Dandelion polyphenols protect against acetaminophen-induced hepatotoxicity in mice via activation of the Nrf-2/HO-1 pathway and inhibition of the JNK signaling pathway

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Available online 20 Feb., 2020

[ABSTRACT] We investigated the liver protective activity of dandelion polyphenols (DP) against acetaminophen (APAP; Paracetamol)-induced hepatotoxicity. Mice were acclimated for 1 week and randomly divided into the following groups (n = 9 per group): Control, APAP, APAP + DP (100 mg·kg−1), APAP + DP (200 mg·kg−1), and APAP + DP (400 mg·kg−1) groups. Mice were pretreated with DP (100, 200, and 400 mg·kg−1) by oral gavage for 7 d before being treated with 350 mg·kg−1 APAP for 24 h to induced hepatotoxicity. Severe liver injury was observed, and hepatotoxicity was analyzed after 24 h by evaluation of biochemical markers, protein expressions levels, and liver histopathology. Pretreatment with DP was able to restore serum liver characteristics (aspartate transaminase, AST; alanine aminotransferase, ALT; alkaline phosphatase, AKP), improve redox imbalance (superoxide dismutase, SOD; glutathione, GSH; malondialdehyde, MDA), and decrease inflammatory factors (tumor necrosis factor-α, TNF-α; interleukin-1β, IL-1β). Pretreatment with DP also significantly inhibited the expression levels of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Furthermore, DP pretreatment could inhibit the apoptosis of liver cells caused by APAP through up-regulation of Bcl-2 and down-regulation of Bax and caspase-9 protein. DP also down-regulated p-JNK protein expression levels to inhibit APAP-induced mitochondrial oxidative stress and up-regulated the expression of Nrf-2 and its target gene HO-1. The histopathological staining demonstrated that DP pretreatment could inhibit APAP-induced hepatocyte infiltration, congestion, and necrosis. Our results demonstrate that DP pretreatment could protect against APAP-induced hepatic injury by activating the Nrf-2/HO-1 pathway and inhibition of the intrinsic apoptosis pathway.

[KEY WORDS] Dandelion polyphenols; APAP-induced liver injury; Oxidative stress; Anti-inflammation; Anti-apoptosis; JNK pathways; Nrf-2/HO-1 pathways

[CLC Number] R965

Available online at www.sciencedirect.com

Introduction

Acetaminophen (APAP; paracetamol) is widely used as an analgesic and antipyretic drug. At therapeutic doses, it can be considered effective and safe, but an overdose may cause severe liver injury, liver failure, and even death [1]. Currently, APAP is the leading cause of drug-induced liver failure in the US and other countries, mainly due to non-abuse-related overdoses of acetaminophen products [2]. Excessive APAP may release superoxide during metabolism via the cytochrome P450 system [3]. Extensive research has determined that mitochondrial oxidative stress and the production of superoxide after APAP overdose is the key to APAP-induced hepatocyte necrosis [4]. APAP hepatotoxicity begins with the metabolism of acetaminophen to N-acetyl-p-benzoquinone imine (NAPQI). The combination of NAPQI and GSH produces covalent adducts that trigger mitochondrial oxidative stress leading to mitochondrial dysfunction [5]. As a precursor of intracellular cysteine and GSH, N-acetyl cysteine (NAC) has been used for many years as the standard antidote for APAP poisoning [6]. However, GSH supplementation is insuf-
efficient to arrest APAP-induced liver damage \[7\]. There is an urgent need to develop preventive or therapeutic alternatives to improve the outcome of APAP poisoning.

The nuclear factor erythroid-2-related factor 2 (Nrf-2)-mediated stress responses have been shown to be an important mechanism for cells to alleviate oxidative stress. In unstressed conditions, Nrf-2 is tethered to its cytoplasmic inhibitor Kelch-like ECH-associated protein 1 (Keap1). Keap1 is an actin-binding protein. Upon oxidative stress, Nrf2 dissociates from Keap1 and translocates into the nucleus, where it heterodimerizes with small Maf-binding proteins and binds to antioxidant element region (ARE), promoting the transcription of its target genes. Heme oxygenase-1 (HO-1) is a target gene of Nrf-2 and activation of the Nrf-2 signaling pathway has been reported to play an important role in APAP-induced hepatotoxicity \[8-9\].

c-Jun N-terminal kinase (JNK) is a member of the mitogen-activated protein kinase (MAPK) superfamily. JNK can activate a variety of signaling cascades not only through phosphorylation of its transcription factors, such as c-Jun \[10\], p53 \[11\], and ATF-2 \[12\], but also through members of the Bcl-2 family \[13\]. Pharmacological inhibition of JNK or silencing of JNK gene expression reduces liver injury after an APAP overdose \[14-19\].

In the past few decades, many natural medicines have been shown to have anti-hepatotoxicity \[18-20\]. Dandelion (Taraxacum mongolicum Hand.-Mazz.) is a perennial herb of the family Asteraceae (Compositae family) \[30\], which is widely distributed in the northern hemisphere \[21\]. Dandelion contains various bioactive substances, including phenolic acids, peptides, flavonoids, oligosaccharides, polysaccharides, alkaloids, and terpenes \[22-24\]. The German physician and botanist Leonhard Fuchs (1543) described its use to medicate gout, diarrhea, blisters, and spleen and liver complaints. In particular, the utilization of dandelion in liver complaints was largely based on the Doctrine of Signatures \[24\]. Its functions of “clearing heat-toxins” and “detumescence and tumor therapy” have been known for the treatment of upper respiratory tract infections, mumps, pharyngitis, acute tonsillitis, acute bronchitis, and urinary tract infections \[20, 24\]. The oligosaccharides and peptides of dandelion have high antibacterial activity \[25\] while the polysaccharides have antibacterial \[26\], antioxidant \[27\], and immune-function-modulating \[28\] activities. Colle et al. have demonstrated that the possible mechanisms may be attributed to scavenger activities against ROS and reactive nitrogen species of phenolic compounds in the extract in vitro \[29\], but the protective mechanism of dandelion leaf extract on liver injury in vivo has not been further studied. Therefore, in the present study, we investigate the protective effects of dandelion polyphenols (DP) against APAP-induced hepatotoxicity in the KM mouse model and describe a potential mechanism, i.e., activation of the Nrf-2/HO-1 pathway and inhibition of the expression of the p-JNK protein.

Materials and Methods

Chemicals and reagents

APAP was available from Yuanye Biotechnology Co., Ltd. (Lot No: B19M8E36136 Shanghai, China). Chlorogenic acid and chicoric acid were purchased from National Institutes for Food and Drug Control. Syringic acid and caffeic acid were purchased from Yuanye Biotechnology Co., Ltd.. Commercial assay kits for AST, ALT, AKP, MDA, SOD, GSH, hematoxylin and eosin (H&E) dye kits and Coomassie brilliant blue protein assay kits were bought from Nanjing Jiang-eng Bioengineering Research Institute (Nanjing, China). We purchased ELISA kits for mouse, TNF-α, IL-2, and IL-1β from Nanjing Jiancheng Bioengineering Research Institute. A Hoechst 33 258 dye kit was purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Rabbit monoclonal antibodies against mouse COX-2 and iNOS were acquired from Servicebio Technology (Wuhan, China). The rabbit monoclonals against mouse caspase-9, Bax, Bcl-2, and β-actin were purchased from Servicebio (Wuhan Servicebio Technology Co., Ltd.). All other chemicals were of the highest grade from a commercial source.

Extraction and purification of DP

Taraxacum mongolicum Hand.-Mazz. (dandelion) were collected from Wuhan, Hubei, China, and identified and authenticated by Prof. LIU Xin-Qiao (School of Pharmaceutical Science, South-Central University for Nationalities, Wuhan, China). Three hundred grams of dry and minced dandelion was extracted with 1800 mL of 80% ethanol solution by a hot reflux extraction for 90 min. Extract of dandelion was obtained by concentrating the extracting solution to dryness by removing the ethanol solvent in a rotary evaporator (Shanghai Yarong Biochemical Instrument Factory, China) at 60 °C.

D-101 macroporous resins were purchased from Bonherb Technology Company (Hebei, China). These resins were soaked with 95% ethanol for 24 h to swell adequately. Subsequently, the resins were eluted by 95% ethanol until white casse disappeared when the eluting reagent was mixed with pure water (1 : 5, \(V/V\)), and then the resins were washed with pure water until the liquor had no alcoholic odor. DP purification was performed according to previous method \[19\].

Total acid contents of DP were measured by the potassium ferricyanide-ferric chloride method using chlorogenic acid as equivalents. Briefly, aliquots of 0.2 mL of samples or standard solutions were mixed with 5 mL of absolute ethanol. Then, 2 mL of 0.3% sodium dodecyl sulfate solution and 2 mL of 1% \(K_2[Fe(CN)_6]\)–1% FeCl\(_3\) (1 : 1, \(V/V\)) were added to the mixture, and 0.1 mol L\(^{-1}\) HCl was added to a final volume of 25 mL, followed by thorough mixing and incubating in the dark for 20 min. Absorbance was detected at 760 nm, and total acid contents were calculated from a calibration curve (\(y = 0.3395x – 0.2204, R^2 = 0.9999\), 1.4–97.6 μg of chlorogenic acid).

Total flavonoids of DP were measured using a modified colorimetric method using rutin as equivalents. Briefly, 1 mL
The liver was fractionated into three parts: one part was fixed in formalin for histological analysis, a second part was stained for histopathology detection and immunohistochemistry (IHC) analysis, and the third was stored in a freezer at −80 °C for further analysis.

**Measurement of biochemical markers in liver tissues**

Serum AST, ALT, and AKP activities were quantified according to the kit criteria. Livers were homogenized in an ice-water bath and then centrifuged at 5000 r min⁻¹ for 10 min. Subsequently, 10% liver tissue homogenates were used to determine the concentrations of MDA, GSH, and SOD.

**Determination of inflammatory markers in liver tissues**

Liver TNF-α, IL-2, and IL-1β contents were determined by commercial ELISA kits in accordance with the manufacturer’s instructions and measured at 450 nm for absorbance in an ELISA reader.

**Histopathological analysis**

Liver tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5-μm serial sections for staining with H&E. Histopathological changes in the liver tissues were observed using a light microscope and representative images are presented.

**TUNEL assay**

The number of apoptotic cells was determined using a TUNEL assay as previously described [31]. Briefly, liver sections were fixed with proteinase K (20 mg mL⁻¹) for 10 min; then the fixed tissue was incubated in methanol containing 3% hydrogen peroxide for 20 min to block endogenous peroxidases. Subsequently, the slides were incubated in equilibration buffer solution and terminal deoxynucleotidyl transferase, and then anti-digoxigenin–peroxidase conjugate was added to the fixed liver tissues. Finally, dianminobenzidine (DAB) was used to stain the peroxidase in liver sections, and hematoxylin was used to counterstain the sections.

**Hoechst 33258 staining**

Apoptosis of cells was detected by Hoechst 33258 staining [32]. Liver tissue (5-μm sections) was fixed in 10% paraformaldehyde and stained with Hoechst 33258 (10 μg mL⁻¹). Three samples were randomly chosen from each group. Stained nuclei were observed using UV excitation after being washed with PBS three times and then photographed with a fluorescent microscope (Leica Microsystems DFC425C, Germany).

**Immunohistochemistry and immunofluorescence analysis**

Immunohistochemical analysis of liver tissues was as previously described [33]. Briefly, we used a series of xylene and aqueous alcohol solutions to dewax and rehydrate the 5-μm-thick paraffin sections, which were then incubated in blocking buffer for 30 min at room temperature. The processed sections were incubated with the primary antibody overnight at 4 °C and then with biotin-labeled goat anti-rabbit IgG-HRP for 1 h at room temperature. Substrate was added to the slices for 30 min followed by DAB staining and hematoxylin counterstaining. Positive expression, which is characterized by a brownish yellow color in the tissue, was observed using an optical microscope (Leica Microsystems DFC425C, Germany).
**Western blot analysis**

Western blot analysis was performed according to the method of Noh *et al.*[33]. In brief, the liver tissue was lysed in RIPA buffer. Next, we used the BCA Protein Assay Kit (Beyotime Biotechnology, China) to determine the total protein. Equivalent amounts of protein extracts (120 μg/lane) were separated by 12% SDS-PAGE and electrotransferred onto a PVDF membrane. Blots were blocked in 10% skimmed milk at 37 °C for 1.5 h, incubated with appropriate primary antibodies overnight at 4 °C, and incubated with secondary antibodies for 1.5 h at room temperature. Target proteins were visualized using. The intensity of protein bands was analyzed using AlphaEase FC software (Alpha Innotech).

**Statistical analysis**

All of the experimental data are presented as the mean ± SD. The statistical significance of the differences between groups was determined by ANOVA in SPSS 18.0. *P*-values < 0.05, < 0.01, or < 0.001 were considered significant. Statistical graphs were prepared using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA).

**Results**

**Total acid and total flavonoid contents**

Total acid and total flavonoid contents of DP were determined (Fig. 1). The contents of total acid and total flavonoid in the extracts were 4.97% and 6.78%. After purification with D-101 macroporous resin, the contents of total flavonoids and total acids were 47.6% and 42.3%, respectively, which together accounts for 89.9% of the content of the DP sample.

Liquid chromatography/mass spectrometry (LC/MS) was used to determine the compound profile of DP, including flavonoids and phenolic acids. Seven compounds were identified according to the retention time of chemical reference substances and characteristic ions as: (1) chlorogenic acid; (2) caffeic acid; (3) syringic acid; (4) quercetin-7-O-hexoside-3-O-(malonyl)hexoside; (5) chicoric acid; (6) quercetin-pentoside hexoside; (7) luteolin-7-glucoside

**Effects of dandelion polyphenols on body weight and organ indices in mice**

As shown in Table 1, there were no changes in the body weight of the mice between the normal and other groups. However, APAP treatment of mice significantly increased the weight of the liver and spleen (Table 1, *P* < 0.05). Organ indices of the liver and spleen of the DP-treated groups were similar to those of the normal group. These data suggest that the damage and inflammation following APAP administration could be prevented by DP treatment.

**Dandelion polyphenols ameliorate APAP-induced liver damage**

In order to estimate the protective effect of DP against APAP-induced hepatotoxicity, serum analyses of ALT, AST, and AKP were performed 24 h after APAP injection (Figs. 2A–2C). There were increased levels of transaminase and alkaline phosphatase in the APAP group after a single APAP dose, which is evidence of severe liver failure (*P* < 0.05, *P* < 0.01, or *P* < 0.001) compared with the control group. However, DP pretreatment prevented the induction of these two transaminases in APAP in a dose-dependent manner (Figs. 2A–2B).

According to the pathological analysis of liver sections, DP pretreatment could reduce liver injury caused by APAP overdose. As shown in Fig. 2D, the hepatic architectures were normal in the normal group. Administration of APAP led to extensive vacuolization, blood congestion in the central vein, and the loss of hepatic architecture. However, pretreatment with DP prevented the pathological changes induced by APAP. At APAP + DP (400 mg·kg⁻¹) groups, the hepatocytes of the mice were similar to the normal group with little cell necrosis. These histopathological observations provide further evidence that DP can protect against liver injury induced by APAP.

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**Table 1 Effects of DP on body weight, and organ indices in mice (mean ± SD, *n* = 9)**

<table>
<thead>
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<th>Treatment (mg·kg⁻¹)</th>
<th>Body weights (g)</th>
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*P* < 0.05, **P** < 0.01, and ***P*** < 0.001 vs normal group; *P* < 0.05, **P** < 0.01, and ***P*** < 0.001 vs APAP group.
Dandelion polyphenols ameliorate APAP-induced hepatotoxicity by increasing the expression of anti-oxidative proteins and Nrf-2 expression

Oxidative stress damage is an important mechanism of APAP-induced acute liver injury \[34\]. The effects of DP on oxidative stress are shown in Figs. 3A–3C. After 24 h of APAP treatment, a significant increase in hepatic GSH and SOD, as well as a decrease in MDA, was observed in the DP pretreatment group compared to the APAP group. ALT, alanine aminotransferase; AST, aspartate transaminase; AKP, alkaline phosphatase

**Fig. 2** Pretreatment with DP protected against APAP-induced liver injury. Levels of ALT (A), AST (B), and AKP (C) were measured in APAP-induced hepatotoxicity. Liver tissues were stained with hematoxylin-eosin (H&E; 100 ×, 400 ×) (D). All data are expressed as the mean ± SD, n = 9. *P < 0.05, **P < 0.01, and ***P < 0.001 vs the normal group; #P < 0.05, ##P < 0.01, and ###P < 0.001 vs the APAP group. ALT, alanine aminotransferase; AST, aspartate transaminase; AKP, alkaline phosphatase

**Fig. 3** Pretreatment with DP protects against APAP-induced liver injury. Levels of SOD (A), MDA (B), and GSH (C) were measured during APAP-induced hepatotoxicity. Effects of DP on the protein expression of HO-1 (D) and Nrf-2 (E). Protein expression was examined by Western blotting in liver tissues. All data are expressed as the mean ± SD, n = 9. *P < 0.05, **P < 0.01, and ***P < 0.001 vs the normal group; ^P < 0.05, ^^P < 0.01, and ^^^P < 0.001 vs the APAP-treated group. GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase
Nrf-2 is the master gene controlling anti-oxidative gene expression, including SOD. We examined the protein expression of HO-1, an Nrf-2 target gene, in the liver following the treatments. As shown in Fig. 3D, HO-1 protein of liver tissue was increased by DP in a dose-dependent manner. In addition, a high dose of DP (400 mg·kg\(^{-1}\)) could also increase Nrf-2 protein expression in the liver tissue (Fig. 3E). These data indicate that the protective effects of DP against APAP-induced hepatotoxicity in mice are likely related to anti-oxidative activity.

**Dandelion polyphenols ameliorate APAP-induced liver inflammation**

APAP overdose leads to an inflammatory response and activates the innate immune cells [36]. TNF-\(\alpha\) and IL-1\(\beta\) are the two most important pro-inflammatory cytokines in APAP-induced hepatotoxicity [36]. In order to determine the anti-inflammatory effects of DP, the serum levels of TNF-\(\alpha\) and IL-1\(\beta\) were assayed. As depicted in Figs. 4A–4B, IL-1\(\beta\), and TNF-\(\alpha\) were increased in the APAP-treated group. However, these levels were markedly decreased by DP treatment. These pro-inflammatory cytokines can increase the expression of iNOS and COX-2, which can further aggravate oxidative stress in the liver. Therefore, we immunohistochemically analyzed the protein expression levels of COX-2 and iNOS in the liver tissue. As shown in Figs. 5A and 5B, a significant decrease was observed in the APAP + DP (400 mg·kg\(^{-1}\)) groups compared with the APAP group.

As depicted in Fig. 4C, compared with the normal group, the IL-2 levels in the APAP group increased significantly, but the overproduction of IL-2 was prevented by pretreatment with DP (\(P < 0.05\) or \(P < 0.01\)). These results suggest that the protective effect of DP may also be due to its anti-inflammatory activity.

**Fig. 4** Pretreatment with DP protected against APAP-induced liver injury. Levels of tumor necrosis factor (TNF)-\(\alpha\) (A), interleukin (IL)-1\(\beta\) (B), and interleukin (IL)-2 (C) in liver tissues were determined by ELISA. All data are expressed as the mean ± SD, \(n = 9\). *\(P < 0.05\), **\(P < 0.01\), and ***\(P < 0.001\) vs the normal group; \#\#\#\#\(P < 0.05\), \#\#\#\(P < 0.01\), and \#\#\(P < 0.001\) vs the APAP-treated group.

Hoechst 33258 staining was used to observe apoptosis in the hepatocytes. As shown in Fig. 6A, significant nuclear fragmentation and concentration of the hepatocyte nuclei can be observed in the APAP-treated group. Most of the hepatocytes in the APAP + DP (400 mg·kg\(^{-1}\)) groups were similar to the normal group, displaying regular uniform fluorescence intensity and normal shape. Hepatocyte apoptosis was further confirmed by TUNEL staining. As indicated in Fig. 6B, almost no positive cells were visualized in normal mice. There are a large number of positive cells were visualized in APAP-treated mice. However, mice pretreated with DP showed fewer apoptotic cells than the APAP group.

To investigate whether DP could inhibit the hepatocyte apoptosis induced by APAP, apoptotic-related signaling proteins (caspase-9, Bax, and Bcl-2) were observed by Western blot analysis. As shown in Figs. 5C and 5D, the number of Bax-positive cells in the APAP-treated group was increased, while the expression of Bcl-2 was decreased. In contrast, down-regulated Bax and up-regulated Bcl-2 were found in the APAP + DP (400 mg·kg\(^{-1}\)) group. The expression level of caspase-9 was increased by APAP treatment and this was prevented by DP pretreatment.

**Discussion**

Acetaminophen is the most commonly used drug for the treatment of pain relief and fevers. However, acute liver injury caused by APAP overdose is the most common cause of drug-induced liver injury worldwide. The molecular basis of the acute liver injury induced by APAP is an increase in oxidative stress, inflammation, and congestion, as well as apoptosis of hepatocytes [37]. Several studies have found that *Taraxacum officinale* protects against lipopolysaccharide-induced acute lung injury by anti-oxidative and anti-inflammatory actions in mice [38] and dandelion leaf extracts and chlorogenic acid protect against APAP-induced liver injury [39]. Here, we continue the exploration of the hepatoprotective effects and the underlying mechanism of dandelion polyphenols DP. Our research clearly demonstrates that DP reduces APAP-induced hepatotoxicity by reducing hepatocyte damage, improving oxidative stress, and inhibiting the expression of the p-JNK protein in all experimental groups was examined by Western blot analysis. As shown in Fig. 6F, p-JNK was down-regulated in the APAP + DP (200 and 400 mg·kg\(^{-1}\)) groups. Compared with the normal group, the expression of p-JNK protein in the APAP group increased significantly (\(P < 0.05\)).
of apoptotic and pro-inflammatory factors. The results indicate that the anti-oxidative property of DP contributes to the protection against APAP-induced hepatotoxicity, and the protective effects are associated with the activation of the Nrf-2/HO-1 pathway and the inhibition of the JNK signaling pathway.

Serum levels of ALT, AST, and AKP—three biochemical markers of liver function [40]—increased significantly in the APAP-treated group. Furthermore, compared with the liver tissue of the untreated group, administration of APAP led
to extensive vacuolization, blood congestion in the central vein, and loss of hepatic architecture. In contrast, pretreatment with DP reduced the histopathological changes and markedly decreased the ALT, AST, and AKP markers. These results were in accordance with the study conducted by previous studies [41].

Oxidative stress can aggravate histopathological changes after APAP challenge [42] and APAP leads to the formation of reactive oxygen species (ROS) in mice [43]. APAP metabolic activation and formation of NAPQI are mediated by cytochrome P450-mediated enzymes, especially CYP2E1, which transforms APAP into NAPQI, followed by GSH depletion that causes the generation of ROS. Hepatic GSH is one of the key molecules for the detoxification of APAP when combined with its metabolic product NAPQI. Our findings showed that the activity of GSH in the liver homogenate of the APAP-treated group was significantly decreased. However, the activity of GSH in the liver was significantly increased after DP pretreatment. Furthermore, oxidative stress contributes to liver injury, which is characterized by lipid peroxidation (LPO) and the accumulation of ROS [44]. Oxidative stress induced by APAP is normally detoxified by the enzymatic antioxidant defense system. Antioxidant enzymes, such as SOD, were depleted by APAP exposure but the decrease in SOD is reversed after DP administration (200 and 400 mg·kg⁻¹), demonstrating the protective effect of DP on APAP hepatotoxicity. The MDA level, an important indicator of LPO, was markedly higher in the APAP group in comparison with the DP group. The direct relationship between the hepatoprotective effect of DP and the activation of cytochrome P450 requires further investigation, but these results indicate that DP significantly attenuates APAP-in-

Fig. 6  Histological examination of morphological changes in liver tissues. (A) Hoechst 33258 staining (200×) and (B, C) TUNEL (400×). Effects of DP on the protein expression of Bax (D), Bel-2 (E), caspase-9 (F), and p-JNK (G). Protein expression was examined by Western blotting analysis in liver tissues. All data are expressed as the mean ± SD, n = 9. *P < 0.05, **P < 0.01, and ***P < 0.001 vs the normal group; #P < 0.05, ##P < 0.01, and ###P < 0.001 vs the APAP-treated group.
duced oxidative stress and the hepatoprotective effect of DP might be related to its antioxidant activity.

Nrf-2 is the major regulator of oxidative stress. It enhances the removal of ROS by regulating the expression of antioxidant genes and phase II detoxifying enzymes, such as heme oxygenase-1 (HO-1), to counteract oxidative stress [45]. In the last few decades, several studies have demonstrated that hepatotoxicity induced by APAP were more serious in Nrf2 knock-out mice than in normal wild-type mice [46-47]. Given the importance of oxidative stress in APAP-induced hepatotoxicity, we speculate that DP, as an Nrf2 activator, might protect against this toxicity. Here, we show that DP could facilitate Nrf2 translocation into the nucleus, which correlated with an increase in the expression of the Nrf-2 target gene HO-1. Both in vitro and in vivo, activation of HO-1 protects cells through anti-inflammatory, anti-oxidant, and anti-apoptotic effects. Our studies indicate that DP supplementation increases the expression of HO-1 protein, which has also been reported in previous studies [48-49].

Keap1 is an actin-binding protein. In the absence of electrophilies or oxidants, Nrf-2 remains in the cytoplasm by binding to its inhibitor Kelch-like ECH-associated protein 1 (Keap1) [11-12]. However, oxidative stress leads to oxidation of key cysteine residues on Keap1, disrupting the Keap1/Nrf-2 interaction. Inactivation of Keap1 also releases Nrf-2, which translocates to the nucleus and activates target gene expression. Mice with a hepatocyte-specific deletion of Keap1 show increased Nrf-2 target gene expression and are resistant to APAP-induced hepatotoxicity. The direct relationship between the hepatoprotective effect of DP and inactivation of Keap1 requires further investigation.

In addition to oxidative stress, the sterile inflammatory response is also an important aspect of APAP-induced hepatotoxicity. From the pathological sections of the liver, we observed inflammatory factors after APAP treatment. Necrosis caused by APAP leads to the recruitment of macrophages and the release of pro-inflammatory factors, such as cytokines and chemokines [50]. TNF-α and IL-1β, two pro-inflammatory cytokines, were increased after APAP treatment (Figs. 4A and 4B). High levels of TNF-α not only induce the accumulation and activation of neutrophils and macrophages but also activate NF-κB and subsequently produce iNOS in the liver. In addition, TNF-α mediates the death receptor pathway and apoptosis. Our results indicate that the expression of TNF-α and IL-1β increased significantly in the APAP-treated group but was similar to the untreated group after DP (100, 200 and 400 mg kg^{-1}) pretreatment. After pretreatment with DP, the expression of iNOS and COX-2 was also reduced in the liver. So we inferred that DP protects liver injury from TNF-α-mediated apoptosis. The direct relationship between the hepatoprotective effect of DP and TNF-α-mediated apoptosis requires further investigation. These results suggest that DP exerted a protective effect against APAP-induced liver inflammation.

The early-phase activated JNK translocates to the mito-
chondria and induces mitochondrial permeability (MPT) by boosting the mitochondrial ROS level and by inhibiting mitochon-
drial respiration, GSH synthesis, and ATP depletion. These further induce necrotic cell death. In the late phase, the injured hepatic cells provoke the immune response cascade by secreting inflammatory cytokines such as TNF-α. Sustained activation of JNK leads to bid/bax signal-mediated mito-
chondrial outer-membrane permeabilization (MOMP) and apoptosis in hepatocytes. Our findings also support the evidence provided by previous studies [51]. Western blotting ana-
lyses clearly revealed that APAP down-regulated p-JNK in hepatocytes.

APAP-induced liver injury is related to apoptosis [18]. There are two major apoptotic pathways: the death receptor pathway (extrinsic pathway) and the mitochondrial pathway (intrinsic pathway). The former is triggered by the activation of caspase-8, and the latter is triggered by the activation of caspase-9 and regulated by anti-apoptotic B-cell leukemia/lymphoma 2 (Bcl-2) and pro-apoptotic Bax [52]. Therefore, we mainly focused on the mitochondrial pathway (intrinsic path-
way), which is regulated by caspase-9, to explore the effect of DP on the APAP-induced apoptosis signaling pathway in the present study. In the mitochondrial pathway, mitochondria-
mediated apoptosis is regulated by Bcl-2 families of proteins, which control mitochondrial membrane permeability. Activation of the mitochondria is accompanied by the translo-
cation of cytochrome C from the mitochondrial intermem-
brane space into the cytoplasm. Cytosolic cytochrome C activ-
tes caspase-9 together with apoptotic protease activator (Apaf)-1, which in turn activates caspase-3 resulting in apoptosis [53]. Immunohistochemistry and Western blotting analyses showed that the expression of caspases-9 and Bax significantly decreased and Bcl-2 increased after DP pretreat-
ment. These data indicate that DP reduced apoptosis of hepa-
tocytes in APAP-induced hepatotoxicity and that the DP protective effect may be through the regulation of the JNK pathway.

**Conclusion**

In summary, This study explicitly shows the alleviating action of DP in APAP hepatotoxicity mouse model. Our results demonstrate that DP could protect against APAP-in-
duced liver injury, which is possibly mediated by the ability of DP to activate the Nrf-2 pathway and preventing the pro-
duction of lipid peroxidation to alleviate oxidative stress level, as well as hepatic antioxidant status. In addition, DP exhibited anti-inflammatory activity through the inhibition of pro-inflammatory cytokines, including TNF-α and IL-1β, during APAP-induced acute hepatotoxicity. Likewise, DP at-
tenuated the activation of JNK-mediated caspase pathway and decreased Bcl-2/Bax expression, which eventually pre-
vented hepatotoxicity caused by APAP-overdose. Thus, DP may provide a new strategy for protection against APAP-in-
duced liver injury.
Abbreviations

APAP, acetylaminoephenth; DP, dandelion polyphenols; ALI, acute liver injury; AST, aspartate transaminase; ALT, alanine aminotransferase; AKP, alkaline phosphatase; GSH, glutathione; SOD, superoxide dismutase; MDA, malondialdehyde; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-2, interleukin-2; COX-2, cyclooxygenase-2; iNOS, nitric oxide synthase; HO-1, heme oxygenase-1; Nrf-2, Nuclear factor (erythroid-derived 2)-like 2; Keap1, Kelch-like ECH-associated protein 1.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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