Protective Effect of Saponins-Enriched Fraction of *Gynostemma pentaphyllum* against High Choline-Induced Vascular Endothelial Dysfunction and Hepatic Damage in Mice

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Choline as a quaternary amine nutrient is metabolized to trimethylamine by gut microbiota and subsequently oxidized to circulating trimethylamine-N-oxide (TMAO), a gut-derived metabolite associated with liver toxicity and cardiovascular disease. The study was to probe the possible vasoprotective and hepatoprotective effects of total saponins of *Gynostemma pentaphyllum* (TSGP) in 3% high-choline water-feeding mice. The purified TSGP was obtained with content of 83.0% saponins, and its antioxidant activities were evaluated in vitro. Furthermore, the mice fed with high choline for 8 weeks significantly expressed vascular endothelial dysfunction and liver oxidative stress (p < 0.01 vs. Normal). Administration of TSGP at 400 and 800 mg/kg body weight (b.w.) significantly lowered the serum total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), endothelin-1 (ET-1) and thromboxane A2 (TXA2) levels, as well as hepatic malondialdehyde (MDA) formation, but effectively elevated the serum nitric oxide (NO), endothelial nitric oxide synthase (eNOS) and prostaglandin I2 (PGI 2) levels, as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST), T-superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in high choline-fed mice. Hematoxylin–eosin (H&E) and oil red O staining also suggested that TSGP could exert the significant protection against endothelial dysfunction and liver injury in high choline-treated mice. These findings suggest that TSGP is of the saponins-enriched extract, and is a good candidate of dietary supplement and therapeutic application in vascular and hepatic oxidative injury.

**Key words**  *Gynostemma pentaphyllum*; saponin; choline; liver damage; vascular endothelial dysfunction

INTRODUCTION

Choline has been considered as an essential nutrient for the human body, which may exert many biological activities. However, recent reports have discovered that the gut microbes-dependent metabolite trimethylamine (TMA) is a gut-derived metabolite associated with liver toxicity and cardiovascular diseases. Several reports have demonstrated that ingestion of some diets with high choline can effectively lead to endothelial dysfunction, and our previous study also showed that dietary ingestion of high choline or TMAO is highly linked to liver oxidative stress injury.

*Gynostemma pentaphyllum* Makino (*G. pentaphyllum*) is a famous edible and medicinal plant in China and some European countries. The principal active constituents of *G. pentaphyllum* are various saponins, which are also known as gypenosides, and more than 100 gypenosides have been segregated and identified from *G. pentaphyllum*. The saponins as the most important active components of *G. pentaphyllum* have been widely used in health care. Studies have also indicated that *G. pentaphyllum* saponins exhibit the benefit effects in treatment of metabolic and vascular diseases. Because the bioactive components of cheap *G. pentaphyllum* was similar to expensive ginseng root, it was named as “the second ginseng,” and hence, the cultures of *G. pentaphyllum* or their extracts for health care have been put into mass production.

To our knowledge, the beneficial protection of *G. pentaphyllum* saponins on the dysfunction of the blood vessel and liver injury caused by high choline intake is still unreported. In this context, the current study was dedicated to isolate the total saponin of *G. pentaphyllum* (TSGP) from *G. pentaphyllum* herb, and its antioxidant activities were evaluated in vitro. Furthermore, we for the first time try to discover whether consumption of TSGP may antagonize the high choline diet-caused vascular functional disorder and liver oxidative stress injury in mice.

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MATERIALS AND METHODS

Isolation of TSGP  G. pentaphyllum was purchased from Wanshou Road Medicine Market (Xi’an, China), and identified by Yonghui Sun from Xi’an Medical College. TSGP was prepared using the previous method with some adjustments. In brief, the dried G. pentaphyllum herb material was shattered and screened, and then the stive (100 g) was extracted in 70% ethanol (1:8 (w/v)) for three times and refluxed 90 min each time at 60°C for three times. After leaching, the filtrate was combined and collected using rotary evaporators under vacuum to volatilize the solvents at 60°C, and the condensed solution was dissolved in deionized water, and then extracted with petroleum ether, ethyl acetate and n-butanol, sequentially. The n-butanol-extracted solution was collected and concentrated. The condensed solution was further purified through D101 resin column chromatography, and eluted with 70% ethanol to get the saponins-enriched fraction solution, followed by drying as TSGP through concentration and evaporation at 60°C.

Determination of Total Saponins in TSGP  The total saponins of TSGP were determined by our the previously described method with slight adjustments. In brief, 100 µL definite TSGP (4.0 mg/mL) was added to 0.2 mL of 5% vanillin in acetic acid and 0.8 mL of 70% perchloric acid. The mixture was incubated at 60°C for 15 min. Ginsenosides Rb1 was viewed as the standard sample. The absorbance was read at 550 nm as the maximum absorbance wavelength after the sample was chilled down at room temperature. A standard curve prepared using the standard ginsenosides Rb1. Namely, ginsenoside Rb1 was separately dissolved in ethanol for an ultimate concentration of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg/mL, and the absorbance at 550 nm was worked out using the standard ginsenosides Rb1. Lin in acetic acid and 0.8 mL of 70% perchloric acid. The mixture was incubated at 60°C for 15 min. Ginsenosides Rb1 was viewed as the standard sample. The absorbance was read at 550 nm as the maximum absorbance wavelength after the sample was chilled down at room temperature. A standard curve was worked out using the standard ginsenosides Rb1. Namely, ginsenoside Rb1 was separately dissolved in ethanol for an ultimate concentration of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg/mL, and the absorbance at 550 nm were determined, respectively.

Assay for in vitro Antioxidant Activities of TSGP 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals-scavenging capacity of TSGP: The radicals-scavenging activity of TSGP was evaluated by previous method. Hydroxyl radicals-scavenging activity of TSGP: The eliminating ability of TSGP against hydroxyl radical (HO·) was evaluated by previous method. Superoxide radicals-scavenging assay of TSGP: The Superoxide radicals-scavenging assay was tested by previous procedure. Ferric-reducing antioxidant ability assay of TSGP: The deoxidizing capacity of TSGP was evaluated by a previous method.

Animals and Experimental Design  Healthy male Kunming mice (body weight 18 ± 2 g) were supplied by the Experimental Animal Center of the Fourth Military Medical University (Xi’an, China). Mice were housed under controlled laboratory conditions (temperature 22 ± 2°C, humidity 60 ± 5% with a 12/12 h light-dark cycle) and permitted free intake of a standard rodent chow and tap water. After acclimation for a week, they were stochastically separated into 5 groups (n = 10). Namely, normal control group, HC control group (3% dietary choline water alone), and three TSGP-treated groups (dose of 200, 400 and 800 mg/kg·b.w., supplemented with 3% dietary choline water, respectively). The mouse was permitted free intake of tap water or 3% dietary choline water. TSGP was dissolved in 0.5% CMC-Na solution and administered intragastrically (i.g.) at 200, 400 and 800 mg/kg·b.w. once daily (0.4 mL) for 8 consecutive weeks, where the dose was designed according the previous results of our pre-experiment in animals. The normal and dietary choline groups of mice were also administered with the same volume of 0.5% CMC-Na solution (0.4 mL, i.g.). All the administrations were conducted between five and six o’clock in the afternoon. The mice were fed with 3% dietary choline water every two days and weighed once a week. Two hours after the last administration, all mice were fasted overnight and were only allowed to drink water freely for 12 h, and then all mice were given isoflurane for complete anesthesia and killed, and Blood, liver and vessels were collected. All samples were disposed and stored according to our previous experiment. All animal experiments were conducted in accordance with the protocols approved by the Committee on Care and Use of Laboratory Animals of the Fourth Military Medical University, China (XJYYLL-2015689).

Assay for Endothelial Nitric Oxide Synthase (eNOS), Nitric Oxide (NO), Endothelin 1 (ET-1), Thromboxane A2 (TXA2) and Prostaglandin I2 (PGI2)  Serum levels of NO, eNOS, ET-1, TXA2 and PGI2 were assessed to reflect the vascular injury of mice, respectively. Assay kits were products from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and the measurements of eNOS, NO, ET-1, TXA2, and PGI2 were performed on the basis of the instructions, and the consequences were expressed as U/L, μmol/L, pg/mL, pg/L and pg/mL, respectively.

Analysis of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Activities for Monitoring Liver Function  The blood of mice was gathered and centrifuged for enzyme activity analysis of the serum ALT and AST as assay kits from Changchun Huili Biotechnology Co., Ltd. (Changchun, China). Serum AST and ALT activities were detected according to the instructions, and the consequences were expressed as U/L.

Determination of Hepatic T-Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Malondialdehyde (MDA)  Detection kits were supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The liver tissue homogenates were prepared in a ratio of 1:9 (w/v, liver: saline), and homogenates were centrifuged at 2000 g for 10 min and gathered for the test of activities of T-SOD, GSH-Px and MDA concentration, and the consequences were expressed as U/mg prot., U/mg prot. and nmol/mg prot., respectively. Determination was performed using the kit instructions. The total protein content in the homogenate was tested by Coomassie bright blue method.

Serum Lipid Measurement and Histopathological Examination  The mouse blood was gathered and centrifuged at 2000 × g for 20 min to collect the supernatant as the isolated serum. Assay kits were supplied by Changchun Huili Biotechnology Co., Ltd. (Changchun, China). Dyslipidaemia was evaluated by testing the TC, TG, high density lipoprotein (HDL)-C and low density lipoprotein (LDL)-C levels according to the instructions of the assay kits. The atherogenic index (AI), an important determinant of cardiovascular risk, was evaluated by the TC/HDL-C ratio. Besides, the histopathological examination of pectoral aortas and liver tissues was done according to our previous procedure.

Statistical Analysis  All experiments were performed in triplicate and the values were expressed as mean ± standard deviation (S.D.). Data analysis was using one-way ANOVA and Duncan’s multiple range tests (DPS 7.05) for differences in data of biochemical parameters among the different groups.
The \( p \)-value <0.05 was considered statistically significant.

RESULTS

Chemical Analysis of Total Saponins Content in TSGP

Total saponins of herbal *G. pentaphyllum* were extracted with 70% ethanol, and 3.9% (w/w) extraction yield of the herb powder was achieved. The TSGP as saponins-enriched fraction was further obtained from the rough extracts by separation on a D101 macroporous resin column. The total saponins content determined using the method of vanillin colorimetry was up to 83.0%. In this work, the regression equation for ginsenoside Rb1 was \( y = 7.8377x - 0.0018 \) with the correlation coefficient \( R^2 \) of 0.9977, where \( y \) and \( x \) was absorbance value at 550 nm and concentration of Rb1, respectively, and \( R \) was linear correlation coefficient. This quantitation demonstrated that TSGP was of a saponins-enriched extract.

In Vitro Antioxidant Activity of TSGP

The assay for *in vitro* antioxidant activities of TSGP through the classical DPPH', HO', \( O_2^- \) and ferric reducing ability was performed. The free radicals-scavenging ability of TSGP on DPPH radicals was 35.5, 65.9, 83.2, 89.5 and 92.0% at concentration of 0.1, 0.5, 1.0, 2.0 and 4.0 mg/mL, respectively (Fig. 1A). Similarly, the reducing ability of TSGP on HO' was 36.5, 47.0, 61.5, 77.1 and 82.0% at various concentrations (0.1–3.0 mg/mL, Fig. 1B). Meanwhile, TSGP also exhibited obvious scavenging activities (22.3, 32.1, 43.1, 69.7 and 72.2%) against \( O_2^- \) at various concentrations (0.1–3.0 mg/mL, Fig. 1C). It was also found that ferric-reducing antioxidant power values of TSGP ranged from 0.08 to 0.57 in 0.1–3.0 mg/mL (Fig. 1D). These findings indicated that TSGP had a strong antioxidant capacity.

In Vitro Effects of TSGP Administration on Body Weight

Table 1 summarized the influences of high choline (HC, model) water with or without TSGP administration on the body weights of the tested mice. It could be seen that the mice significantly gained weight after intake of 3% dietary HC water for 8 weeks in comparison with the normal control.
group \( (p < 0.05) \). Interestingly, administration of TSGP at 400 and 800 mg/kg·b.w. effectually decreased the body weight gain caused by HC feeding \( (p < 0.05) \), particularly at the last two weeks. It was worth noting that there was no obvious discrepancy in average ingestion of food and water observed in the daily food intake (data not shown). All these results suggest that the mice with ingestion of HC water can gain the body weight and the protective administration of HC-fed mice with TSGP could effectively prevent this weight gain.

**Effects of TSGP on Serum eNOS, NO and ET-1 Levels**

NO exerts a markedly protective effect on vassal integrity and endothelial function, which is synthesized by the eNOS.\textsuperscript{28} In this study, the levels of eNOS and NO were decreased by 25.9 and 59.0\% after the normal mice were given with HC water, respectively \( (p < 0.01, \text{Figs. 2A, B}) \). But the decline of eNOS and NO levels could be effectually antagonized by consumption of TSGP at 400 and 800 mg/kg·b.w. \( (\text{Figs. 2A, B}) \). In contrast, it is well known that the elevated release of ET-1 is one of the endothelial dysfunctional characters.\textsuperscript{29} Interestingly, HC feeding led to a 32.0\% increase in ET-1 levels of mice, relative to the normal feeding control \( (p < 0.01, \text{Fig. 2C}) \), but administration of TSGP at 400 and 800 mg/kg·b.w. could inhibited the HC-induced increase of the serum ET-1 release, respectively \( (p < 0.05, p < 0.01) \). Although a mild elevation in eNOS level and a slight reduction in ET-1 release were observed following the administration of TSGP at 200 mg/kg·b.w., there was not the statistical significance when compared to HC feeding control, respectively \( (\text{Figs. 2A–C, } p > 0.05) \). These findings suggested that feeding of 3\% HC water in mice caused endothelial dysfunction clearly, and TSGP might significantly suppress the endothelial damage in a dose-dependent manner.

**Effects of TSGP on Serum TXA\textsubscript{2} and PGI\textsubscript{2} Levels**

TXA\textsubscript{2} and PGI\textsubscript{2} are the most common prostanoids in cardiovascular system, which display extensive modification against endothelial damage and atherosclerosis.\textsuperscript{30} Herein, we further measured the serum levels of TXA\textsubscript{2} and PGI\textsubscript{2} of the tested mice with or without HC feeding. In particular, the serum TXA\textsubscript{2} level in HC-treated mice was markedly enhanced to 546.8 ± 47.8 pg/mL with an enhancement of above 4.0-fold \( (p < 0.01, \text{Fig. 2D}) \). Meanwhile, the serum PGI\textsubscript{2} level was reduced to 19.4 ± 3.4 pg/mL in HC-treated mice from 50.7 ± 5.2 pg/mL of the normal control group \( (p < 0.01, \text{Fig.} \)
As expected, following administration of mice with TSGP at 200 mg/kg·b.w., the TXA₂ level was significantly decreased to 211.5 ± 34.8 pg/mL (p < 0.01), and TSGP could dose-dependently inhibit the elevation of TXA₂ level with different degrees at 400 and 800 mg/kg·b.w., respectively (p < 0.01). Conversely, PGI₂ level was significantly enhanced to 24.3 ± 4.3 pg/mL in the mice following administration of HC-fed mice with 400 mg/kg·b.w. TSGP (p < 0.05), and a further increase could be seen at 800 mg/kg·b.w. TSGP (p < 0.01) (Fig. 3).

### Effects of TSGP on Enzymatic Activities of Serum AST and ALT

AST and ALT were deemed to be valid biochemical indexes of early liver injury. As illustrated in Table 2, the levels of the serum TC, TG, and LDL-C in HC-fed mice had a sharp elevation by 46.9% (p < 0.01), 75.0% (p < 0.01) and 50.0% (p < 0.01), and HDL-C had a significance decline by 45.5% (p < 0.01) when compared to the normal mice, respectively. Meanwhile, AI, an important determinant index of cardiovascular risk, had an obvious increase by 168.9% in HC-feeding mice in comparison with the normal group (p < 0.01). However, the elevation of TC, TG and LDL-C levels and the reduction of HDL-C concentration in HC-fed mice were effectively attenuated by treatment of the mice with 400 and 800 mg/kg·b.w. of TSGP, respectively (p < 0.05, p < 0.01). A slight change (p > 0.05) in TC, TG, LDL-C and HDL-C levels was also observed following administration of TSGP at 200 mg/kg·b.w. in comparison with HC control. As a result, TSGP might return to normal status for the dyslipidemia of HC-induced mice.

### Histological Observation for Thoracic Aortas of TSGP-Treated Mice

To further provide the evidence of the biochemical assay, the mouse thoracic aorta was stained through chemical assay, the mouse thoracic aorta was stained through H&E (Magnification 400×).

![Figure 3](image)

(A) Representative histological section of the aorta of the normal mice, high choline-induced mice, low-, middle- and high-doses of TSGP-treated mice. The medial thickness was typically showed by the arrows (values followed at the bottom) and analyzed with image pro-plus 6.0 software. (B) Representative intima-media/lumen ratio of the thoracic aorta of the tested mice in the normal group, high choline-induced group, low-, middle- and high-doses of TSGP-treated group. Data denoted were means ± S.D. (n = 10). *p < 0.05, **p < 0.01, vs. the normal group. *p < 0.05, **p < 0.01, compared to high choline-treated mice.

### Table 2. Effects of High-Choline (HC) Ingestion and/or TSGP Administration on the Serum TC, TG, HDL-C and LDL-C Levels of the Tested Mice (n = 10)

| Parameters       | TC    | TG    | LDL-C | HDL-C | AI    |
|------------------|-------|-------|-------|-------|-------|
| Normal           | 3.2 ± 0.6 | 0.8 ± 0.1 | 1.2 ± 0.2 | 1.1 ± 0.2 | 2.9 ± 0.1 |
| Choline          | 4.7 ± 0.3** | 1.4 ± 0.1** | 1.8 ± 0.1** | 0.6 ± 0.1*** | 7.8 ± 0.2*** |
| HC + TSGP (200 mg/kg·b.w.) | 4.2 ± 0.4 | 1.2 ± 0.2 | 1.6 ± 0.2 | 0.6 ± 0.1 | 7.0 ± 0.1 |
| HC + TSGP (400 mg/kg·b.w.) | 3.7 ± 0.7** | 0.9 ± 0.1* | 1.4 ± 0.2* | 0.9 ± 0.1** | 4.1 ± 0.1** |
| HC + TSGP (800 mg/kg·b.w.) | 3.5 ± 0.3** | 0.8 ± 0.1** | 1.3 ± 0.2** | 1.0 ± 0.1** | 3.5 ± 0.1** |

Data are expressed as mean ± S.D. of 10 mice in each group. Values of serum TC, TG, HDL-C and LDL-C levels are all expressed in mmol/L, respectively. Atherogenic index (AI) = TC/HDL-C. **p < 0.01, relative to normal group. *p < 0.05 and **p < 0.01, relative to the HC group.
hematoxylin–eosin (H&E). As shown in Fig. 3A, the histopathological alteration suggested the proliferation of the vascular wall or incassation of media of the pectoral aorta of HC water-fed mice occurred. Nevertheless, the damage extent of vascular endothelium in HC-treated mice together with TSGP treatment had the significant improvement, where the tissue thickness of the thoracic aorta had the remarkable decrease in a dose-dependent manner. Furthermore, we analyzed the ratio of intima-media/lumen, suggesting that TSGP effectively steadied the vessels and reduced the wall thickness ratio against HC-caused damage. As shown in Fig. 3B, the intima-media/lumen ratio in HC feeding mice had an elevation by 91.1% as compared to the normal control group (*p < 0.01), indicating that HC consumption severely caused the damage of the vascular structure. However, administration of mice with the lower levels of TSGP at 200 and 400 mg/kg·b.w. could also effectually enhance the T-SOD and GSH-Px activities, and reduced MDA levels, respectively (*p < 0.05, **p < 0.01).

Hepatic Protection of TSGP against Oxidative Stress or Histopathologic Injury The enzyme activities of T-SOD and GSH-Px, and the formation of MDA were considered to be important indicators of liver tissue damage.25) Figures 4C–E exhibited the alteration of these biochemical parameters of hepatic damage in mice. It was found that TSGP remarkably alleviated the oxidative stress injury of HC feeding mice, where treatment of HC-fed mice with TSGP at 800 mg/kg·b.w. remarkably elevated T-SOD activity by 144.4% (**p < 0.01, Fig. 4C) and GSH-Px activity by 11.2% (**p < 0.01, Fig. 4D), but significantly decreased the MDA concentration by 35.9% (**p < 0.01, Fig. 4E), respectively. Notably, administration of mice with the lower levels of TSGP at 200 and 400 mg/kg·b.w. could also effectually enhance the T-SOD and GSH-Px activities, and reduced MDA levels, respectively (*p < 0.05, **p < 0.01).

As shown in Figs. 5A–E, the histological change of the cellular structure in the liver tissue section of the normal mice was not observed. However, the severe parenchymal disarrangement with characteristics of degeneration of cells, cellular karyopyknosis, cytoplasmic vacuolation, massive steatosis, necrosis and the loss of cellular boundaries appeared following HC treatment in mice for 8 weeks (Fig. 5B). As expected, administration of TSGP obviously ameliorated the hepatic lesions (Figs. 5C–E), especially at 800 mg/kg·b.w., where the
confirmed that TSGP exerted the good hepatoprotection and together with biochemical analysis, histopathologic examination (Figs. 6C–E). TSGP at 400 and 800 mg/kg·b.w. had effective protection against liver damage caused by HC consumption (Figs. 6C–E). The green arrows indicate the enlarged sinusoids between the plates of hepatocytes. (F) Representative hepatocytes stained area of the tested mice in the normal group, high choline-induced group, low-, middle- and high-doses of TSGP-treated group. Data denoted were means ± S.D. (n = 10). **p < 0.01, *p < 0.05, vs. the normal group. +p < 0.05, **p < 0.01, compared to high choline-treated mice.

hepatic tissues of the tested mice exhibited nearly normal appearance with well-preserved cytoplasm, prominent nuclei and legible nucleoli. As depicted in Fig. 6 with photomicrographs of Oil Red O-stained liver tissues in the tested mice, feeding of 3% HC water led to the extensive lipid droplets in parenchymal cells, and the boundaries were ambiguity, relative to the normal mice. However, TSGP dose-dependently displayed effective protection against liver damage caused by HC consumption (Figs. 6C–E). TSGP at 400 and 800 mg/kg·b.w. had more valid protection, in comparison to 200 mg/kg·b.w.. Together with biochemical analysis, histopathologic examination confirmed that TSGP exerted the good hepatoprotection and remission of oxidative stress in HC-fed mice.

Fig. 5. Effects of TSGP on Histopathological Changes of the Liver Hepatocytes Stained with H&E in High Choline-Induced Mice (Original Magnification of 400×)

(A) Normal group, (B) high choline-induced group, (C) 200 mg/kg·b.w. TSGP (low-dose) + 3% choline, (D) 400 mg/kg·b.w. TSGP (medium-dose) + 3% choline, (E) 800 mg/kg·b.w. TSGP (high-dose) + 3% choline. The green arrows indicate normal cellular architecture with clear hepatic cell nucleus. The red arrows indicate the degenerative cell necrosis. The yellow arrows indicate the enlarged sinusoids between the plates of hepatocytes.

DISCUSSION

CVD is one of the major diseases threatening worldwide human health, and its intervention with naturally occurring bioactive ingredients is of a hotspot in current studies. Metabolic syndrome, such as obesity, liver dysfunction, dyslipidemia and vascular endothelial damage, is one of the key dangerous factors of CVD. Interestingly, TMA-containing nutrients, such as choline, lecithin, l-carnitine, are rich in a Western diet with high consumption, and human gut microbial TMA lyases can use these nutrients as substrates to produce TMA, which is absorbed to the liver via the portal circulation, and is further converted into TMAO by host hepatic flavin monoxygenases (FMOS), where TMAO can lead to vascular and liver injury. Herein, our result demonstrated that TSGP as saponins-enriched fraction exerted the protective effect against l-carnitine-induced vascular endothelial damage, which was in accordance with previous investigation. A similar report has shown that oolong tea extract or citrus peel polymethoxylflavonones may decrease vascular inflammation in l-carnitine feeding mice by reducing TMAO formation and hepatic FMO3 mRNA levels. Therefore, we speculate that the protective effects of TSGP on endothelial damage may be related to gut microbiota-derived TMAO formation of dietary l-carnitine ingestion in mice. Recently, naturally occurring saponins from G. pentaphyllum herb have been found to be effective in the prevention and treatment of metabolic and vascular diseases. The cheaper G. pentaphyllum is generally named as “the second ginseng” because its bioactive components are similar to chemical composition of ginseng roots which have potentially value in treating cardiovascular diseases. The active compounds of G. pentaphyllum saponins for protecting CVD have been reported to be Gypenoside LVI, Gypenoside XVII, Gypenoside XLIX and so on.35–37) Gypenoside LVI attenuated the ox-LDL-induced foam cell formation mainly by promoting cholesterol efflux and inhibiting inflammatory response, and the protective effect of Gypenoside XLIX against cardiovascular disease was involved in the inhibition of cytokine-induced vascular cell adhesion molecule-1 expression.35,36) Gypenoside XVII prevented atherosclerosis by attenuating endothelial apoptosis and oxidative stress, and alleviated atherosclerosis via the Erk-mediated phosphatidylinositol 3-kinase (PI3K)/Akt pathway. However, there are no reports on the beneficial effects of G. pentaphyllum saponins with prophylaxis and treatment of vascular diseases and liver damage resulted from the intake of high dietary choline.

In this study, TSGP as saponins-enriched fraction was extracted from G. pentaphyllum and subsequently separated with D101 macroporous resin column chromatography. The total saponins content determined using the method of vanillin columnometry was up to 83.0% in TSGP, indicating that the non-saponin compounds occupied 17.0%, which was coincident with previous studies regarding that G. pentaphyllum contained saponins, flavonoids, polysaccharide, sterol and other chemical compositions. In this regard, the 17.0% non-saponin compounds derived from nonpolar D101 macroporous resin column might be sterol, flavonoids and other chemical ingredients, but the significantly lower concentration of flavonoids and sterols in TSGP was possibly insufficient to show biological activities, relative the saponins-enriched fraction, although sterol and flavonoids was approved to exert the pro-
tective effects against atherosclerosis and other benefits. Furthermore, antioxidant activities of TSGP were verified by DPPH, O2−, HO− and ferric reducing ability of plasma, indicating that TSGP had a significant antioxidant function (Fig. 1). Moreover, the mice receiving HC water showed the obvious vascular and liver damage by oxidative injury and lipid peroxidation, which was in accordance with the recent researches. Besides, it was of interesting that TSGP exhibited the high protective effect against hypercholine-induced the vascular and liver injury in mice.

It is widely recognized that endothelial cells exist on the internal surface of blood vessel to control the balance between vasodilation and vasoconstriction for holding vasal tension and structure equilibrium. However, when suffered oxidative stress and inflammation, the endothelium undergoes structural and functional changes and finally loses its protective role, which is known as endothelial dysfunction. Endothelial dysfunction is characterized by decreasing the NO secretion from eNOS, which plays a key role in protecting the vascular injury, and releasing constrictor molecules such as ET-1 and TXA2, resulting in an increase in vascular permeability. In our hands, feeding of mice given 3% HC water remarkably resulted in the reduction in the NO and eNOS levels (p<0.01), demonstrating that vascular damage occurred in HC-treated mice. However, following the mouse ingestion of TSGP for 8 weeks, the eNOS and NO levels were sharply enhanced in HC-fed mice, respectively (Fig. 2), indicating that TSGP might effectually protect endothelial damage by promoting the NO synthesis. On the other hand, ET-1 has been distinguished as the most potent endogenous vasoconstrictor and a mitogen in vascular smooth muscle cells. In our hands, ET-1 levels were significantly elevated in the model mice, but the administration of TSGP remarkably antagonized the increase in ET-1 levels (Fig. 2C). NO, PGI2 and ET-1 as vascular mediators can maintain the relaxation of vascular tension and a certain degree of oxidative injury. Herein, TSGP was demonstrated to effectively elevate the NO level and inhibit the ET-1 release, suggesting its protection against vascular dysfunction.

TXA2 and PGI2 are two main prostaglandins for coordinating cardiovascular system homeostasis, which are mainly produced by thrombocytes and vasal endothelial cells. TXA2 can cause platelet activation and vascular smooth muscle contraction, which is blocked in high cardiovascular risk patients, while PGI2 is the most effective endogenous vascular protector and plays a crucial role in the prevention of atherosclerosis and other cardiovascular diseases. Our results clearly demonstrated that after ingestion of HC water for consecutive 8 weeks, circulating TXA2 concentration was obviously enhanced, while PGI2 level was dramatically decreased as compared to the normal mice (Fig. 2), indicating that the vascular system homeostasis injury occurred by exposure of 3% dietary choline to the tested mice. However, management with TSGP could restrain the elevation in the serum TXA2 levels and the decline in the serum PGI2 levels. In addition, histopathological examination indicated that the aortic vascular lesion was significantly remitted after TSGP treatment (Fig. 3A), and the intima-media/lumen ratio of mouse pectoral aorta and pty of the tunica media of the main artery were reduced (Fig. 3B) in comparison with HC intake in mice. All the alterations indicated that TSGP has a beneficial effect on the damaged endothelium.

Dyslipidaemia is one of the risk factors of CVD, accompanied by chronic liver disease, and systemic lipid disorder involved in abnormal TG, TC and LDL-C/HDL-C alteration was used to estimate cardiovascular diseases and atherosclerosis process. As showed in Table 2, the elevation of the serum TG, TC and AI (TC/HDL-C) was observed after HC feeding in mice, showing that high dietary choline consumption caused circulating lipid disorders. However, the blood lipid abnormality was ameliorated following TSGP management, indicating that TSGP obviously improved the lipid disorders to protect cardiovascular and hepatic function. The oil red O-stained liver tissues in mice further demonstrated that...
TSGP could preserve the liver from HC-induced histopathological change (Fig. 6).

In spite of this, we also evaluated the oxidative stress injury of the liver in HC-fed mice. AST and ALT are viewed as significantly biochemical indexes for indicating inchoate liver damage since these enzymes may seep into the bloodstream from the liver tissue when hepatic cell structure is damaged. Herein, the AST and ALT activities elevated through feeding of mice with 3% HC. However, administration of TSGP could obviously reduce the elevation in AST and ALT activities of HC feeding mice (Fig. 4), suggesting the protective effects of TSGP by conservation of the integrated structure of the hepatocellular membrane. Production of endogenous ROS as oxidative stress molecules is well known to cause endothelial dysfunction or/and liver damage through covalent binding and lipid peroxidation. However, the free radicals-scavenging enzymes of SOD and GSH-Px can exert protective effects on oxidative stress and display the significant effect in ROS detoxification. Meanwhile, MDA is a mediator of lipid peroxidation. In our work, tissue injuries and failure of antioxidant defense system to prevent the formation of excessive ROS could be reflected in the decreased GSH-Px and T-SOD activities and the increased MDA formation in HC feeding mice. However, treatment with TSGP had a significant improvement in mitigating the damage status, indicating that TSGP might effectually protect the hepatic cells against the oxidative toxic effects to HC feeding in mice (Fig. 4). Moreover, the result from the H&E staining assay for the mouse liver histological section was in accordance with biochemical parameters mentioned earlier. This is the first research with the precise demonstration that TSGP may inhibit the high choline diet-oriented hepatic oxidative damage in mice, and our discoveries offered a novel perception to the benefit usage of TSGP.

CONCLUSION

The results presented in this study demonstrated that the ingestion of dietary high choline was linked to vascular and liver disruption in mice, and TSGP exerted the significantly beneficial effect against the HC-induced vascular and hepatic oxidative stress damage. All these findings provided great potentials for the use of TSGP as a promising functional food and medicine in the prevention or therapy of vascular and liver disorders.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Wang Z, Kliffell E, Bennett BJ, Koeth R, Levison BS, DuGar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Luais AJ, Hazen SL. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature, 472, 57–63 (2011).
2) Alejandra MW, Roger AD, Timothy JG, Xu ZM, Susan IB, Sheila MI, David DK. Variations in plasma choline and metabolite concentrations in healthy adults. Clin. Biochem., 60, 77–83 (2018).
3) Wang Z, Tang WH, Buffa JA, Fu XM, Britt EB, Koeth RA, Levison BS, Fan YY, Wu YP, Hazen SL. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. Eur. Heart J., 35, 904–910 (2014).
4) Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat. Med., 19, 576–585 (2013).
5) Anwar S, Bhandari U, Panda BP, Dubey K, Khan W, Ahmad S. Trigonelline inhibits intestinal microbial metabolism of choline and its associated cardiovascular risk. J. Pharm. Biomed. Anal., 159, 100–112 (2018).
6) Kang JS, Lee WK, Lee CW, Yoon WK, Kim N, Park SK, Lee HS, Park HK, Han SB, Yun J, Lee K, Lee KH, Park SK, Kim HM. Improvement of high-fat diet-induced obesity by a mixture of red grape extract, soy isoflavone and L-carnitine. Implications in cardiovascular and non-alcoholic fatty liver diseases. Food Chem. Toxicol., 49, 2453–2458 (2011).
7) Ottiger M, Nickler M, Steuer C, Bernasconi L, Hubar A, Christ-Crain M, Henzen C, Hoes H, Thormann R, Zimmerli W, Mueller B, Schuetz P, Gut, microbiota-dependent trimethylamine-N-oxide is associated with long-term all-cause mortality in patients with exacerbated chronic obstructive pulmonary disease. J. Nutr., 45, 135–141 (2018).
8) Jia MF, Ren DY, Nie Y, Yang XB. Beneficial effects of apple peel polyphenols on vascular endothelial dysfunction and liver injury in high choline-fed mice. Food Funct., 8, 1282–1292 (2017).
9) Li D, Ren DY, Luo YY, Yang XB. Protective effects of ursoic acid against hepatotoxicity and endothelial dysfunction in mice with chronic high choline diet consumption. Chem. Biol. Interact., 258, 102–107 (2016).
10) Ren DY, Liu YF, Zhao Y, Yang XB. Hepatotoxicity and endothelial dysfunction induced by high choline diet and the protective effects of phloretin in mice. Food Chem. Toxicol., 94, 203–212 (2016).
11) Wu PK, Tai WCS, Choi RCY, Tsim KWK, Zhou H, Liu X, Jiang ZH, Wendy HWL. Chemical and DNA authentication of taste variants of Gynostemma penthaphyllum herbal tea. Food Chem., 128, 70–80 (2011).
12) Bai MS, Gao JM, Fan C, Yang SX, Zhang G, Zheng CD. Bioactive dammarane-type triterpenoids derived from the acid hydrolysate of Gynostemma penthaphyllum saponins. Food Chem., 119, 306–310 (2010).
13) Ly T, Yang XB, Zhao Y, Ruan Y, Yang Y, Wang ZZ. Separation and quantification of component monosaccharides of the tea poly saccharides from Gynostemma penthaphyllum by HPLC with indirect UV detection. Food Chem., 112, 742–746 (2009).
14) Sunantaruk S, Yoonan N, Panagkird N, Woraasuttayangkurn L, Nookabkhaew S, Satayavivad J. Immunomodulatory effects of cadmium and Gynostemma penthaphyllum herbal tea on rat splenocyte proliferation. J. Agric. Food Chem., 56, 9305–9311 (2008).
15) Xie ZH, Huang HQ, Zhao Y, Shi HM, Wang SK, Wang TY, Chen P, Yu LL. Chemical composition and anti-proliferative and anti-inflammatory effects of the leaf and whole-plant samples of diploid and tetraploid Gynostemma penthaphyllum (Thunb.) Makino. Food Chem., 132, 125–133 (2012).
16) Chen MH, Wang QF, Chen LG, Shee JJ, Chen JC, Chen KY, Chen SH, Su JJ, Liu YW. The inhibitory effect of Gynostemma
pentaphyllum on MCP-1 and type I procollagen expression in rat hepatic stellate cells. J. Ethnopharmacol., 126, 42–49 (2009).

17) Cheok CY, Salanik SA, Sulaiman R. Extraction and quantification of saponins: a review. Food Res. Int., 59, 16–40 (2014).

18) Yan J, Wu ZL, Zhao YL, Jiang CS. Separation of tea saponin by two-stage foaming fractionation. Separ. Purif. Technol., 80, 300–305 (2011).

19) Zhou CS, Xiao WJ, Tan ZL, Salem AZM, Geng MM, Tang SX, Wang M, Han XF, Kang JH. Effects of dietary supplementation of tea saponins (Ixodes kudingcha C. J. Tseng) on ruminal fermentation, digestibility and plasma antioxidant parameters in goats. Anim. Feed Sci. Technol., 176, 163–169 (2012).

20) Kao TH, Huang SC, Stephen Inbaraj B, Chen BH. Determination of flavonoids and saponins in Gynostemma pentaphyllum (Thunb.) Makino by liquid chromatography-mass spectrometry. Anal. Chim. Acta, 626, 206–211 (2008).

21) He NW, Zhao Y, Guo L, Shang J, Yang XB. Antioxidant, anti-inflammatory, and pro-apoptotic activities of a saponin extract from the roots of panax notoginseng Burk. J. Med. Food, 15, 350–359 (2012).

22) Wang J, Cheung W, Leung D. Determination of pesticide residue transfer rates (percent) from dried tea leaves to brewed tea. J. Agric. Food Chem., 62, 966–983 (2014).

23) Zhao Y, Xie ZH, Nie YG, Shi HM, Chen P, Yu LL. Chemical compositions, HPLC/MS fingerprinting profiles and radical scavenging properties of commercial Gynostemma pentaphyllum (Thunb.) Makino samples. Food Chem., 134, 180–188 (2012).

24) Lu XS, Zhao Y, Sun YF, Yang S, Yang XB. Characterisation of polysaccharides from green tea of Huangshan Maofeng with antioxidant and hepatoprotective effects. Food Chem., 141, 3415–3425 (2013).

25) Zhang RJ, Zhao Y, Sun YF, Lu XS, Yang XB. Isolation, characterisation, and hepatoprotective effects of the raffinose family oligosaccharides from Rehmannia glutinosa Libosch. J. Agric. Food Chem., 61, 7786–7793 (2013).

26) Weng Y, Yu L, Cui J, Zhu YR, Guo C, Wei G, Duan JL, Yin Y, Guan Y, Wang YH, Yang ZF, Xi MM, Wen AD. Antihyperglycaemic, hypolipidemic and antioxidant activities of total saponins extracted from Aralia taibaiensis in experimental type 2 diabetic rats. J. Ethnopharmacol., 152, 553–560 (2014).

27) Fernández-Musoles R, Salom JB, Martínez-Maqueda D, López-Díez JJ, Recio I, Manzanares P. Antihypertensive effects of black currant Anthocyanins on the activation of endothelial nitric oxide synthase (eNOS) in vitro in human endothelial cells. J. Agric. Food Chem., 59, 8616–8624 (2011).

28) Hsu WH, Lee BH, Lu H, Pan TM, Akanalavim and monosaccharide degradation of endothelial adhesion molecules and endothelial NO synthase (eNOS) expression induced by tumor necrosis factor-α (TNF-α) and high choline-fed mice. J. Agric. Food Chem., 60, 1666–1672 (2012).

29) Yuhki K, Kojima F, Kashiwagi H, Kawanishi J, Fujino T, Naramiya S, Ishikawa S, Kikuchi H. Roles of prostanoids in the pathogenesis of cardiovascular diseases: Novel insights from knockout mouse studies. Pharmacol. Ther., 129, 195–205 (2011).

30) Xiao HF, Xie G, Wang JW, Hou XF, Wang X, Wu WQ, Liu XB. Cholic acid pretreatment of obesity by attenuating hepatic steatosis, inflammation and oxidative stress in high-fat diet-fed mice. Food Res. Int., 54, 345–353 (2013).

31) Zou W, Buffa JA, Wang Z, Warrier M, Schugar R, Shih DM, Gupta N, Gregory JC, Org E, Fu X, Li L, DiDonato JA, Lusis AJ, Brown JM, Hazen SL. Flavon monoxygenase 3, the host hepatic enzyme in the metaorganismal trimethylamine N-oxide-generating pathway, modulates platelet responsiveness and thrombosis risk. J. Thromb. Haemost., 16, 1857–1872 (2018).

32) Wu XY, Chen LJ, Zeb F, Huang YZ, Jing A, Ren JI, Yang F, Feng Q. Regulation of circadian rhythms by NEAT1 mediated endothelial proliferation: A protective role of asparagin. Exp. Cell Res., 382, 111451 (2019).

33) Chen PY, Li SM, Koh YC, Wu JC, Yang MJ, Ho CT, Pan MH. Oolong tea extract and citrus peel polymethoxyflavones reduce transformation of L-carnitine to trimethylamine-N-oxide and decrease vascular inflammation in L-carnitine feeding mice. J. Agric. Food Chem., 67, 7869–7879 (2019).

34) Shen CY, Jiang JG, Huang LH, Wei Z. Gyneposides LVI attenuates foam cell formation by promoting cholesterol export and inhibiting inflammation response. J. Funct. Foods, 59, 71–77 (2018).

35) Huang TH, Tran VH, Roufogalis BD, Li YH. Gyneposides XLIX, a naturally occurring PPAR-α activator, inhibits cytokine-induced vascular cell adhesion molecule-1 expression and activity in human endothelial cells. Eur. J. Pharmacol., 565, 158–165 (2007).

36) Yang K, Zhang H, Luo Y, Zhang J, Wang M, Liao P, Cao L, Guo F, Sun G, Sun X. Gyneposide XVII prevents atherosclerosis by attenuating endothelial apoptosis and oxidative stress: Insight into the ERK-mediated PI3K/Akt pathway. Int. J. Mol. Sci., 18, 97–105 (2017).

37) Zhang Q, Lei ZN, Li XY, Yan XY, Wang SY, Yang WL, Nie QH, Zhang Q, Bao H, Yu JY, Jin HG. Characterization and identification of the chemical constituents of Gynostemma pentaphyllum using high performance liquid chromatography-electrospray ionization – quadrupole time-of-flight tandem mass spectrometry (HPLC-ESI-QTOF-MS/MS). Anal. Lett., 41, 1–14 (2019).

38) Wang ZC, Gao S, Liu XY, Chen PZ, Lu WB, Yang FZ, Li HF, Sun Q, Zhang H. Efficient production of polysaccharide by Chaetomium globosum CGMCC 6882 through co-culture with host plant Gynostemma pentaphyllum. Bioprocess Biosyst. Eng., 42, 1731–1738 (2019).

39) Marino A, Elberti M, Cataldo A. Sterols from Gynostemma pentaphyllum. Biol. Soc. Ital. Biol. Spec., 65, 314–319 (1989).

40) Kaneko T, Baba N. Protective effect of flavonoids on endothelial cells against linoleic acid hydroperoxide-induced toxicity. Bioch. Biotechnol. Biochem., 63, 323–328 (1999).

41) Jia MF, Ren DY, Nie Y, Yang XB. Beneficial effects of apple polyphenols on vascular endothelial dysfunction and liver injury in high choline-fed mice. Food Funct., 8, 1282–1292 (2017).

42) Shimizu K, Sato M, Zhang YZ, Kouguchi T, Takahata Y, Morimitsu F, Shimizu M. The bioavailable octapetide Gly-Ala-Hyp-Gly-Leu-Hyp-Gly-Pro stimulates nitric oxide synthesis in vascular endothelial cells. J. Agric. Food Chem., 58, 6960–6965 (2010).

43) Wilbert-Lampen U, Trapp A, Modrzik M, Fiedler B, Straube F, Plass A. Effects of corticosteroid-releasing hormone (CRH) on endothelin-1 and NO release, mediated by CRH receptor subtype 2: A potential link between stress and endothelial dysfunction? J. Psychosom. Res., 61, 453–460 (2006).

44) Russo G, Leopold JA, Loscalzo J. Vasoactive substances: nitric oxide and endothelial dysfunction in atherosclerosis. Vascul. Pharmacol., 38, 259–269 (2002).

45) Sittia S, Tomasoni L, Atzeni F, Ambrosio G, Cordiano C, Catapano A, Tramontana S, Perticone F, Naccarato P, Camici P, Picano E, Cortigiani L, Bevilacqua M, Milazzo L, Cusi D, Barlassina C, Arlott P, Pisani P, Tramer S. Resistance of human endothelial cells to aortic thrombosis by flavonoids: antioxidant properties of pyridostatin. Rev. Physiol. Biochem. Pharmacol., 141, 235–321 (2010).

46) Cherdon C, Robin S, Hanson J, Ooms A, Level L, Drion P, Michels C, Pirotte B, Masereel B, Sakalihassan N, Defraigne JO, Dogné JM. 59, 61–74 (2014).

47) Navarro-Nunez L, Castillon J, Lozano ML, Martinez C, Benavente-Garcia O, Vicente V, Rivera J. Thrombaxone A2 receptor antagonism by flavonoids: structure–activity relationships. J. Agric. Food Chem., 66, 2205–2212 (2018).

48) Cortigiani L, Bevilacqua M, Milazzo L, Cusi D, Barlassina C, A, Tramontana S, Perticone F, Naccarato P, Camici P, Picano E, Cortigiani L, Bevilacqua M, Milazzo L, Cusi D, Barlassina C, Arlott P, Pisani P, Tramer S. Resistance of human endothelial cells to aortic thrombosis by flavonoids: antioxidant properties of pyridostatin. Rev. Physiol. Biochem. Pharmacol., 141, 235–321 (2010).
Mohite A, Chillar A, So SP, Cervantes V, Ruan KH. Novel mechanism of the vascular protector prostacyclin: regulating microRNA expression. Biochemistry, 50, 1691–1699 (2011).

Chatrath H, Vuppalanchi R, Chalasani N. Dyslipidemia in patients with nonalcoholic fatty liver disease. Semin. Liver Dis., 32, 22–29 (2012).

Scicchitano P, Cameli M, Maiello M, Modesti PA, Muiesan ML, Novo S, Palmiero P, Saba PS, Pedrinelli R, Ciccone MM. Nutraceuticals and dyslipidaemia: Beyond the common therapeutics. J. Funct. Foods., 6, 11–32 (2014).

Zhang X, Wu ZF, Weng PF. Antioxidant and hepatoprotective effect of (−)-eigallocatechin 3-O-(3-O-methyl) gallate (EGCG3Me) from Chinese Oolong Tea. J. Agric. Food Chem., 62, 10046–10054 (2014).

Liu Q, Tian GT, Yan H, Geng XR, Cao QP, Wang HX, Ng TB. Characterization of polysaccharides with antioxidant and hepatoprotective activities from the wild edible mushroom Russula vinosa lindblad. J. Agric. Food Chem., 62, 8858–8866 (2014).

Li WX, Li YF, Zhai YJ, Chen WM, Kurihara H, He RR. Theacrine, a purine alkaloid obtained from camellia assamica var. kucha, attenuates restraint stress-provoked liver damage in mice. J. Agric. Food Chem., 61, 6328–6335 (2013).

Yang XS, Dong C, Ren GX. Effect of soyasaponins-rich extract from soybean on acute alcohol-induced hepatotoxicity in mice. J. Agric. Food Chem., 59, 1138–1144 (2011).

Lee TT, Ciou JY, Chiang CJ, Chao YP, Yu B. Effect of Pleurotus eryngii Stalk residue on the oxidative status and meat quality of broiler chickens. J. Agric. Food Chem., 60, 11157–11163 (2012).