Stachyose increases absorption and hepatoprotective effect of tea polyphenols in high fructose-fed mice

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Scope: The consumption of tea polyphenols (TP) and stachyose contributes to preventive effects on hepatic injury. This study examined the effects of stachyose on absorption and hepatoprotective effects of TP in mice.

Methods and results: GC-MS measurement showed that stachyose significantly increased serum total phenolic, ECG and EGCG contents in mice. The mice fed with high fructose (HF)-diet for 56 days exhibited oxidative stress observed by an increase in hepatic MDA levels and decreases in GSH-Px and SOD activities. Serum TC, TG, LDL-C and CRP levels, and ALT and AST activities were increased, while HDL-C concentrations were decreased following HF diet. Co-supplementation of stachyose and TP more effectively improved all parameters mentioned above when compared to administration of stachyose or TP alone. Histological observations of hepatic tissues also confirmed the beneficial effects of co-administration of stachyose and TP.

Conclusion: Our results suggest that stachyose enhances absorption and hepatoprotective effects of TP, and combined ingestion of stachyose and TP is a novel strategy for alleviating HF diet-induced hepatic injury.

Keywords: Absorption / High fructose / Liver damage / Stachyose / Tea polyphenols

1 Introduction

Fructose has been an important part of the human diet for many thousands of years, and its consumption has increased over the past decades due to the fact that high-fructose corn syrup (HFCS) as a cost-effective sweetener is widely used in food industry [1, 2]. Meanwhile, over 10% daily calories for a large number of children and adults come from the added sweetener HFCS, which contains 55–90% fructose [3]. Given the substantial participation of HFCS into food industry and the increase of its consumption in our everyday diet, obesity has increased to epidemic levels [4, 5]. Previous studies have documented that high-fructose (HF) diet can cause the increases of body weight, hepatic steatosis, intrinsinc antioxidant defense disorder, hypohepatia and depletion of hepatocyte population [5,6]. Besides, HF diet also results in cholesterol and lipid dysregulation, which is associated with the development of a few types of liver diseases [7]. Accordingly, effective preventive strategy of HF diet-induced liver injury needs to be established urgently.

Diet plays a significant role in the prevention of metabolic syndrome induced by HF [8]. Interestingly, fruits and vegetables have been used for prevention of degenerative disease which is caused by dietary structure imbalance. Functional oligosaccharides and flavonoids are widely distributed in the plant kingdom, and have a plenty of nutritional effects. Among functional oligosaccharides, stachyose is one of the most abundant phytochemicals in Soybean (Glycine max L.), Rehmannia glutinosa Libosch and Lycopus lucidus Turcz [9–11], which are widely used as raw material of stachyose in China. Our previous study suggests that stachyose-enriched extract can ameliorate CCl₄-induced abnormal hepatic lipid metabolism, damage of antioxidant defense system and depletion of hepatocyte population [11]. Similarly, catechin is one of the most abundant flavonoids in tea, and catechin supplementation has been shown to attenuate HF diet-induced liver oxidative stress and hepatic

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSTFA, N,O-bis(trimethylsilyl)trifluoroacetamide; CA, caffeic acid; CRP, C-reactive protein; ECG, epicatechin gallate; EC, epicatechin; EGC, epigallocatechin; EGCg, epigallocatechin gallate; FI, fat index; GC-MS, gas chromatography-mass spectrometry; GSH-Px, glutathione peroxidase; HDL-C, high-density lipoprotein-cholesterol; HF, high fructose; LDL-C, low-density lipoprotein-cholesterol; MDA, malonaldehyde; NAFLD, non-alcoholic fatty liver disease; SOD, superoxide dismutase; TC, total cholesterol; TG, total triglyceride; TP, tea polyphenols

Colour online: See the article online to view Figs. 1 and 2 in colour.
In addition, tea polyphenols have also been approved to improve insulin sensitivity, suggesting that their supplementation may help cope with the metabolic stress caused by unhealthy dietary patterns [8]. However, there is very low absorption in the body by oral administration of epigallocatechin gallate (EGCG), which is the most abundant catechin in green tea with strong physiological activities [13]. Interestingly, some nondigestible saccharides, such as fructo-oligosaccharides, have been recently shown to enhance bioavailability of genistein, daidzein, α-G-rutin and α-glucosyl isoquercitrin, and difructose anhydride III promotes absorption of α-G-rutin [14–18]. These findings suggest that tea polyphenols (TP) and stachyose together may have positive synergistic effects to reduce liver dysfunction. Therefore, in current study we used GC-MS to investigate the efficacy of stachyose in promoting absorption of TP in mice. Furthermore, liver protective effect of administration of stachyose or TP or in combination was also studied in HF-fed mice with the purpose to confirm whether stachyose could enhance the effect of TP in HF-fed mice.

2 Materials and methods

2.1 Materials and chemicals

The stachyose (pure>80%) was extracted from soybean residue, which is byproduct of soymilk. TP (pure>70%) consists of epigallocatechin gallate (EGCG, 522.36 mg/g), epicatechin gallate (ECG, 117.40 mg/g), epigallocatechin (EGC, 40.46 mg/g), epicatechin (EC, 31.06 mg/g) and caffeic acid (CA, 7.02 mg/g) identified by GC-MS analysis (Fig. 1A, B) and was purchased from the Yongqi Biology Co., Ltd. Food grade fructose was purchased from Senbo Biology Co., Ltd. (Xi’an, China). Pure standards of CA (98%), catechins (98%) and genistein (98%) were obtained from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany). N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Adamas Reagent, Ltd. (Shanghai, China). β-Glucuronidase (G0251-100KU, EC 3.2.1.31) and sulfatase (S9626-10KU, EC 3.1.6.1) were purchased from Sigma-Aldrich China (Shanghai, China). Assay kits of total cholesterol (TC), total triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were purchased from Huili Biotechnology Co. Ltd. (Changchun, China). Detection kits of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malonaldehyde (MDA) were obtained from Jiancheng Bioengineering Institute (Nanjing, China). ELISA kits of C-reactive protein (CRP) were also purchased from Jiancheng Bioengineering Institute (Nanjing, China).

2.2 Animals and experiment design

Male Kunming mice (weight 18–22 g) were purchased from the Experimental Animal Center of the Fourth Military Medical University. They were housed at ambient temperature (22 ± 2°C) in a 12-h light/dark cycle and a minimum relative humidity of 60 ± 5%. They were allowed free access to tap water and a standard rodent chow which was also purchased from the Experimental Animal Center of the Fourth Military Medical University. One week after acclimatization to laboratory environment, the mice were divided randomly into five groups with eight mice each. Group N: a normal group, in which the mice received daily distilled water and a standard diet, and were administered intragastrically (ig, 0.4 mL) with physiological saline during the experimental period once daily. Group M: high-fructose model group of the mice receiving 30% high-fructose water and daily physiological saline (ig, 0.4 mL) by gavage. Group S: stachyose-treated group of the mice receiving 30% high-fructose water and being administered with stachyose at 400 mg/kg-bw (ig, 0.4 mL). Group TP: the mice received 30% high-fructose water and were administered with TP at 400 mg/kg-bw (tea polyphenol-treated group, ig, 0.4 mL). This dose in mice is approximately equivalent to 62 g dry tea/day for a 70 kg-person. Group STP: stachyose and tea polyphenol-treated group, where the mice received 30% high-fructose water and were administered with mixed solution including stachyose and TP at 400 mg/kg-bw, respectively (ig, 0.4 mL). During the experimental period, mice were allowed free access to food and tap water or 30% fructose water. All administrations were conducted for consecutive 8 weeks, and tap water and 30% fructose water were renewed every other day and the body weight of all the groups was measured once a week. Additionally, food and water intake was also recorded twice per week. All the animals were fully anesthetized by the inhalation of isoflurane and weighed, and then sacrificed to obtain blood, livers and intra-abdominal fats after fasting for 12 h at the end of the last administration. The experimental procedure was carried out according to the Guidelines of Experimental Animal Administration published by the State Committee of Science and Technology of People’s Republic of China, and the experimental protocol used in this study was approved by the Committee on Care and Use of Laboratory Animals of the Fourth Military Medical University (SYXK-007-2007), China.

2.3 Determination of serum catechins contents

Serum catechins contents were tested by GC-MS with a modified version of previously described method [19,20]. One hundred and fifty μL serum was thawed and an additional 5 μL phosphate-buffered ascorbic acid (200 mg/mL vitamin C and 62.5 mg/mL NaH₂PO₄) was added, and then 5 μL internal standard (genistein, 38.33 μg/mL) and 50 μL 66.59 mg/mL CaCl₂ were added. Serum was incubated at 37°C in water bath for 60 min containing 100 U sulfatase and 2500 U β-glucuronidase dissolved in 200 μL water. After incubation, the serum was extracted twice with ethyl acetate (1.0 mL each time). The combined ethyl acetate extract was vacuum dried at
Figure 1. The GC-MS chromatograms of silane derivatives of authentic polyphenolic standard compounds (A) and the component polyphenolic compounds of the tested commercial tea polyphenols preparation (TP, B). Effects of stachyose on serum total polyphenols contents (C), CA (D), EC (E), EGC (F), ECG (G) and EGCG (H) concentrations in 30% fructose water-fed mice for consecutive 8 weeks. Values are expressed as means ± SD of 8 mice in each group.
45°C (Rikakikai Co., Ltd, Tokyo, Japan), and subsequently re-
dissolved in 40 μL pyridine and derivatized with 60 μL BSTFA
at 70°C for 2 h. The 1 μL of derivatized extract was injected for
GC-MS analysis. The chemical composition of purchased TP
was determined by GC-MS after derivative according to the
method mentioned above.

The GC-MS analysis of TMS derivatives was performed on an
Rxi-5Sil MS Cap. column (30 m × 0.25 mm id, 0.25 μm
film thickness, Risetech Technology Co., Ltd, Beijing, China).
Helium was used at 1.0 mL/min, and the column tempera-
ture was kept at 70°C for 4 min, and increased at 4 °C/min
to 100°C where it was held for 3 min, and then increased at
30 °C/min to 260°C where it was held for 20 min and fur-
ther increased at 10 °C/min to 310°C where it remained for
30 min. Major fragmentation ions and molecular ions for CA
(m/z = 396), EC (m/z = 368), EGC (m/z = 456), ECG (m/z =
560), EGCG (m/z = 648) and IS (m/z = 471) were monitored
in selective ion monitoring mode.

2.4 Serum and hepatic biochemical assays

The samples of blood were centrifuged at 1500 × g for 15 min
and stored at 4°C for further analysis. Serum TC, TG, HDL-C,
LDL-C, AST, ALT and CRP levels were estimated by corre-
spending commercially available diagnostic kits, and the re-
results were expressed in mmol/L, mmol/L, mmol/L, mmol/L,
U/L, U/L and ng/mL, respectively. Hepatic tissue (∼0.5 g)
was homogenized in ninefold frozen normal saline in volume
and then centrifuged at 3000 × g for 10 min. The supernatant
was used for the assay of hepatic MDA, T-SOD and GSH-Px
levels, which were performed with commercially available di-
agnostic kits following the manufacturer’s instruction, and the
results were expressed as nmol/mg prot, U/mg prot and
U/mg prot, respectively.

2.5 Histological examination

A portion of the mouse liver from the left lobe was sec-
tioned into blocks and fixed in 4% paraformaldehyde for
histopathological analysis [11, 21]. Fixed tissues were dehy-
drated through a graded series of ethanol and embedded in
paraffin, cut into slices (6 μm) and stained with hematoxylin
eosin (H&E) [11, 21]. For Oil Red O staining, hepatic tis-
sue was processed using cryostat (CM1950, Leika, Germany)
and then fixed and stained [5]. An Olympus light microscope
was employed for observation and photograph.

2.6 Statistical analysis

Results of polyphenol contents, weights of mouse body and
mouse fat, serum and hepatic biochemical parameters were
expressed as means ± SD, and were analyzed with Excel
2013 software. Statistical significance for the results of phenol
contents, serum and hepatic biochemical test results were
determined by one-way analysis of variance followed by Least-
Significant Difference (LSD) test. ANOVA were performed
using SPSS 20 (IBM) and p < 0.05 was considered statistically
significant.

3 Results

3.1 Stachyose enhanced absorption of TP in mice

To evaluate interactions between tea polyphenols (TP) and
stachyose, focusing on absorption and efficacy to increase liver
function, the effects of stachyose on absorption of TP in
HF-fed mice administrated by stachyose or TP alone or in
combination was investigated. Serum phenolic compounds
were detected in sulfate-, glucuronide- and un-conjugated
forms in the mice given experimental diets for 56 days
(Fig. 1C–H). As shown in Fig. 1C, when TP was administrated
with stachyose, serum levels of total polyphenols in
HF-fed mice was significantly higher (p < 0.05) than when
TP was administrated alone. However, circulating concentra-
tion of serum CA (Fig. 1D), an important phenolic acid in
the TP, was not significantly different when TP was taken
alone or together with stachyose. Similarly, serum levels of
EC (Fig. 1E) and EGC (Fig. 1F), two unesterified flavonoids
in gallate-form, were also not prominently increased by ad-
ministration of stachyose+TP as compared with the admin-
istration of TP alone. Interestingly, two primary catechin-
gallates, ECG (p < 0.05, Fig. 1G) and EGCG (p < 0.05,
Fig. 1H) reached noteworthy high concentrations in the
serum of the mice administrated TP together with stachyose
than when TP was administrated alone. These results demon-
strate that supplementation of the stachyose together with TP
strongly increases absorption of TP as catechin-gallates form
in vivo.

3.2 Effects of co-supplementation of stachyose and
TP on body and fat weight

As shown in Table 1, food intake of HF-fed mice was lower
than that of normal diet-fed mice (p < 0.05). Water intake of
HF-fed mice was slightly higher than that of normal diet-fed
mice despite there was no significant difference (p > 0.05),
and food plus fructose intake of mice was similar in HF, and
HF+stachyose and/or TP groups over the whole feeding pe-
period (cumulative intake, p > 0.05). However, feeding of the
tested mice with 30% fructose water for 56 days caused a sig-
ificant increase in body weight (p < 0.05), intra-abdominal
fat weight (p < 0.05) and fat index (FI, p < 0.05) as compared
to the mice fed with vehicle (deionized water). However, this
elevation was not observed (p > 0.05) when TP was ingested
alone for HF-fed mice. In parallel, it was also noted that the
mice administrated with TP caused the remarkable decreases
in body weight, fat weight and FI (p < 0.05). Excitingly, when
stachyose was administrated together with TP in HF-fed mice,
Table 1. Food intake, water intake, body weight, fat weight, fat index and serum lipid profile of mice at the end of week 8

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>M</th>
<th>S</th>
<th>TP</th>
<th>STP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/d)</td>
<td>11.1 ± 2.7a</td>
<td>8.7 ± 1.9b</td>
<td>8.7 ± 2.0b</td>
<td>8.5 ± 1.8b</td>
<td>8.5 ± 1.9b</td>
</tr>
<tr>
<td>Water intake (mL/d)</td>
<td>6.3 ± 2.3a</td>
<td>7.1 ± 2.2b</td>
<td>7.0 ± 2.3a</td>
<td>7.1 ± 2.3a</td>
<td>7.2 ± 2.1a</td>
</tr>
<tr>
<td>Fructose intake (g/day)</td>
<td>—</td>
<td>—</td>
<td>2.1 ± 0.5b</td>
<td>3.6 ± 0.5a</td>
<td>3.7 ± 0.6a</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>37.54 ± 2.19b,c</td>
<td>44.58 ± 4.54a</td>
<td>42.82 ± 3.15b,c</td>
<td>37.13 ± 2.00b,c</td>
<td>34.23 ± 3.79c,d</td>
</tr>
<tr>
<td>Fat weight (g)</td>
<td>0.91 ± 0.28c,d</td>
<td>1.54 ± 0.28a</td>
<td>2.80 ± 0.36b,c</td>
<td>1.00 ± 0.47c,d</td>
<td>0.67 ± 0.31c,d</td>
</tr>
<tr>
<td>Fat Index (FI)</td>
<td>0.023 ± 0.006c,d</td>
<td>0.038 ± 0.006b,c</td>
<td>0.038 ± 0.008a,b,c</td>
<td>0.028 ± 0.012b,c,d</td>
<td>0.019 ± 0.008d</td>
</tr>
<tr>
<td>Serum TC (mmol/L)</td>
<td>3.54 ± 0.46a</td>
<td>4.77 ± 1.12a</td>
<td>4.21 ± 0.37b,c</td>
<td>3.88 ± 1.11b,c,d</td>
<td>2.43 ± 0.83b,c,d</td>
</tr>
<tr>
<td>Serum TG (mmol/L)</td>
<td>1.08 ± 0.12b</td>
<td>1.46 ± 0.13a</td>
<td>1.42 ± 0.15b,d</td>
<td>0.92 ± 0.27b,c,d</td>
<td>0.78 ± 0.21b,c,d</td>
</tr>
<tr>
<td>Serum LDL-C (mmol/L)</td>
<td>1.41 ± 0.15b</td>
<td>1.81 ± 0.23a</td>
<td>1.75 ± 0.42b,c</td>
<td>1.58 ± 0.20b,h</td>
<td>1.40 ± 0.28b,c,d</td>
</tr>
<tr>
<td>Serum HDL-C (mmol/L)</td>
<td>0.30 ± 0.09b,c</td>
<td>0.17 ± 0.08a</td>
<td>0.20 ± 0.08b,c,d</td>
<td>0.24 ± 0.09b,c,d</td>
<td>0.37 ± 0.09c,d</td>
</tr>
</tbody>
</table>

Fat Index: Fat weight (g)/body weight (g)
Values were expressed as means ± SD of eight mice in each group and different letters in the same row indicated significant differences at p < 0.05. N: a normal group; M: high-fructose model group; S: stachyose-treated group of the mice; TP: the mice were administered with TP diet; STP: stachyose and tea polyphenol-treated group mice. a,b,c,d Mean values with different alphabetical letters denote significant differences (p < 0.05) among all the groups.

A significant decrease occurred in the body weight, fat weight and FI (p < 0.05) as compared to HF-fed mice, and the mice administered stachyose or TP alone, respectively. These data indicate that stachyose has the capacity to enhance the efficacy of TP in controlling body weight gain of the mice via inhibiting fat generation.

3.3 Additive effects of stachyose and TP on regulation of lipid homeostasis

Dyslipidemia is a main risk factor for nonalcoholic fatty liver disease (NAFLD), which is generally together with excessive weight gain by long-term feeding of HF diet [4, 22]. As shown in Table 1, continuous 8 weeks ingestion of 30% fructose water in mice prominently led to an increase in serum TC, TG and LDL-C levels by 34.7% (p < 0.05), 35.2% (p < 0.05) and 28.4% (p < 0.05), respectively, whereas the HDL-C concentration was significantly decreased by 43.3% (p < 0.05), which suggested the significant dyslipidemia in mice. Unfortunately, no significant changes in serum TC, TG, LDL-C and HDL-C levels of HF-fed mice treated with stachyose at 400 mg/kg.bw were observed. As depicted in Table 1, the HF-elevated TC and LDL-C levels of the mice treated with TP alone were slightly decreased, and interestingly, this TP administration could significantly reduce TG levels, and elevated HDL-C levels in HF-treated mice, respectively (p < 0.05). Similarly, the administration of stachyose alone less obviously relieved abnormal lipid profile induced by HF diet in mice. However, it is worth noting that HF-induced abnormal lipid profile in mice, including TC, TG, LDL-C and HDL-C levels, was markedly ameliorated by co-administration of stachyose and TP, suggesting that stachyose enhanced the hypolipidemic effect of TP by improving TP absorption in mice.

3.4 Synergistic effects of stachyose and TP on antioxidant capacity in HF-fed mice

A significant increase (p < 0.05) in hepatic MDA levels was observed in the mice exposed to high fructose diet, and administration of stachyose together with TP observably reduced hepatic MDA concentration (p < 0.05, Fig. 2A). It was also found that hepatic MDA levels in HF-fed mice after administration of both stachyose and TP were lower than after only stachyose or TP was administered alone (p < 0.05). Furthermore, as shown in Fig. 2B and C, HF treatment caused a significant decrease in SOD and GSH-Px activities by 7.7% and 20.6%, related to the normal mice, respectively (p < 0.05). Administration of TP or stachyose alone in mice elevated SOD (Fig. 2B) and GSH-Px (Fig. 2C) activities, which was higher than the vehicle-treated mice (p < 0.05). As expected, co-treatment of stachyose and TP together showed clear and sustained increases in hepatic GSH-Px and SOD activities with significantly higher values than other three groups (i.e., model, TP or stachyose).

3.5 Stachyose in combination with TP decreased liver toxicity

The degree of liver injury was usually estimated by serum enzyme activities of ALT and AST [12]. The AST (Fig. 2D) and ALT (Fig. 2E) activities in the serum of HF-treated mice were dramatically increased by 18.14 and 6.34% in comparison with the normal mice, respectively (p < 0.05). As expected, the decreased trend of serum ALT and AST activities was observed for all the protective treatments, and significant changes of AST activities were observed for individual TP and mixed stachyose+TP administration when compared to the HF-fed mice (p < 0.05). In addition, serum ALT activity was also significantly reduced in the mice treated with
Figure 2. Effects of stachyose and tea polyphenols (TP) on hepatic MDA (A), SOD (B) and GSH-Px (C) levels, serum AST (D) and ALT (E) enzymatic activities, and serum CRP levels (F) in the mice fed 30% high-fructose water for consecutive 10 weeks. The effects of stachyose and TP on histopathological changes (G), liver hepatocytes stained with Oil Red O (a, b, c, d and e) and H&E (f, g, h, i and j) in 30% high-fructose water-fed mice (original magnification of 400×). Values of biochemical parameters are expressed as means ± SD of 8 mice in each group. a,b,c,d) Mean values within a figure with different alphabetical letters denote significant differences (p < 0.05) among all the groups.

stachyose+TP, compared to that in model group (p < 0.05). It was found that when co-treatment of stachyose and TP in the HF-fed mice, serum AST activities showed prominent improvement as compared to that in individual TP treated mice (p < 0.05).

Moreover, CRP, a marker of inflammation [23], is commonly used as indicator for organism injury occurred in hepatotoxicity [24], and its serum concentrations were also evaluated in current research and the results were shown in Fig. 2F. Long-term HF diet induced a significant increase of serum CRP level in mice, relative to the untreated normal mice (p < 0.05). However, the treatment with stachyose, TP or stachyose+TP in HF-fed mice caused the remarkable decrease in serum CRP levels (p < 0.05, versus HF-fed mice). Although there was no statistical difference among treatments of stachyose or TP alone and in combination, the administration of stachyose together with TP caused the decrease by 12 and 10% in serum CRP levels, compared to administration of stachyose and TP alone, respectively.
3.6 Histopathological observations of mouse liver

As depicted in Fig. 2G, the histological observations of Oil Red O staining of livers supported the results of biochemists about hepatotoxicity, oxidative stress and dyslipidemia. In comparison with hepatic deposition of lipid droplets of the mouse tissues from the normal mice (Fig. 2Ga), the liver sections in HF-treated mice showed widespread fat accumulation inside the parenchyma cells (Fig. 2Gb). However, as shown in Fig. 2Gb–d, the livers of stachyose or TP alone-treated mice exhibited the significant improvement in fat deposition as compared to that in the mice of model group. Additionally, the situation of hepatic fat accumulation was more obviously improved by administrating a diet of stachyose together with TP than ingestion of individual stachyose or TP diet.

Figure 2Gf–j show photomicrographs of H&E-stained liver specimens. The liver slices of HF-fed mice showed degenerative changes, including ballooning degeneration, granuloma inflammatory disorders and the loss of cellular boundaries. However, protective administration of stachyose or TP alone changed the hepatic lesions caused by HF. Interestingly, the liver of mice treated with stachyose+TP showed near normal appearance with well-preserved cytoplasm, prominent nuclei and legible nucleoli (Fig. 2Gj). Consistent with biochemical tests, histopathological study demonstrated that stachyose combining with TP could more effectively protect liver tissue from HF-induced fatty liver and hepatic damage as compared to the pretreatment of stachyose or TP alone in this study.

4 Discussion

Chronic fructose consumption at high levels contributes to obesity, NAFLD, dyslipidemia, diabetes and cardiovascular diseases [22]. Previous studies have demonstrated that natural antioxidants have potential effects in the treatment and prevention of hepatic injury induced by HF diet via additive, synergistic or antagonistic response in vivo [5, 25]. Stachyose and TP are common in our everyday diet and both have been considered to possess beneficial hepatic effects [11, 12]. To our knowledge, this is first study to report the interaction between stachyose and TP. In this research, the nondigestible stachyose promoted absorption of TP in HF-fed mice. With regard to the interaction between stachyose and TP, our findings show here that stachyose can enhance the absorption of two catechin-gallates, ECG and EGCG after administration of TP, which is similar to previous studies [14–17]. However, absorption of some other phenols in unesterified form was not markedly improved after co-administration of stachyose and TP as compared to treatment with TP alone. The potential mechanism may be due to the fact that nondigestible saccharides suppress degradation of water-soluble flavonoid glycosides [15, 18], and in this regard, the tested catechins in nongallate-esterified form have a lower water solubility than its esterified form, which caused prolonging of absorption of water-soluble catechins as the intact form in intestine. Our results further demonstrated that the co-administration of stachyose and TP markedly ameliorated liver injury induced by HF diet which was better than independent administration of stachyose or TP. This protective effect was associated with the reduced hepatic lipid accumulation and decreased HF-mediated oxidative stress.

Previous report indicated that chronically high consumption of HF contributed to the well-established link with obesity [22]. The characteristics of obesity in rodents caused by HF are an increase of body weight, body fat and dyslipidemia [1, 22]. In agreement with other studies [5, 26, 27], the present study also showed that HF caused elevations of body and fat weights, F1, and serum TC and TG levels in mice. These data once again indicated that long-term feeding of HF caused obesity and hypertriglyceridemia, which was a key factor of NAFLD in early stage. Interestingly, co-administration of stachyose and TP was more effective to reduce body and fat weights, F1, and serum TC and TG levels than independent administration of stachyose or TP in HF-fed mice. In accordance with the report of Bettaiab and colleagues [8], our data also showed that no obvious difference in food or fructose intake was observed in HF water, HF+stachyose, HF+TP and HF+TP+stachyose treated mice, while food intake of normal diet-fed mice was higher than the others (Table 1). It was also found that food plus fructose intake was not different in all the HF-treated mice over the whole feeding period. This finding suggests that the decreases of body and fat weights in all the HF-fed mice is not due to the reduction of fructose intake, and administrations of stachyose or TP or in combination can contribute to the effect. HF-induced dyslipidemia is not only associated with the increases of serum TC, TG and LDL-C levels, but is also relevant to the decrease of HDL-C levels [28]. In fact, in this study HF could induce significant increase and decrease in serum LDL-C and HDL-C levels in mice, respectively. Bettaiab and colleagues have also indicated that epicatechin may mitigate the HF-induced abnormalities of plasma HDL-C and LDL-C levels [8]. Interestingly, our research indicated that stachyose could increase the beneficial effects of TP on serum HDL-C and LDL-C recovery in HF-fed mice.

The “second hit” of NAFLD is proposed to be associated with oxidative stress and lipid peroxidation [4]. Generally, reactive oxygen species (ROS) has a great potential to react with lipids, particularly polyunsaturated fatty acids, which can result in cell membrane damage. Thus, MDA has been widely used as indicator of lipid peroxidation and a marker for the status of oxidative stress which can be markedly elevated after administration of HF in both rodents and humans [4, 29]. Our results showed that the hepatic MDA levels in this mice model were increased as a result of the lipid peroxidation. Previous studies showed that stachyose or TP treatments decreased the generation of MDA in mouse liver [11, 12]. Moreover, two antioxidant enzymes, SOD and GSH-Px, are capable of scavenging ROS and lipid peroxidation products and they play a crucial role in the body’s defense mechanism to prevent oxidative stress [30]. In this work, long-term consumption
of HF diet reduced SOD and GSH-Px activities, suggesting that HF diet weakened defenses of natural antioxidant system in vivo. However, co-administration of stachyose and TP in HF-fed mice more effectively inhibited lipid peroxidation, and increased antioxidant enzyme activities than the treatment with stachyose or TP alone. This synergism effect might be caused by the fact that stachyose could promote the observed absorption of polyphenols (Fig. 1C–H), which could strengthen interactions with biological systems via inhibiting lipid peroxidation [31, 32].

It is well known that ROS may stimulate the release of inflammatory cytokine and amplify the inflammation response to cause further disturbance in liver function and CRP metabolism [23]. It was found that chronic HF administration induced increase of serum CRP level, suggesting that inflammation was caused by HF in mice, which was similar with previous report [27]. Fortunately, stachyose, TP and stachyose+TP treatments inhibited the HF-caused inflammatory response in mice, and the administration of stachyose+TP was more effective than two other treatments. It is also known that an elevation in liver transaminases, ALT and AST, is encountered as a result of hepatocellular damage with necrosis, loss of cell membrane integrity and function [33]. Herein, it was found that HF-fed mice presented a significant increase in serum ALT and AST activities, whereas the increase was effectively attenuated by administration of stachyose together with TP. Although application of individual stachyose or TP in HF-fed mice also reduced the elevation of serum ALT and AST activities when compared to HF-fed mice, stachyose+TP further inhibited the enzymatic activities in mice that was significantly lower than the effect induced by stachyose or TP alone. Furthermore, histological studies also demonstrated the biochemical changes mentioned above. As shown in Fig. 2G, the mice treated with HF exhibited remarkable histopathological changes, but co-supplementation of stachyose and TP resulted in the marked improvement in the hepatic histopathology including lipid deposition, parenchyma dilatation, necrosis and leukocytic infiltration, suggesting that co-administration of stachyose and TP is a valid strategy for hindering development of hepatic oxidative stress damage in mice.

In conclusion, with regard to the interaction between stachyose and TP, we showed here that serum TP, especially in the form of gallic acid esters, was largely increased by stachyose administration on day 56, and stachyose enhanced the hepatoprotective effect of TP, and the combination of stachyose and TP possesses the potential for the management or prevention of HF-induced hepatic injury by reversing dyslipidemia and inhibiting lipid peroxidation in mice. These findings suggest that co-ingestion of nondigestible oligosaccharides and polyphenols as normal diet is a promising potential strategy for managing or reducing the risk of hepatic injury, which can have extensive application in development of healthy food.

W.L. performed the design of the study, carried out the mouse experiments and GC-MS analysis of serum samples, analyzed the data and wrote the manuscript. D.H. and A.G. performed the biochemical analysis of serum and liver samples, carried out histopathological examination of liver tissue, assisted in mouse experiments. X.Y. planned and reviewed all experiments, and reviewed the manuscript. All authors read and approved the final manuscript.

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5 References


