19b	4.63±0.13a	4.50 ±0.44a	3.42±0.23b	3.69±0.23b

Data were analyzed using one-way ANOVA. Differences between treatment groups were further analyzed using the Student-Newman-Keuls test after a significant effect was detected. Results are means±S.E.M. (n = 10). Values bearing different superscript letters (a, b, c) are different ($p < 0.05$).

Table 2. Serum TG, TC, HDL-C and LDL-C in rats of every group

Time	Parameters (mmol/L)	Group				
		NG	HF	PS	TE	PS+TE
4th week	TC	2.4±0.14b	2.88±0.14a	2.7±0.12ab	2.76±0.13ab	2.73±0.16ab
	TG	1.6±0.16c	2.7±0.12a	2.6±0.17a	1.96±0.07bc	2.31±0.26ab
	HDL-C	1.53±0.11a	1.08±0.05bc	0.98±0.08c	1.27±0.08ab	1.14±0.12bc
	LDL-C	0.43±0.05c	0.83±0.09ab	0.66±0.09b	0.87±0.05a	0.76±0.05ab
8th week	TC	2.18±0.15	2.36±0.17	2.16±0.09	2.23±0.16	2.17±0.15
	TG	1.35±0.19ab	1.38±0.11ab	1.51±0.17a	1.06±0.09b	1.09±0.12b
	HDL-C	1.59±0.11a	1.30±0.07b	1.14±0.05b	1.32±0.08b	1.18±0.10b
	LDL-C	0.26±0.04b	0.71±0.14a	0.66±0.07a	0.90±0.11a	0.65±0.09a
12th week	TC	2.17±0.12ab	2.50±0.11a	2.07±0.07b	2.12±0.16b	1.97±0.15b
	TG	1.37±0.18ab	1.58±0.14a	1.42±0.20ab	0.96±0.12b	1.01±0.13b
	HDL-C	2.09±0.16a	1.92±0.07ab	1.75±0.11ab	1.64±0.13b	1.63±0.12b
	LDL-C	0.33±0.03b	0.45±0.03a	0.37±0.02ab	0.40±0.04ab	0.33±0.04b

Data were analyzed using one-way ANOVA. Differences between treatment groups were further analyzed using the Student-Newman-Keuls test after a significant effect was detected. Results are means±S.E.M. (n = 10). Values bearing different superscript letters (a, b, c) are different ($p < 0.05$).

Compared with HF, TE showed a non-significant decrease before the 11th week and a significant decrease at the 12th week. Since on the 2nd week, PS+TE showed a significant decrease in the average body weight. At the end of the experimental period, TE alone and PS+TE remarkably decreased the body weight by 12.8% (496.9±14.9 vs 570±40.2) and 14.0% (489.8±9.7 vs 570±40.2), respectively, compared with that of the HF control group ($p < 0.05$).

3.2. Organ Weights The relative weights of heart, liver, spleen, lung, kidney, epididymal and perirenal fat pad of rats for every group are displayed in Table 1. As for the relative weight of heart, spleen, lung and kidney, there was no significant difference among all groups. The relative weight of liver in HF rats was significantly higher than that of the NG rats. PS alone produced a slight reduction in the relative liver weight, TE and PS+TE remarkably decreased the relative liver weight by 14.5% ($p < 0.05$) and 13.4% ($p < 0.05$), respectively. The relative weights of epididymal and perirenal fat in HF rats were significantly higher than that of NG rats. Supplementation of TE and PS+TE could significantly reduce the relative weights of epididymal and perirenal fat by 26.1% ($p < 0.05$) and 20.3% ($p < 0.05$), respectively, in comparison with the HF rats.

3.3. Serum Lipid Levels Serum TG, TC, HDL-C and

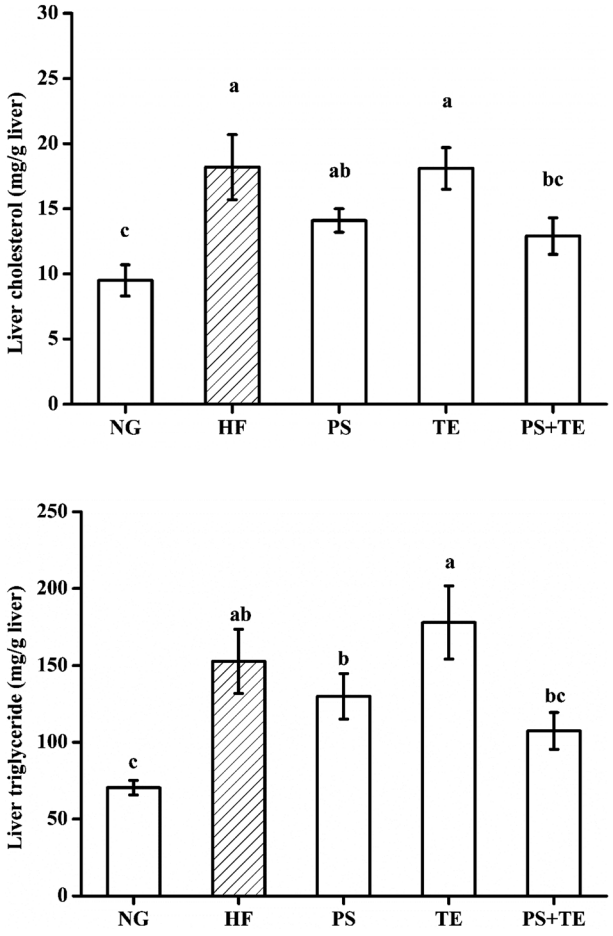


Fig. 2. Liver cholesterol and triglyceride levels in rats of every group. Data were analyzed using one-way ANOVA. Differences between treatment groups were further analyzed using the Student-Newman-Keuls test after a significant effect was detected. Results are means±SEM (n = 10). Values bearing different superscript letters (a, b, c) are different ($p < 0.05$).

LDL-C levels of rats at 4th, 8th and 12th week are displayed in Table 2. Five groups of rats had similar serum lipid profile at the beginning of the experiment. At week 4, HF rats had significant elevations of serum TG, TC and LDL-C concentrations and significant reduction of serum HDL-C concentration compared with that of NG (Table 2). And Serum TG level of TE rats was significantly decreased by 27.4 % when compared with HF group. At week 12, PS and TE alone or in combination significantly decreased serum TC level of rats by 17.2 %, 15.2 % and 21.2 %, respectively. Serum TG level was slightly decreased by 10.1 %, but that in TE and PS+TE rats were dramatically decreased by 39.2 % and 36.1 %, respectively. Both PS and TE alone slightly decreased the serum LDL-C level by 17.8 % and 11.1 %, respectively, whereas PS+TE significantly decreased serum LDL-C level by 26.7 %.

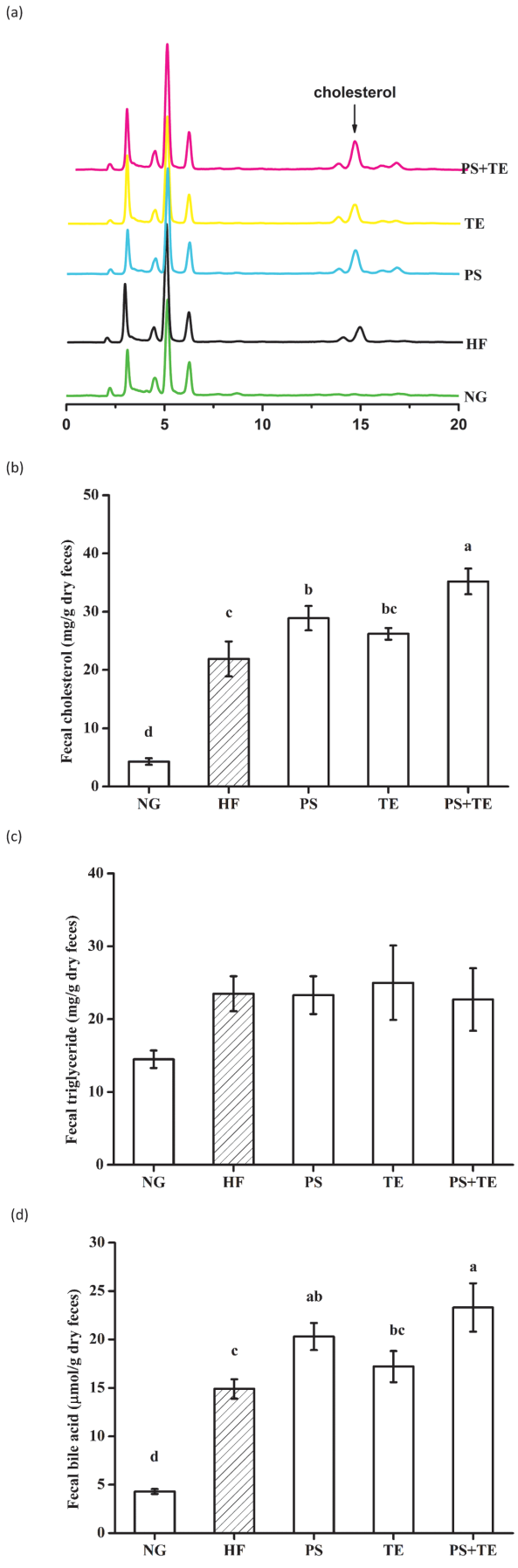
3.4. Liver Lipid Levels Liver cholesterol and triglyceride concentrations of rats for every group are shown in Fig. 2. Consuming PS and TE slightly but not significantly changed liver cholesterol content, whereas PS+TE significantly reduced by 29.1 % (12.9±1.4 vs 18.2±2.5), in comparison with that of HF group. TE alone did not affect liver triglyceride level, but PS and PS+TE slightly decreased liver triglyceride contents.

3.5. Serum Biomarkers for Liver and Renal Injury Serum biomarker levels for liver injury (ALT, AST, TBA) and renal injury (UA, CREA) are displayed in Table 3. Compared with HF group, the ALT activity was significantly decreased by 20.5 % ($p < 0.05$), 25.8 % ($p < 0.05$) and 29.5 % ($p < 0.05$) after oral administration of PS, TE and PS+TE. Similarly, PS, TE and PS+TE significantly decreased the AST activity by 40.3 % ($p < 0.05$), 47.6 % ($p < 0.05$) and 41.5 % ($p < 0.05$). However, no significant difference in serum TBA level was observed between the HF control and treatment groups. Consuming PS and TE slightly decreased serum CREA level by 8.4 % ($p > 0.05$) and 9.2 % ($p > 0.05$), whereas PS+TE significantly reduced by 30.7 % ($p < 0.05$), in comparison with that of HF controls. Similarly, PS and TE slightly decreased serum UA level by 1.3 % ($p > 0.05$) and 12.5 % ($p > 0.05$), whereas PS+TE significantly reduced by 33.0 % ($p < 0.05$).

3.6. Fecal Lipid Levels Fecal cholesterol, triglyceride and total bile acid levels are shown in Fig. 3. Compared with HF group, PS and TE alone increased fecal cholesterol by 32.0 % (21.9±3.0 vs 28.9±2.1, $p < 0.05$) and 19.6 % (21.9±3.0 vs 26.2±1.0, $p > 0.05$), respectively, whereas PS+TE significantly increased by 60.7 % (21.9±3.0 vs 35.2±2.2, $p < 0.05$) (Fig. 3a, 3b). Compared with PS group, fecal cholesterol level was significantly increased by 21.8 % (28.9±2.1 vs 35.2±2.2, $p < 0.05$) when combined supplementation of PS and TE for 12 weeks. Fecal triglyceride contents were slightly changed by 0.9 % (23.5±2.4 vs 23.3±2.6), 6.4 % (23.5±2.4 vs 25.0±5.1) and 3.4 % (23.5±2.4 vs 22.7±4.3) after oral administration of PS, TE and their combination (Fig. 3c). PS and PS+TE significantly increased fecal bile acids contents by 36.2 % (20.3±1.4 vs 14.9±1.0) and 56.4 % (23.3±2.5 vs 14.9±1.0), respectively, when compared with that of HF rats (Fig. 3d).

Discussion

The lipid-lowering effects of phytosterols and green tea (or tea extract) alone have been proved by many researchers (Afzal *et al*., 2015; Chen *et al*., 2008; Hunter and Hegele, 2017; Johnston *et al*., 2017; Lee *et al*., 2015; Li *et al*., 2006; Liu *et al*., 2015). In this study, PS and TE either alone or in combination showed different lipid-lowering action. Supplementation of PS alone significantly decreased serum TC level. This is in line with the result of most animal and clinical studies concerning the consumption of phytosterols and their derivatives (He *et al*., 2011; He *et al*., 2013; Orem *et al*., 2017). Currently, there still remained inconsistent in TC-lowering effect of TEs. In this study, consumption of TE alone significantly decreased serum TC level of rats. And PS+TE displayed slight advantage in lowering serum TC over either



PS or TE alone. In addition, PS also showed a non-significant decrease of serum TG level. In some previous literatures, phytosterols showed different levels of TG-lowering effect in animal model and human (Rideout *et al*., 2010; Rideout *et al*., 2015). This may be associated with the initial baseline concentration of TG (Demonty *et al*., 2013; Orem *et al*., 2017). In a clinical study by Plat and Mensink, a daily consumption of a low-fat yogurt enriched with 2 g of phytostanols for 8 weeks decreased plasma TG by 27.5 % in subjects with dyslipidemic metabolic syndrome, but had no effect on plasma TG in normolipidemic subjects with the same study (Plat and Mensink, 2009). In this study, serum TG level was significantly reduced in PS+TE when compared with HF, indicating that serum TG-lowering effect of PS was further strengthened when combined with TE. PS and TE alone produced a slight but not significant decrease in serum LDL-C, whereas PS+TE possessed a significant decrease, suggesting the presence of additive effect.

We also examined the effect of PS and TE alone or in combination on body weight, relative liver weight, relative epididymal and perirenal fat of rats. Compared with HF, PS alone had little effect on body, liver and adipose weight, which was in agreement with most previous studies (Jia *et al*., 2008; Liu *et al*., 2015; Moghadasian *et al*., 2016). TE alone showed a significant decrease in body, liver and adipose tissue weight. PS+TE also displayed significant decrease in body, liver and adipose tissue weight. PS did not reduce body and adipose weight, but they could effectively prevent the formation of obesity when combined with TE. Similarly, a previous study demonstrated that combined supplementation of phytosterols and conjugated linoleic acid decreased fat mass and promoted weight loss (Furlan *et al*., 2013). The results showed that PS+TE could prevent obesity formation.

Serum biomarker levels for liver and renal function were also examined. PS or TE alone slightly decreased serum UA and CREA levels, while PS+TE significantly reduced UA and CREA levels. Similarly, serum ALT and AST levels were significantly reduced by PS and TE alone or in combination. Similar results were also observed in previous studies (Jia *et al*., 2008; Lee *et al*., 2015; Song *et al*., 2017; Suzuki *et al*., 2013). Song *et al*. found that serum UA and ALT level of rats was significantly decreased by phytosterol esters treatment (Song *et al*., 2017). Suzuki *et al*. found that green tea extract could decrease serum ALT, AST levels of C57BL/6J mice fed high-fat diet (Suzuki *et al*., 2013). These results suggested that supplementation of PS and TE either alone or in combination

Fig. 3. Fecal cholesterol, triglyceride and bile acid levels in rats of every group. Data were analyzed using one-way ANOVA. Differences between treatment groups were further analyzed using the Student-Newman-Keuls test after a significant effect was detected. Results are means±SEM (n = 10). Values bearing different superscript letters (a, b, c, d) are different ($p < 0.05$).

Table 3. Serum biomarker levels for liver and renal injury in rats of every group

	Group				
	NG	HF	PS	TE	PS+TE
Serum biomarkers for liver injury					
ALT (U/L)	46.5±1.0a	47±2.7a	37.38±1.9b	34.86±2.6b	33.13±2.3b
AST (U/L)	308.3±15.6a	340.2±36.4a	203.1±23.1b	178.4±27.6b	198.9±14.7b
TBA (μmol/L)	9.28±1.3b	14.74±1.0a	13.9±1.7ab	14.73±1.7a	12±1.8ab
Serum biomarkers for renal injury					
CREA (μmol/L)	39.6±2.4b	46.6±2.3a	42.7±1.4ab	42.3±3.7ab	32.3±1.9b
UA (μmol/L)	100.8±6.7ab	123.5±7.4a	121.9±13.6a	108.1±16ab	82.8±8.4b

Data were analyzed using one-way ANOVA. Differences between treatment groups were further analyzed using the Student-Newman-Keuls test after a significant effect was detected. Results are means±S.E.M. (n = 10). Values bearing different superscript letters (a, b) are different ($p < 0.05$).

for 12 weeks did not cause liver and kidney damage of rats.

The results from liver lipid analysis showed that PS supplementation showed slight decrease in liver cholesterol and triglyceride content. A study by Song *et al*. (2017) found that phytosterol esters could prevent non-alcoholic fatty liver disease of rats by decreasing hepatic cholesterol, triglyceride and free fatty acid levels. In this study, TE had no any lowering-effect of liver lipids. PS+TE displayed a significant decrease in both liver cholesterol and triglyceride levels. Although the exact mechanism is not clear, the combination of PS and TE may attenuate hepatic steatosis induced by high-fat diet.

Regarding fecal lipid excretion, PS alone showed a significant increase of fecal cholesterol and bile acid level when compared with HF. TE alone displayed a non-significant increase in fecal lipids. Compared with PS or TE, PS+TE resulted in a significant increase of fecal cholesterol and bile acids, suggesting that synergistic effect was produced in promoting fecal cholesterol and bile acid excretion when combined use of PS and TE. Therefore, the lipid-lowering effect of PS or TE may be further enhanced when used in combination by promoting fecal lipid excretion.

Based on the current results, PS+TE were effective in decreasing fat mass, serum and liver lipids and promoting fecal lipid excretion, thus indicating that PS+TE could prevent the formation of both single and mixed hyperlipidemia. Phytosterols possess lipid-lowering effect mainly through interfering intestinal cholesterol absorption, increasing cholesterol removal or modifying the expression of the related genes of cholesterol metabolism (Chen *et al*., 2008). Several mechanisms have been suggested for the hypolipidemic effect of TEs, such as suppressing intestinal TG absorption, inhibiting cholesterol and fatty acid biosynthesis (Chen *et al*., 2014). Currently, the exact mechanism of PS+TE still remained unclear in regulating lipid metabolism, but it may be related to the lipid-lowering mechanism of PS or TE alone.

In conclusion, PS and TE alone retained its own lipid-lowering profiles in rats fed high-fat high-cholesterol diet. PS was effective in reducing liver lipids and promoting fecal lipid excretion. TE was effective in decreasing fat mass and controlling body weight gain. PS and TE, when used in combination, produced a complementary effect. These results suggested that PS+TE could prevent the formation of obesity, single and mixed hyperlipidemia. Therefore, PS+TE could be considered as a potential nutraceutical or functional ingredient to prevent cardiovascular diseases and its related complications. Future studies should be performed to understand the mechanism of action and potential clinical outcome of PS+TE.

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Notes

The authors declare no competing financial interest.

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