A mix of apple pomace polysaccharide improves mitochondrial function and reduces oxidative stress in the liver of high-fat diet-induced obese mice

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Scope: Apple pomace polysaccharides (APP), a free radical scavenger, is one of the major compounds derived from apple pomace. However, whether APP has beneficial effects on metabolic disorders is still unknown.

Methods and results: In the present study, water-soluble APP was isolated from the pomace of the locally abundant “Qinguan” apple and chemically characterized. Then, APP was orally administrated to high-fat diet (HFD)-induced obese mice. We found that APP significantly reduced HFD-induced body weight gain and ameliorated HFD-induced hepatic metabolic disorders and oxidative stress. In a palmitate-loaded HepG2 cell model, APP protected the cells from palmitate-induced insulin resistance and loss of viability by suppressing mitochondrial reactive oxygen species and rescuing mitochondrial respiratory function.

Conclusion: Our work suggests that APP, a promising bioactive food component, successfully improved obesity-associated hepatic metabolic disorder, most likely though the activation of hepatic mitochondrial function and the suppression of mitochondrial oxidative stress.

Keywords: Apple pomace polysaccharides / Hepatic metabolic disorder / High-fat diet / Mitochondrial function / “Qinguan”

1 Introduction

China is the largest apple juice concentrate producer and supplier in the world, accounting for 60% of the global trade volume [1]. During apple juice processing, one abundant waste product is pomace [2], with approximately 1 million tons produced every year in China. The pomace represents a rich source of potentially bioactive apple ingredients [3], including polyphenols and flavonoids [4]. However, a major class of phytochemicals commonly found in apple pomace is the polysaccharides, which play an important role as free radical scavengers in the prevention of oxidative damage in living organisms and can be exploited as novel antioxidants [5, 6].

Numerous epidemiological studies have indicated that consumption of apple and apple products is correlated with a lower prevalence of chronic diseases and diabetes [7–9], which is believed to be partially due to oxidative stress reduction, weight management, and improvement in insulin resistance [5, 10, 11]. Hepatic metabolic disorder is one of the most common chronic liver diseases in the world [12–15]. It is closely related to the increased frequency of obesity, insulin resistance, nonalcoholic fatty liver disease,

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and diabetes [16, 17]. The unprecedented urbanization of China has led to rapid changes in lifestyle and a consequent increase in the prevalence of metabolic disorder. The Investigation Reports of Chinese Residents Nutrition and Chronic Diseases (2015) revealed that 30.1% of adults and 9.6% of children are overweight, 11.9% of adults and 6.4% of children are obese, 9.7% of adults have type 2 diabetes mellitus, and 25.2% of adults have high blood pressure (http://news.xinhuanet.com/health/2015-06/30/c_127968555.htm). The increasing prevalence of obesity, coupled with type 2 diabetes mellitus and hypertension, has rendered a large proportion of the Chinese population at a risk for developing metabolic disorder in the coming decades. Polysaccharides are a major ingredient in apple pomace, but whether they affect hepatic metabolic disorder remains to be explored. In the present study, we analyzed the monosaccharide profiles of apple pomace polysaccharides (APPs) isolated from “Qinguan” and demonstrated the protective effects of APP against high-fat diet (HFD)-induced hepatic metabolic disorder in mice. We further found that APP improved palmitate-induced mitochondrial dysfunction and insulin resistance in HepG2 cells.

2 Materials and methods

2.1 Materials and chemicals

The apple pomace used in this study was collected from Shanxi Normal University, Xi’an, Shaanxi province, China. Insulin, adiponectin, leptin, and fatty acid synthase (FAS) were measured using commercial ELISA kits according to the manufacturer’s protocols (Shanghai Guduo Biological Technology Co., Ltd., China). Detection kits for total cholesterol (TC), triglycerides (TGs), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), total antioxidant capacity (T-AOC), fungal catalase (CAT), myeloperoxidase (MPO), hexokinase (HK), pyruvate kinase (PK), FAS, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), superoxide dismutase (SOD), glutathione peroxidase, and malondialdehyde (MDA) were from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Oligomycin, FCCP, and antimycin A were from Sigma (St. Louis, MO, USA). Anti-p-AKT and anti-AKT antibodies were purchased from Santa Cruz Biotechnology. All other reagents were purchased from Invitrogen.

2.2 Extraction and purification of APP

Based on our previous report [18], APP was extracted and purified, with some modifications. The apple pomace was dried at 55°C and crushed into a powder (60 mesh) by a disintegrator. The powder was extracted with 95% ethanol (1:10, w/v) and refluxed at 80°C for 8 h to remove impurities and small lipophilic molecules. Subsequently, the powders were dried and extracted with hot water (1:40, w/v) at 90°C for 2 h for three cycles. The combined aqueous extract was concentrated to 25% of the original volume by a rotary vacuum evaporator at 60°C and then centrifuged at 4500 × g for 20 min. The supernatant was collected and precipitated for three cycles by adding five times the volume of 95% ethanol at 4°C for 24 h. After centrifuging, the separated precipitate was completely dissolved in an appropriate volume of water and intensively dialyzed for 3 days against ultrapure water (cutoff Mw 8000 Da) to remove the small molecular compounds. The remaining protein was deproteinized using a freeze-thaw process, which was repeated ten times in a plastic bottle, followed by filtration. The extracts were centrifuged at 3000 × g for 10 min to remove insoluble material, and the supernatant was lyophilized in the freeze-dry apparatus (Sihuan Co., China) to obtain the refined APP, which was brown and fluffy.

2.3 Chemical characterization of the polysaccharides

The monosaccharide composition of APP was analyzed by HPLC as described in Lv et al. [18, 19]. Briefly, 20 mg of APP sample was hydrolyzed with 2 mL of 3 M trifluoroacetic acid at 100°C for 8 h in an ampoule (5 mL) sealed under a nitrogen atmosphere to release constituent monosaccharides, and derivatization was then carried out with 5-Methyl-2-phenyl-1,2-dihydropyrazol-3-one (PMP). The analysis of PMP-labeled monosaccharides was performed using a reversed-phase HPLC column (4.6 μm id × 250 mm, 5 μm, Venusil, USA) on a Shimadzu LC-2010A HPLC system equipped with a UV–Vis detector and a Shimadzu Class-VP 6.1 chromatography workstation (Shimadzu, Japan). The mobile phase A was ACN, and B was 3.3 mM TEA (pH 7.5), with a gradient elution of 95-95-90-90% B by a linear decrease from 0-5-30 min, respectively. The wavelength for UV detection was 250 nm.

2.4 Animals and experimental design

Ninety Kunming mice (weight 18–22 g, half male and half female, and approximately 45 days of age) were provided by the Experimental Animal Center, Lanzhou University (Lanzhou, China). The mice were housed in a temperature (22–28°C) and humidity (60 ± 5%)-controlled animal room and maintained on a 12-h light/12-h dark cycle (light from 06:00 a.m. to 06:00 p.m.) with food and water provided during the experiments. After 1 wk of acclimatization, the mice were randomly distributed into the following five groups: (i) control mice fed a standard chow diet (12% kcal fat content; KEAO, Beijing, China); (ii) mice fed an HFD (45% kcal fat content; KEAO); (iii) mice fed an HFD and administered a daily oral gavage of low-dose APP (200 mg/kg/day); (iv) mice fed an HFD and administered a daily oral gavage of middle-dose APP (400 mg/kg/day); and (v) mice fed an HFD and administered a daily oral gavage of high-dose APP (800 mg/kg/day). After 30 days of feeding, the mice were fasted overnight and...
sacrificed. All procedures were performed in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People’s Republic of China.

2.5 Blood samples and biochemical measurements

At the end of the experimental period, all mice were fully anesthetized by the inhalation of ether, weighed, and then sacrificed to obtain blood and liver samples. The blood samples were centrifuged at 1200 × g for 20 min and stored at 4°C, while the livers were frozen at −80°C. On the basis of body weight and corresponding liver weight of every mouse, we calculated the hepatosomatic index (HI) according to the following formula: 

\[ HI = \frac{\text{liver weight}}{\text{body weight}} \times 100\% \]

The levels of TG, TC, LDL-C, T-AOC, AST, ALT, MDA, LDH, GSH-PX, SOD, CAT, MPO, HK, and PK were analyzed using automated biochemical analyzer (Nanjing Jiancheng Bioengineering Institute, China). Serum levels of insulin, adiponectin, leptin, and FAS were measured using commercial ELISA kits according to the manufacturer’s protocols (Shanghai Guduo Biological Technology Co., Ltd., China).

2.6 HepG2 cell culture

HepG2 cells were cultured in DMEM supplemented with 10% fetal calf serum, 100 U/mL penicillin G sodium, and 100 μg/mL streptomycin sulfate at 37°C in a humidified incubator with 5% CO₂, and the experiments were initiated once the cells reached 70% confluence [20].

2.7 Assessment of cell viability

The effect of APP on the viability of HepG2 cells was analyzed in vitro using MTT assays [21]. The cells were seeded at a density of 5 × 10⁴ cells/mL in 96-well plates and incubated with APP at concentrations of 0, 1, 2, 5, 10, 25, 50, 100, and 200 μg/mL for 24 h. After incubation, 100 μL of MTT-DMEM solution (1:9) was added to each well. The plates were further incubated for 4 h followed by addition of 100 μL of DMSO to each well. The absorbance was measured at 490 nm on the Bio-Rad model 680 Microplate Reader (PA, USA).

2.8 JC-1 assay for mitochondrial membrane potential

HepG2 were cultured at a density of 5 × 10⁴ cells/mL in 96-well plates. After treatment, mitochondrial membrane potential (MMP) was measured with the lipophilic cationic probe 5, 5′, 6, 6′-terachloro-1, 1′, 3, 3′-tetraethyl-imidacarbocyanineiodide (JC-1). Cells were washed with PBS once after JC-1 staining and scanned with a microplate fluorometer (Fluoroskan Ascent, Thermo Fisher Scientific, Inc.) at 488 nm excitation and 538 and 590 nm emission wavelengths to measure green and red JC-1 fluorescence, respectively. The red/green fluorescence intensity ratio reflects the MMP [22].

2.9 Determination of reactive oxygen species

HepG2 were cultured at a density of 2 × 10⁵ cells/mL in six-well plates. After treatment, the intracellular reactive oxygen species (ROS) generation was determined by measuring the fluorescence of 2, 7-dichlorofluorescein diacetate (DCFH-DA). Briefly, DCFH2-DA at a final concentration of 10 μM was incubated with HepG2 cells in serum-free medium for 30 min, and cells were washed twice and collected with PBS. After centrifugation at 1000 × g for 1 min at 4°C, cells were suspended with PBS. Cells were analyzed by flow cytometry (BD Bioscience, Franklin Lakes, NJ, USA) [22].

2.10 Mitochondrial ROS measurement

HepG2 were cultured at a density of 5 × 10⁴ cells/mL in 12-well plates. After treatment, the generation of mitochondrial ROS was determined by the MitosOX Red Mitochondrial Superoxide Indicator (Thermo Fisher, USA), and the mitochondria were assessed with MitoSOX Red for mitochondrial superoxide (Thermo Fisher, USA). Briefly, MitoSOX and MitoTracker Green FM at a final concentration of 10 μM were incubated with HepG2 cells in serum-free medium for 30 min. After washing with PBS, the cells were visualized by confocal microscopy (Zeiss, Jena, Germany).

2.11 Western blot analyses

Samples were lysed with Western and IP lysis buffer (Beyotime). The lysates were homogenized, and the homogenates were centrifuged at 13 000 × g for 15 min at 4°C. The supernatants were collected, and the protein concentrations were determined with Pierce™ BCA protein assay kit (Thermo Fisher, USA). Equal aliquots (20 μg) of the protein were separated by 10% SDS-PAGE, transferred to pure nitrocellulose membranes (PerkinElmer Life Sciences), and blocked with 5% nonfat milk in tris-buffered saline and tween 20 (TBST) buffer. The membranes were incubated with anti-AKT and anti-p-AKT (1:1000) antibodies at room temperature for 1.5 h. Chemiluminescent detection was performed using an ECL Western blotting detection kit (Thermo Fisher, Rockford, IL, USA). The results were analyzed by Quantity One software (Bio-Rad, Shanghai, China) to obtain the optical density ratio of the target proteins relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
Figure 1. The HPLC chromatograms of 13 standard monosaccharides (A) and the component monosaccharides released from APP (B). Peaks: (1) fucose; (2) glucosamine; (3) rhamnose; (4) arabinose; (5) galactosamine; (6) galactose; (7) glucose; (8) xylose; (9) mannose; (10) fructose; (11) ribose; (12) galacturonic acid; and (13) glucuronic acid.

Table 1. The monosaccharides in APP

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>Molar contents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>32.52</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>29.96</td>
</tr>
<tr>
<td>Galactose</td>
<td>20.60</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>7.33</td>
</tr>
<tr>
<td>Xylose</td>
<td>6.61</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.98</td>
</tr>
</tbody>
</table>

2.12 Cell oxygen consumption rate measurement

HepG2 cells were seeded at a density of $1.5 \times 10^4$ cells/mL in XF 24-well microplates (Seahorse Bioscience, Billerica, MA, USA). After treatment, oxygen consumption was measured using extracellular flux analysis (Seahorse Bioscience). The final concentrations of the mitochondrial inhibitors were 1 μM antimycin A, 0.5 μM Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), and 1 μM oligomycin. Basal respiration is the baseline oxygen consumption reading before the compounds are injected. Maximal respiration represents the maximum oxygen consumption rate (OCR) measurement value after the FCCP injection. Spare respiratory capacity is calculated by recording the OCR response to FCCP and dividing that number by the basal respiration to obtain a percentage. After detection, cell protein was calculated, and the OCR was adjusted accordingly.

2.13 Statistical analysis

Data are presented as the mean ± SD. Statistical analysis was performed using one-way ANOVA followed by an LSD post hoc analysis (Graphpad Prism 6, USA). In all comparisons, the level of significance was set at $p < 0.05$.

3 Results

3.1 Chemical characterization of APP

APP was extracted from apple pomace using a multistep purification procedure, including hot-water extraction and repeated ethanol precipitation, and the yield of APP was approximately 5.13% w/w of the dried apple pomace. The total carbohydrate content of APP was determined to be 65.5% by the phenol-H$_2$SO$_4$ assay. In addition, the APP did not react with the Folin–Ciocalteu reagent, suggesting that the small molecular phenolic compounds in APP had been removed by dialysis processing against distilled water in the purification of the macromolecular polysaccharides.

Chromatographic analysis was employed to identify and quantify the major monosaccharides present in APP. As shown in Fig. 1A, 13 PMP-labeled standard monosaccharides were rapidly separated in 32 min. The peaks were identified in the order of fucose, glucosamine, rhamnose, arabinose,
Table 2. Effects of APP on body weight and HI of mice subjected to HFD treatment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0</th>
<th>200</th>
<th>400</th>
<th>800 (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>19.51±0.30</td>
<td>19.62±0.40</td>
<td>20.14±0.20</td>
<td>20.06±0.30</td>
<td>17.69±0.40</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>32.23±0.25</td>
<td>36.70±0.34</td>
<td>33.81±0.43</td>
<td>32.21±0.32</td>
<td>31.88±0.45</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>12.72±0.13</td>
<td>17.08±0.24</td>
<td>13.57±0.23</td>
<td>12.15±0.32</td>
<td>14.19±0.40</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>1621.28</td>
<td>1541.57</td>
<td>1567.89</td>
<td>1583.86</td>
<td>1605.85</td>
</tr>
<tr>
<td>HI (%)</td>
<td>4.61±0.056</td>
<td>5.26±0.078</td>
<td>4.87±0.172</td>
<td>4.71±0.183</td>
<td>4.49±0.014</td>
</tr>
</tbody>
</table>

Date are shown as means ± SD (n = 18).

a) p < 0.05, as compared with the normal group.
b) p < 0.05, as compared with the high-fat group.
c) p < 0.01, as compared with the high-fat group.

galactosamine, galactose, glucose, xylose, mannose, fructose, ribose, galacturonic acid, and glucuronic acid by matching their retention times with those of monosaccharide standards under the same analytical conditions. The monosaccharide compositions of APP are shown in Fig. 1B, and the quantified constituents are shown in Table 1. APP was composed of arabinose, galacturonic acid, galactose, rhamnose, xylose, and glucose with molar percentages of 32.52, 29.96, 20.60, 7.33, 6.61, and 2.98%, respectively.

3.2 The effects of APP administration on body weight and HI in HFD-induced obese mice

An HFD mice model was used to investigate the potential effects of APP on obesity-associated hepatic metabolic disorder. Obesity was induced by the administration of an HFD over a 30 day period. APP was administered by oral gavage at dosages of 200, 400, and 800 mg/kg/day during the HFD treatment. As shown in Table 2, the HFD
significantly \( (p < 0.05) \) increased body weight. The APP treatment effectively reduced body weight (Table 2). Compared with the normal control group, the HFD-treated mice showed an increased HI \( (p < 0.05) \), which could be significantly \( (p < 0.05) \) decreased by pretreatment with APP at the tested doses of 200, 400, and 800 mg/kg/day in a dose-dependent manner.

### 3.3 The effects of APP on serum parameters in HFD-induced obese mice

The HFD-induced obesity model is usually accompanied by hyperlipidemia and impaired sensitivity. Figure 2 shows the serum levels of TC, TG, and LDL-C, which were increased by HFD and effectively blocked by all low-, middle-, and high-dose APP treatments; only high-dose APP did not significantly inhibit the LDL-C induced by the HFD. The HFD-induced decrease in adiponectin and HDL-C levels was significantly restored by all the three APP treatments. In addition, a significantly higher level of fasting insulin was induced by HFD and was effectively blocked by all three doses of APP.

### 3.4 The effects of APP on hepatic injury in HFD-induced obese mice

It is well known that liver damage with the leakage of cellular enzymes into plasma is a sign of hepatic injury [23]. The enzymatic activities of serum ALT, AST, and LDH are considered sensitive indicators of hepatic function [24, 25]. As shown in Fig. 3A–C, the HFD treatment induced acute hepatotoxicity in mice, as indicated by the increases in serum ALT, AST, and LDH levels relative to the control group \( (p < 0.01) \). As expected, APP supplementation successfully restored all these enzymatic activities to normal levels. The lipid biosynthesis level in the liver was assessed by measuring FAS, which was increased by the HFD [26]. As expected, APP treatment significantly decreased FAS levels \( (p < 0.05) \) at low doses (Fig. 3D).

### 3.5 The effects of APP on liver parameters in HFD-induced obese mice

Lipid peroxidation level in the liver was assessed by measuring MDA [5, 27]. In the normal control group, the liver MDA level was 8.60 ± 1.30 nM/mg protein. However, the hepatic MDA levels sharply increased to 21.70 ± 4.30 nM/mg protein \( (p < 0.01) \) after HFD administration. As expected, this HFD-induced increase was effectively attenuated by pretreatment with APP at the tested doses of 200, 400, and 800 mg/kg/day in a dose-dependent manner (Fig. 4A). The HFD treatment dramatically elevated MPO levels (Fig. 4B) relative to the control group \( (p < 0.01) \). As expected, APP supplementation successfully restored MPO to normal levels, and this protective effect was dose-dependent. The acute administration of HFD to mice caused characteristic hepatotoxicity that affected the antioxidant parameters of liver tissue, as indicated by a significant decrease in glutathione peroxidase (Fig. 4C), SOD (Fig. 4D), CAT (Fig. 4E), GSH (Fig. 4F), T-AOC (Fig. 4G), and ASAFA (Fig. 4H) levels relative to the normal control mice \( (p < 0.01) \). As expected, APP supplementation successfully restored all these markers to control levels. The HFD treatment dramatically elevated leptin (Fig. 4I) relative to the control group \( (p < 0.01) \). As expected, APP supplementation...
reduced leptin to normal levels, especially at low doses. The energy production level in the liver was assessed by measuring glycogen (Fig. 4J), HK (Fig. 4K), and PK (Fig. 4L). In contrast to the control mice, the levels of these markers were obviously reduced after administration of the HFD \(( p < 0.01)\), and pretreatment with APP considerably increased the level of HFD-reduced markers.

3.6 APP protects against palmitate-induced mitochondrial dysfunction and insulin resistance in HepG2 cells

As shown in Fig. 5A, compared to the cells cultured in the H-DMEM medium with no APP, cell growth was significantly stimulated by APP over the entire tested concentration range from 1 to 200 \( \mu \text{g/mL} \). These results showed that APP was not toxic to the HepG2 cells. When the polysaccharide concentration was lower than 25 \( \mu \text{g/mL} \), APP promoted cell proliferation in a dose-dependent manner.

Free fatty acid-induced lipotoxicity plays a pivotal role in the pathogenesis of hepatic metabolic disorder [20]. To elucidate the protective effects of APP against FFAs and to determine whether the effects of APP on liver mitochondrial function were due to amelioration of oxidative stress, we used an in vitro HepG2 model with palmitate treatment to alter FFAs. After a 24 h treatment, 600 \( \mu \text{M} \) palmitate inhibited insulin signal transduction by decreasing p-AKT levels under insulin stimulation, and this inhibition was diminished by APP treatment (Fig. 5B and C). Additionally, palmitate induced significant mitochondrial dysfunction, including cell apoptosis (Fig. 5D), increased ROS production (Fig. 5E–G), and loss of the MMP (Fig. 5H). As expected, treatment with APP effectively restored mitochondrial function, indicating that APP might also be an effective nutrient. Further investigation of mitochondrial oxygen consumption and the electron transport chain complex activities was conducted with APP. The basal OCR levels were less than control in those PAL-treated cells (Fig. 5I). To determine whether the APP affected mitochondrial respiration, we treated HepG2 cell with APP, PAL, and APP + PAL. Under these conditions, an increase in basal OCR levels was observed indicating that the cells are using glucose oxidation and consuming more oxygen (Fig. 5I). Subsequent addition of oligomycin showed that the levels of ATP-linked respiration were attenuated in control cells or cells treated with PAL (Fig. 5J). To determine the maximal
respiratory capacity, the mitochondrial uncoupler FCCP was injected into the media. The stimulation of mitochondrial respiration with FCCP after oligomycin was substantially greater in the presence of 10 μg/mL APP compared to the control. Injection of mitochondrial complex III inhibitor antimycin A significantly inhibited respiration (Fig. 5I). A comparative study of effects of APP, PAL, and APP + PAL on the OCR levels of HepG2 cells showed that palmitate abolished the mitochondrial respiratory capacity, including basal respiration, ATP production, maximal respiration, and spare respiratory capacity, all of which were significantly improved by APP treatment.

4 Discussion

Several studies have addressed the link within dietary apple, apple product consumption, and benefits in humans [8, 28, 29]. Apple pomace could be a valuable and easily accessible source for bioactive compounds [3, 30], which include chemopreventive agents that can inhibit the development of serious chronic diseases and complications [5, 28, 31, 32]. Here, we isolated the polysaccharides from the apple pomace of “Qinguan.” APP was characterized as an acidic heteropolysaccharide, rich in arabinose (32.52%), galactose (20.60%), and galacturonic acid (29.96%), which accounted for up to 83.08% of all the quantitative monosaccharides, and was richer in uronic acids than “Fuji” apple peel polysaccharides and apple flesh polysaccharides [5]. Bioactive polysaccharides have been found in a large number of natural resources, including plants, fungi, and bacteria, and have attracted more and more attention in the biochemical and medical areas. Among them, apple pectin has demonstrated a better cholesterol-lowering effect than other pectins [31] and polysaccharide of apple pomace on antioxidative stress [3, 4, 29]. In the current study, we found that APP treatments, significantly inhibited the development of obesity and restored the basal serum parameters (Table 2 and Fig. 2), indicate that APP had beneficial effects on HFD-induced oxidative stress.

HFD induced severe liver injury [33], as reflected by the markedly elevated enzymatic activities of serum AST, ALT, and LDH (Fig. 3A–C), while administration of APP significantly reduced the levels of these serum enzymes, which may be responsible for its hepatoprotective action by scavenging and destroying lipid peroxyl radical and ROS such as...
the superoxide anion (O$_{2}^-$), the hydrogen peroxide (H$_{2}$O$_{2}$), and the hydroxyl radical (OH) [5, 8, 34–36]. Oxidative stress occurs when there is an imbalance between cellular oxidant species production and antioxidant capability [20], and therefore, antioxidant supplementation to inhibit the free radical-induced damage has become an attractive therapeutic strategy for reducing the risk of liver disease [37]. The intracellular redox balance depends on both oxidants and the antioxidant defense system in cells, including GSH-PX, SOD, and CAT, and also small molecular antioxidants, such as GSH. The results showed that APP possessed a variety of antioxidant and free radical-scavenging activities, which exhibited beneficial effects against the oxidative liver damage induced by HFD. Other studies have previously shown that metabolic oxidative stress increases systemic and leptin levels over and above the levels found in obesity [38]. Increased leptin affects myriad pathways, causing dysregulation of immune and metabolic functions [39]. As expected, the HFD-induced increased levels of leptin and markedly reduced enzymatic activities of PK and HK as well as hepatic glyco- gen concentration (Fig. 4) were completely normalized by APP.

Cellular defenses that protect the hepatocyte against oxidative stress have been proposed to be an important way to reduce the liver injury [20]. In this study, APP markedly suppressed the decrease of cell viability resulting from palmitate and the cell viability showed significant increased cell viability resulting from APP in a dose-dependent manner up to 10 μg/mL compared with media-treated cells. These results indicate that APP exerts a protective effect by inhibiting palmitate-induced cell death.

There are mounting evidences that HFD-induced oxidative stress resulting from the overproduction of ROS is a major factor contributing to the development and progression of liver damage. The collapse of the MMP results in the rapid release of cytochrome C into the cytoplasm [40]. In the present study, palmitate-treated HepG2 cells showed a marked increased generation of mitochondrial ROS (Fig. 5E)
Figure 5. Continued.
and F) and a loss of MMP (Fig. 5H). However, pretreatment with APP attenuated the increase in ROS level and prevented the loss of MMP.

The mitochondria are a major source of ROS, which is particularly susceptible to oxidative stress [41]. Previous studies have shown that Astragalus and Cornus polysaccharides inhibit mitochondrial injury caused by the continuous production of free radicals and selective oxidative damage [42, 43], and Ganoderma atrum polysaccharides protect mitochondrial by scavenging ROS [40, 44, 45], increasing the activity of antioxidant enzymes, and Astragalus and ginseng polysaccharides ameliorating mitochondrial dysfunction, which ultimately improved energy metabolism [46–48]. In the present study, we found that palmitate-treated HepG2 cells showed marked changes in several parameters of the mitochondrial function (Fig. 5H–J). Interestingly, APP restoring all these parameters to normal levels, as evidenced by enhancing the basal OCR levels, maximal respiration, and maintaining MMP (Fig. 5H and J), indicating that APP effectively improves mitochondrial function in HepG2 cells under palmitate insult.

Taken together, our results demonstrated that APP successfully ameliorated the HFD-induced oxidative stress, which may be attributed to APP scavenging mitochondrial ROS and improving mitochondrial function. As an abundant and inexpensive compound derived from apple pomace, APP is a promising bioactive food ingredient that may prevent hepatic metabolic disorder.

L.C., L.L., and L.H. have carried out animal studies; L.C., C.L., R.L., M.Z., and D.Z. have carried out cell studies; L.C., F.S., and R.L. have isolated apple pomace polysaccharides studies; and L.C., J.L., Y.G., and J.L. have written the manuscript.

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The authors have declared no conflict of interest.

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