



Protective Effect of Dandelion Leaf Water Extracts on APAP-Induced Liver Injury in Rats and Its Mechanism

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ABSTRACT

With the advent of a large number of drugs in recent years and the aggravation of human aging, drug-induced liver injury is increasing year by year. The protective effect of dandelion extract on acetaminophen (APAP) - induced drug-induced liver injury in rats and its specific mechanism was studied by in vitro cell culture. For this aim, twenty healthy SD rats with the same physiological status were divided into model group and normal group, with 10 rats in each group. The drug-induced liver injury model was made by intragastric administration of 1 g/kg APAP for 14 days. The liver function lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were detected to verify the success of the model. After that, the liver tissues were aseptically isolated from the normal group and APAP model group, and the primary hepatocytes were cultured. They were divided into control group (control), liver injury model group (model), medium-dose dandelion extract group (1mm dlwe) and high dose dandelion extract group (2mm dlwe). The cell proliferation activity was detected by CCK-8 cell proliferation activity kit. Cell samples were collected at 72 hours to detect the contents of AST and ALT in cell supernatant. The contents of oxidative stress-activated oxygen (ROS), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) were detected by colorimetry, and the apoptosis was detected by flow cytometry. Inflammatory factors, key genes of liver injury, drug metabolic enzymes cytochrome P450 2E1 (CYP2E1), mitogen-activated protein kinase (MAPK) and nuclear transcription factors were detected by RT-PCR- κ B (NF- κ B) P65 signaling pathway-related gene expression level. Finally, the expression of CYP2E1, MAPK and NF-κB signaling pathways were analyzed by Western blot. Results showed that Serum ast, ALT and LDH increased ($P < 0.05$), suggesting that the liver injury model was successful. The hepatocytes in the normal group were oval, flat, evenly distributed and well adhered to the wall. The liver injury model group had more suspended cells, pseudopodia, polygonal and poor growth state. The cells in the medium-dose dandelion extract group (1mm dlwe) and high dose dandelion extract group (2 mm dlwe) adhered well, mostly oval, similar to the normal group and grew well. CCK8 found that the cells in the model group decreased significantly, the proliferation activity decreased significantly, the ast, alt, LDH and ROS in the cell supernatant of the model group increased compared with other groups ($P < 0.05$), and the contents of GSH and GSH PX decreased ($P < 0.05$). Apoptotic cells in the model group increased, and TNF in the model group-α, COX-2, CYP2E1, MAPK, JNK and NF KB p65 increased ($P < 0.05$). CYP2E1, MAPK and NF KB p65 increased in the model group ($P < 0.05$).

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Introduction

The liver is a vital organ that regulates the synthesis, metabolism, storage and redistribution of carbohydrates, proteins and lipids (1), and it also plays an important role in detoxification. During the process of removing exogenous drugs and toxins, these toxic substances will cause various liver diseases, including alcoholic liver injury (2). Acetaminophen (APAP) is one of the most widely-used analgesic antipyretics in the world, which is considered as a safe drug at normal therapeutic doses. However, the overdose of

APAP is the known cause of acute hepatic necrosis (3). In the United States and Europe, APAP is overused usually because it is easily available, and APAP-induced liver injury is the most common form of acute liver failure (4). APAP-induced liver injury is mainly mediated by cytochrome P450. After hepatotoxicity occurs due to the application of high-dose APAP, the level of glutathione (GSH) in the liver will decline. According to existing data, mitochondrial dysfunction may be one of the important mechanisms of APAP-induced hepatotoxicity (5). The overdose of

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APAP produces excessive N-acetyl-p-benzoquinone imine (NAPQI) to deplete GSH and bind to cellular proteins, triggering the injury process and ultimately leading to necrosis of hepatocytes (6). N-acetylcysteine (NAC) has always been the main antidote to APAP poisoning. NAC offers cysteine as the precursor for GSH synthesis, so it improves the APAP-induced hepatotoxicity by supplementing GSH in the early administration after APAP (7,8). However, its efficacy is related to the time delay between overdose of APAP and treatment, only limited to the early stage of APAP poisoning (9). In addition, the long-term high-dose NAC treatment may damage the liver regeneration after APAP-induced liver injury (10). In the absence of reliable liver-protecting drugs in allopathy, searching for a kind of new, effective and safe drug to prevent liver diseases is worthy of concern. Therefore, it is of great value to develop more effective and safer drugs in the prevention and treatment of APAP-induced hepatotoxicity.

Clinical studies in the present century have confirmed the therapeutic effects of several plants on liver diseases. In recent years, the anti-oxidative stress effect of natural products extracted from plants, such as polyphenols, observed in APAP-induced liver injury due to the diversity of pharmacological properties, including antioxidant capacity, has attracted considerable attention (11,12). Antioxidants are considered as compounds acting through one or more of the following mechanisms: chemical reduction activity, free radical scavenging, potential complexation of pro-oxidant metals, and quenching of singlet oxygen (13). In addition, they can enhance the endogenous antioxidant defense and regulate the cellular redox state (14). Many gentians possess the antioxidant property and many other biological properties, such as anti-ulcer, anti-diabetes and anti-proliferation (15). Dandelion, a member in the composite family, is a kind of perennial herb native to the northern hemisphere (16). In China, dandelion is not only tasty food but also a traditional Chinese herbal medicine with choleric, diuretic, anti-rheumatic, anti-diabetic and anti-inflammatory effects. Studies have shown that dandelion extract (DLWE) has a variety of pharmacological effects. For example, dandelion root extracts can reduce alcohol-induced oxidative stress (17), dandelion leaf extracts can

alleviate non-alcoholic fatty liver disease induced by a high-fat diet (18), and dandelion flower extracts can scavenge reactive oxygen species (ROS) and protect DNA in the ROS-induced injury in vitro. In addition, the dandelion polysaccharide has antioxidant and anti-inflammatory effects. To sum up, exploring the protective effect of DLWEs on the liver and its specific mechanism of action are of great significance in the prevention of APAP-induced liver injury.

The specific molecular mechanism of DLWEs in the treatment of APAP-induced liver injury has not been fully clarified. In the present study, therefore, it was proposed that DLWE could exert a protective effect on APAP-induced liver injury in rats through oxidative stress and apoptosis. The cells were cultured and added with DLWEs at different concentrations, and then the oxidative stress was detected via enzyme-linked immunosorbent assay (ELISA), the cell proliferation and apoptosis were observed, and the changes in cellular pathway molecules were detected via gene and protein assays, so as to clarify the therapeutic effect of DLWE on liver injury and its specific mechanism of action, thereby providing an experimental basis for the subsequent research and the development of new drugs and new treatment means.

Materials and methods

Animal modeling

Twenty male SD rats were free to drink water. After one week of adaptive feeding, refer to the APAP-induced liver injury mouse model method of Zhao Hui et al. (19). Use APAP for induction, dissolve PAAP in 0.2% carboxymethyl cellulose (CMC) solution, and give rats a single dose by gavage. Once a day, five times a week, 1g/kg each time, for two consecutive weeks, the control group was given the same dose of normal saline, 10 rats in each group, and the follow-up test was started after the successful model building.

Dandelion root and leaf extract was provided by Xi'an Tianrui Biotechnology Co., Ltd.

Detection of liver function

After the experiment, the venous blood was routinely drawn from the caudal vein, placed at room temperature for 30 min and centrifuged at 1000 g for 10 min. The supernatant was collected to detect the liver function indexes, and whether there were

changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were observed to further indicate the development of liver injury. Whether the animal model was successfully established was verified by detecting liver injury indexes, so as to provide important references for early diagnosis, predict the occurrence of diseases in advance, and prepare for subsequent experiments.

Separation, culture and morphological observation of primary hepatocytes

Rats in each group were injected with 300 U heparin sodium saline. After routine skin preparation and disinfection, the liver was taken aseptically with surgical instruments and moved to a sterile ultra-clean bench. The liver was perfused with the pre-heated 37°C perfusate containing type IV collagenase till softening and outflow of turbid liquid. The liver was fully digested with trypsin if the capsule was separated from the parenchyma and there were obvious granules on the liver surface. Then the liver perfused was quickly placed in a sterile culture dish, and the digestion of hepatocytes was terminated using the RPMI 1640 medium. The liver parenchyma tissues were cut into pieces and the cell suspension was filtered using 60-mesh and 200-mesh sieves, followed by centrifugation at 1000 rpm for 5 min. Among them, the primary hepatocytes isolated and cultured from the mice in the control group are used as the control group. The primary hepatocytes of the model group are divided into the liver injury model group (model), the medium-dose dandelion extract group (1mM DLWE), high-dose dandelion extract group (2mM DLWE). Among them, the medium-dose dandelion extract group (1mM DLWE) and the high-dose dandelion extract group (2mM DLWE) were given interventions of 1mM DLWE and 2mM DLWE, respectively. Change the culture medium every 24 hours, observe the cell growth morphology under an inverted microscope and take pictures on time.

CCK-8 cell proliferation assay

The cells in the logarithmic growth phase in each group were inoculated into a 96-well plate and cultured in an incubator with 5% CO₂ at 37°C for 0, 24, 48 and 72 h. Add 100µTMB chromogenic solution to each well in the 96-well plate (provided by KPL,

USA) After incubating for 1 hour in a 37°C incubator, the absorbance (450nm) of each group was measured with an ultraviolet spectrophotometer, and a broken line graph was made to reflect the proliferation activity of the cells.

Detection of liver function indexes in each group

After stimulation, the cells in good growth status in the incubator were selected in each group, the medium was discarded, and the cells were collected using the cell scraper. Then the cells were lysed with RIPA lysis buffer (strong), placed at room temperature for 5 min and centrifuged at 1000 g for 10 min. The supernatant was collected to detect the liver function indexes, and whether there were changes in AST, ALT and LDH were observed to further indicate the development of liver injury.

Detection of oxidative stress indexes in each group

After cell stimulation, select three groups of cells in good growth condition from the incubator, discard the culture medium, collect the cells and supernatant of each group with a cell scraper, lyse the cells with RIPA strong lysate, and collect the supernatant by centrifugation. A colorimetric assay kit (manufacturer: Qingdao Jieshikang Biotechnology Co., Ltd., product number: T3023) was used to detect the levels of cell ROS, GSH, and GSH-Px. The specific operation steps are carried out in accordance with the actual and the instructions, and finally, the absorbance of each group is detected under the microplate reader.

Detection of apoptosis in each group via flow cytometry

The liver injury model of primary hepatocytes was treated with moderate-dose DLWEs (1 mM DLWE) and high-dose DLWEs (2 mM DLWEs) for 24 h and then washed twice with PBS, and the precipitate at the bottom of the tube was collected. Three replicates were set in each group. According to the instructions, 500 µL of binding buffer was added to suspend the precipitate, 5 µL of Annexin V-binding buffer and 5 µL of propidium iodide (PI) dye were also added, and the mixture was placed at room temperature for 15 min. Finally, the apoptosis rate was detected using a flow cytometer according to the procedure.

Detection of expressions of apoptosis and pathway-related genes via RT-PCR

RNA was extracted from the collected cells, and DNA was synthesized using TaKaRa kit. Please refer to the manual for specific operation steps. Then amplify into single-stranded cDNA according to the conventional reaction system, and put it at -20°C for use in PCR amplification reaction. The sample is amplified with primers for the gene to be detected and the internal reference gene, with 3 replicates for each reaction. Establish a 20 μL amplification system according to cDNA (2 μL), qPCR mix (10 μL), primer (2 μL), and ddH₂O (6 μL) to perform PCR amplification reaction. The target gene and GAPDH internal reference primer sequence were designed according to the sequence on GenBank, and the tumor necrosis factor- α (TNF- α) and cyclooxygenase-2 (COX-2) were detected by the qRT-PCR method., mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinases (JNK), nuclear factor- κB (nuclear factor-Kb, NF- κB) p65, drug metabolism enzyme cytochrome P450 2E1 (cytochrome P450 2E1, CYP2E1) and other target gene expression. The specific primer sequence is shown in Table 1

Table 1. Primer sequence

target gene	Primer sequence (F-R, 5-3)
GAPDH	GGGACATGCCCGCTGGAGAAAC AAAGCCCAGGATGCCCTTAGT
TNF- α	CCCTCCTGGCCAACGGCATG TCGGGGCAGCCTTGTCCTT
COX2	GGGCTCAGCCAGGCAGCAAAT GCACTGTGTTTGGGGTGGGCT
MAPK	CCAGATGCCGAAGATGAACT GGGCTGCTGTGATCCTCTTAT
JNK	TTCCATTGTGGGTAGGTGG CTTACAGCTTCCGCTTCAG
NF-KB p65	ATACGTCGGCCGTGTCTAT GGAAGTGTGATCCGTGTAGG
CYP2E1	CCAGTGGAGTCTACATTCT TTCATTCTGTGTTCTAACTGG

Western blotting

All the original data obtained during the experiment were processed with SPSS 20.0 analysis software, the validity of the original data was preserved, and the data was multiple-compared. The experimental results obtained are expressed as mean \pm standard deviation ($\bar{x}\pm\text{SD}$), one-way analysis of variance was used for comparison among multiple groups, and the SNK-q test was used for pairwise comparison. For statistically significant differences, use $P<0.05$. Graphpad prism 7.0 is used to draw the histogram.

Results and discussion

Detection of liver function

After successful modeling, the venous blood was drawn from the caudal vein and centrifuged, and the supernatant was collected to detect the content of biochemical indexes ALT, AST and LDH. As shown in Table 2, the levels of serum AST, ALT and LDH were significantly increased in the model group ($p<0.05$), suggesting the successful establishment of the liver injury model, and the subsequent experiments could be performed.

Table 2. Content of ALT, AST and LDH (U/L)

Group/index	ALT	AST	LDH
Normal group	10.23 \pm 0.58	50.78 \pm 1.59	5.23 \pm 0.23
Model group	149.88 \pm 1.26 ^a	199.21 \pm 2.47 ^a	120.56 \pm 2.56 ^a

Note: The levels of serum AST, ALT and LDH are significantly increased in model group. ^a $p<0.05$ vs. normal group

Morphological observation of primary hepatocytes

The specific changes in adherent growth of hepatocytes in each group were observed under an inverted microscope (Figure 1). In the control group, the hepatocytes were in an oval shape, tiled, adhered well to the wall and distributed evenly, with obvious intercellular space, and the cell growth status was good like slab stones (Figure 1A). In the model group, there were more suspended cells, the pseudopods grew, and the cells were in a polygonal shape with poor growth status (Figure 1B). In the 1 mM DLWE group and 2 mM DLWE group, the cells adhered well to the wall, and they were mostly in an oval shape with good growth status, similar to the control group (Figures 1C & 1D).

CCK-8 cell proliferation assay

CCK-8 cell proliferation assay was performed to detect the absorbance of cells in each group at different time points. As shown in Figure 2, at 24, 48, and 72 h, the proliferation ability of hepatocytes was significantly stronger in the control group than that in other groups ($p<0.05$), it was weaker in the model group ($p<0.05$), and the ability in 1 mM DLWE group and 2 mM DLWE group was close to that in the control group, indicating that DLWEs are not toxic to hepatocytes and can promote the proliferation activity of hepatocytes.

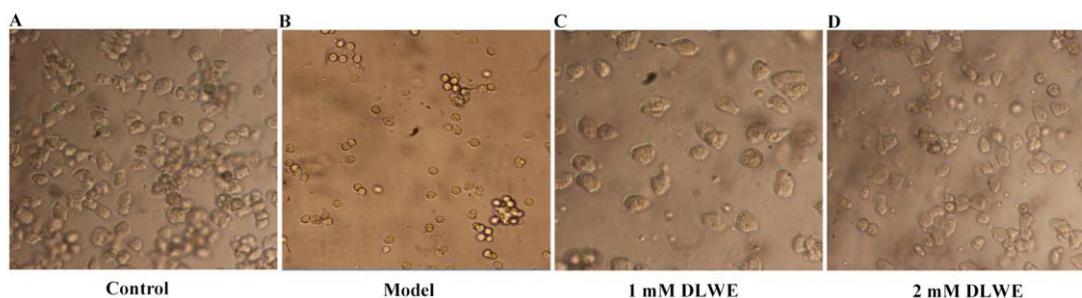


Figure 1. Morphological observation of hepatocytes in each group. A: Control group, B: Model group, C: 1 mM DLWE group, D: 2 mM DLWE group. In the control group, the hepatocytes are in an oval shape, tiled, adhere well to the wall and distributed evenly, with obvious intercellular space, and the cell growth status is good (1A, $\times 40$). In the model group, there are more suspended cells, the pseudopods grow, and the cells are in a polygonal shape with poor growth status (1B, $\times 40$). In 1 mM DLWE group and 2 mM DLWE group, the cells adhere well to the wall, and they are mostly in an oval shape with good growth status, similar to the control group (1C & 1D, $\times 40$).

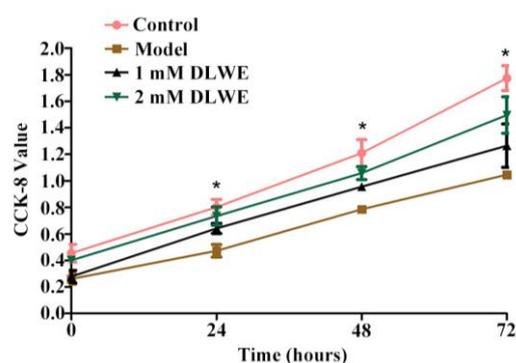


Figure 2. CCK-8 assay. At 24, 48, and 72 h, the proliferation ability of hepatocytes is significantly stronger in the control group than that in other groups ($p < 0.05$), it is weaker in the model group ($p < 0.05$), and the ability in 1 mM DLWE group and 2 mM DLWE group is close to that in the control group. * $p < 0.05$.

Liver function indexes in each group

After treatment for 24 h, the supernatant in each group was collected to detect the content of biochemical indexes ALT, AST and LDH. As shown in Table 3, the content of LDH, ALT and AST was significantly increased in the model group ($p < 0.05$), while it significantly declined in 1 mM DLWE group and 2 mM DLWE group compared with that in the model group ($p < 0.05$), suggesting that 1 mM DLWE and 2 mM DLWE can significantly improve liver injury.

Table 3. Content of LDH, ALT and AST in cells (U/L)

Group/index	ALT	AST	LDH
Control	8.23 \pm 0.54	15.78 \pm 1.59	5.41 \pm 0.23
Model	49.88 \pm 1.26 ^a	99.21 \pm 2.47 ^a	90.56 \pm 2.85 ^a
1 mM DLWE	20.56 \pm 0.23 ^b	40.05 \pm 2.61 ^b	30.21 \pm 0.58 ^b
2 mM DLWE	12.47 \pm 0.58 ^b	30.21 \pm 2.47 ^b	10.28 \pm 0.97 ^b

Note: The content of LDH, ALT and AST is significantly increased in model group ($p < 0.05$), while it significantly declines in 1 mM DLWE group and 2 mM DLWE group compared with that in model group ($p < 0.05$). ^a $p < 0.05$ vs. control group, ^b $p < 0.05$ vs. model group

Oxidative and antioxidant factors in each group

The changes in the concentration of oxidative stress indexes in each group were detected via ELISA. The results revealed that compared with those in the control group, the concentration of oxidative index ROS was increased ($p < 0.05$), while the concentration of antioxidant indexes GSH and GSH-Px declined in the model group ($p < 0.05$). In the 1 mM DLWE group and 2 mM DLWE group, the changes in ROS, GSH and GSH-Px were opposite to those in the model group ($p < 0.05$). The above findings demonstrate that 1 mM DLWE and 2 mM DLWEs can ameliorate the redox imbalance in liver injury (Figure 3).

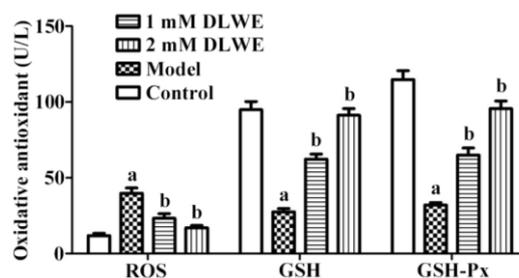


Figure 3. Detection of oxidative antioxidant factors. The concentration of oxidative index ROS is increased ($p < 0.05$), while the concentration of antioxidant indexes GSH and GSH-Px obviously declines in the model group ($p < 0.05$). In 1 mM DLWE group and 2 mM DLWE group, the changes in ROS, GSH and GSH-Px are opposite to those in the model group ($p < 0.05$). ^a $p < 0.05$ vs. control group, ^b $p < 0.05$ vs. model group.

Apoptosis in each group detection via flow cytometry

In this study, the apoptosis level in each group was detected via flow cytometry. In the control group, there were few apoptotic cells, and they almost could not be observed (Figure 4A). In the model group, the

number of positive cells was larger than that in the control group (Figure 4B). In 1 mM DLWE group and 2 mM DLWE group, there were fewer apoptotic cells

than model group (Figures 4C & 4D) ($p < 0.05$), suggesting that 1 mM DLWE and 2 mM DLWE can inhibit APAP-induced apoptosis of hepatocytes.

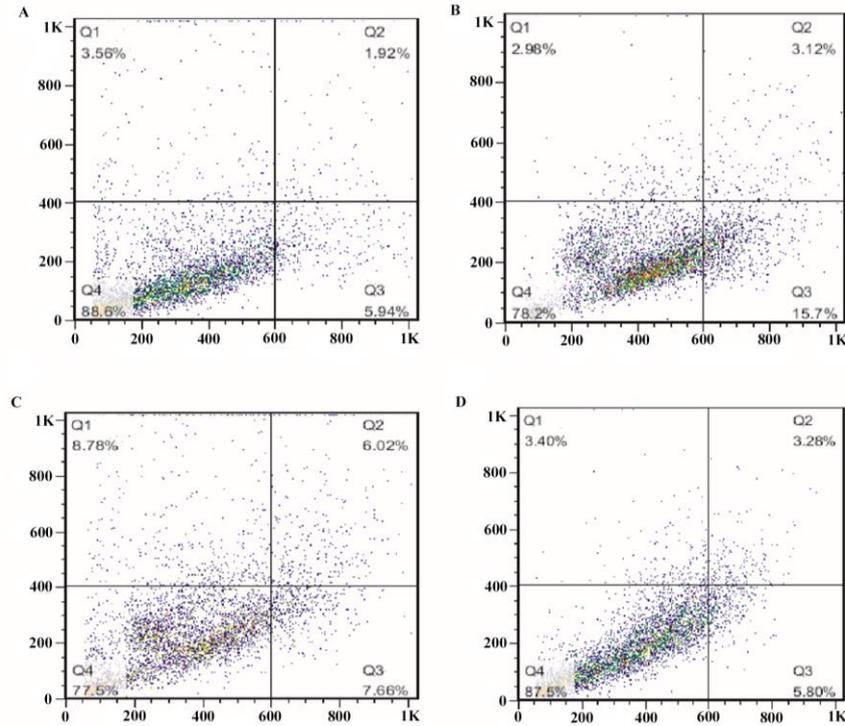


Figure 4. Apoptosis level in each group. A: Control group, B: Model group, C: 1 mM DLWE group, D: 2 mM DLWE group. In the control group, there are few apoptotic cells, and they almost cannot be observed. In the model group, the number of positive cells is obviously larger than that in the control group. In 1 mM DLWE group and 2 mM DLWE group, there are fewer apoptotic cells than the model group ($p < 0.05$). ^a $p < 0.05$ vs. control group, ^b $p < 0.05$ vs. model group.

Related gene expressions detection via RT-PCR

According to the results of RT-PCR (Figure 5), the levels of TNF- α , COX-2, CYP2E1, MAPK, JNK and NF- κ B p65 were remarkably higher in the model group ($p < 0.05$), while they were remarkably lower in 2 mM DLWE group, close to those in the control group. The above results indicate that the DLWE intervention suppresses the expressions of inflammatory factors, liver injury molecules and inflammatory pathways, and inhibits the occurrence of APAP-induced liver injury.

Western blotting

The protein detection results manifested that the model group had remarkably increased levels of CYP2E1, MAPK and NF- κ B p65 ($p < 0.05$), while 1 mM DLWE group and 2 mM DLWE group had the opposite levels to those in model group ($p < 0.05$) (Figure 6), suggesting that DLWE intervention inhibits the expressions of MAPK and NF- κ B p65, further suppressing liver injury.

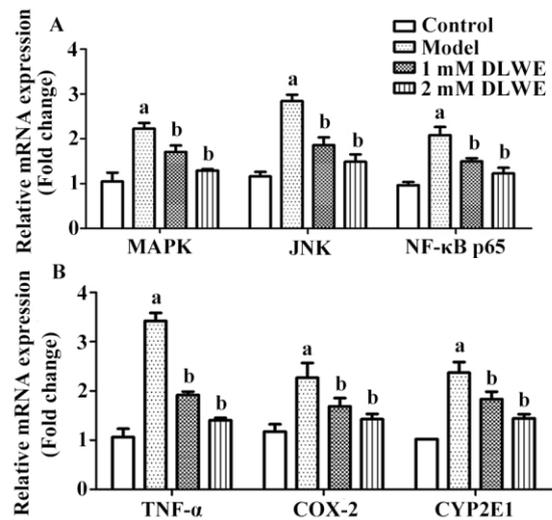


Figure 5. Gene expression levels. A: The levels of MAPK, JNK and NF- κ B p65. B: The levels of TNF- α , COX-2 and CYP2E1. The levels of TNF- α , COX-2, CYP2E1, MAPK, JNK and NF- κ B p65 are remarkably higher in the model group ($p < 0.05$), while they are remarkably lower in 2 mM DLWE group, close to those in the control group. ^a $p < 0.05$ vs. control group, ^b $p < 0.05$ vs. model group.

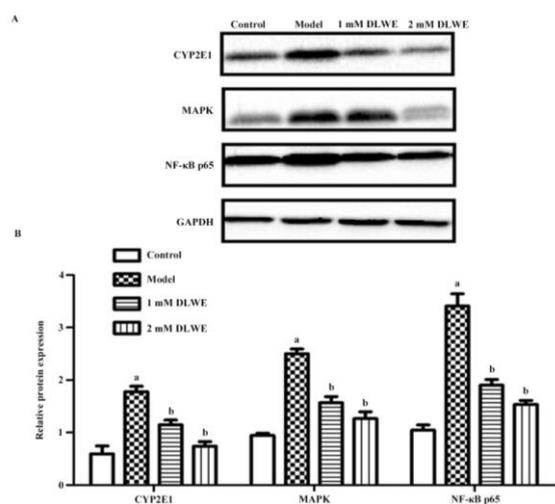


Figure 6. Protein expression. A: Western Blot of protein expression. B: Relative protein expression. The model group has remarkably increased levels of CYP2E1, MAPK and NF- κ B p65 ($p < 0.05$), while 1 mM DLWE group and 2 mM DLWE group have the opposite levels to those in the model group ($p < 0.05$). ap<0.05 vs. control group, bp<0.05 vs. model group

The liver is the main site of the body's detoxification, as well as the main target for the drug contact in the body. Therefore, drug-induced liver injury is a serious public health problem (20). APAP is a commonly-used analgesic antipyretic, which is usually safe with good tolerance at therapeutic doses. However, the overdose of APAP is the most common cause of drug-induced acute liver failure (21). The toxicity of APAP is related to the biological activity of cytochrome P450 to electrophilic metabolite NAPQI. Under the excessive therapeutic dose, both glucuronidation and sulfation pathways will become saturated, and the liver GSH is depleted by extensive bioactivation of APAP, leading to oxidative stress (22). Oxidative stress may trigger the signaling pathways through mitochondrial toxicity, ultimately causing cell death. There is a large amount of evidence that oxidative stress may be involved in the toxicity of APAP (23). Several studies have demonstrated that antioxidants and anti-inflammatory agents can effectively protect the body from acute hepatotoxicity caused by APAP overdose (24). Therefore, this experiment studies the protective effect of dandelion extract on APAP-induced drug-induced liver injury in rats and its specific mechanism. First, an animal modeling experiment was used to observe whether the liver function-related indicators would change after modeling. It further

indicates the development of liver damage and proves whether the model animals are successful, in order to provide an important reference for early diagnosis, predict the occurrence of diseases in advance, and prepare for follow-up experiments. The clinical application of liver function tests is of great value in the diagnosis, treatment, and prognosis of liver diseases. Among them, AST, ALT, and LDH are common indicators for clinical evaluation of liver damage. The results of this experiment to detect AST, ALT, and LDH indicate that the index level is found. The content of LDH, ALT and AST in the model group was significantly increased, indicating that the APAP-induced rat model of liver injury was successful, and subsequent experiments can be carried out. After separating the primary hepatocytes from the normal group and the model group of animals and adding dandelion intervention, the status of the hepatocytes in each group was observed under an inverted microscope. In the normal group, the hepatocytes were oval, flat, and adherent well, the cells were evenly arranged, the intercellular spaces were obvious, and the cells grew in good condition and were paving stone-like. The liver injury model group has a large amount of cell suspension, pseudopodia, a polygonal shape, and poor growth. The medium-dose dandelion extract group (1mM DLWE) and the high-dose dandelion extract group (2mM DLWE) adhered well to the cells, mostly oval, similar to the normal group, and grew well. Subsequently, we used the CCK-8 proliferation experiment to detect the absorbance of each group of cells at different times. The results showed that at 24, 48, and 72 h, the proliferation ability of hepatocytes in the normal group was significantly higher than that in the other groups, and the model group had lower cell proliferation. The 1mM DLWE and 2mM DLWE groups were similar to the control group. It shows that dandelion extract is not toxic to liver cells and can promote the proliferation activity of liver cells. In addition, the detection results of ALT, AST and LDH in the cell culture supernatant showed that the LDH, ALT, and AST contents of the model group were significantly increased, and the contents of the 1mM DLWE and 2mM DLWE groups were significantly reduced compared with the model group. It shows that the dandelion extract in the 1mM DLWE and 2mM DLWE groups can significantly improve liver

damage. This is consistent with previous studies (25-26).

GSH-Px is a major peroxide detoxifying enzyme that catalyzes the intercellular reductive GSH, which is a hydrogen donor for the production of oxidized GSH, and it also scavenges H₂O₂ and catalyzes the reduction of peroxides in hepatocytes (27). In addition, studies have found that TMC can effectively protect the GSH level in the liver in APAP-treated mice. GSH is an important ROS scavenger that can be involved in the ROS scavenging by reducing peroxides in the presence of GSH-Px (28). In hepatocytes, high-dose APAP (500 mg/kg) leads to the depletion of GSH and results in the binding of toxic NAPQI to essential hepatocyte proteins, causing acute liver injury. Besides, maintaining the GSH level is of great importance for protecting thiols and other nucleophilic groups in proteins from toxic metabolites in APAP-induced liver injury (29). In the present study, the concentration of oxidative index ROS was increased, while the concentration of antioxidant indexes GSH and GSH-Px declined in the model group. In the 1 mM DLWE group and 2 mM DLWE group, the changes in ROS, GSH and GSH-Px were opposite to those in the model group. The above findings demonstrate that 1 mM DLWE and 2 mM DLWE can ameliorate the redox imbalance in liver injury. Apoptosis can promptly scavenge the garbage generated during the maintenance of the body's vital activities, which, as the body's defender, supplies energy for the production of subcellular structure and metabolism, and maintains the cell stability (30). The mechanism and pathway of apoptosis in the physiological metabolism of organisms can serve as important treatment guidelines for a variety of diseases, such as tumors and liver injury. In this experiment, there were few apoptotic cells, and they almost could not be observed in the control group. In the model group, the number of positive cells was larger than that in the control group. In the 1 mM DLWE group and 2 mM DLWE group, there were fewer apoptotic cells than the model group, suggesting that 1 mM DLWE and 2 mM DLWE can inhibit APAP-induced apoptosis of hepatocytes.

Studies have shown that the lack of glutathione in APAP-induced liver injury enhances the expression of TNF- α and induces cell damage. In addition to pro-inflammatory cytokines, APAP can also induce

overexpression of inflammatory COX-2 in the liver of mice (31), among them, NF- κ B p65 and MAPK signal pathways are common signal pathways in the liver injury-related research. F- κ B is activated in liver injury and achieves liver protection through anti-inflammatory and antioxidant effects. MAPK-JNK can regulate the expression of inflammatory factors such as TNF- α and IL-1. The activation of MAPK-JNK is considered to be an important signal element of APAP-induced hepatotoxicity, which further promotes the development of drug-induced liver injury (32), NF- κ B is an important transcription factor in the inflammatory process. It can regulate the expression of genes related to the inflammatory cascade and participate in the inflammatory response and oxidative stress response. Other studies have shown that NF- κ B plays an important role in inducing liver damage induced by APAP, and its content can be used to indicate the severity of liver damage (33). Studies have found that CYP2E1 knockout mice are resistant to APAP-induced liver damage, and down-regulation of this gene can lead to a decrease in reactive metabolites. In addition, APAP increases the expression of CYP2E1, while DLWE can significantly inhibit the expression of CYP2E1, thereby improving oxidative stress (34-36). Genetic testing found that TNF- α , COX-2, CYP2E1, MAPK, JNK, and NF- κ B p65 were significantly increased in the model group, and the gene expression in the 2mM DLWE group was the opposite and close to the control group. It shows that dandelion extract inhibits the expression of inflammation, liver injury indicator molecules and inflammatory pathways after the intervention, and inhibits the occurrence of APAP-induced liver injury. The protein results showed that CYP2E1, MAPK, and NF- κ B p65 increased significantly in the model group. The expression changes of the 1mM DLWE and 2mM DLWE groups were opposed to the model group. It further shows that dandelion extract inhibits the expression of MAPK and NF- κ B p65 after the intervention, and further inhibits the occurrence of liver injury. This is consistent with the above research results. This study further demonstrated that dandelion extract has a protective effect on APAP-induced drug-induced liver injury in rats by detecting oxidative stress, apoptosis, and inflammation pathways. More genes and proteins can be included in the follow-up to further verify this

effect. In this regard, it is suggested to study other extracts (37-40).

In conclusion, DLWEs may inhibit the occurrence of oxidative stress and apoptosis through suppressing MAPK and NF- κ B pathways, thereby resisting APAP-induced liver injury. In the future, such an effect can be explored using more techniques. The experimental results enrich and improve the theoretical basis of the protective effect of DLWE on APAP-induced liver injury and its effects on MAPK and NF- κ B signaling pathways, which also provides a theoretical basis for the development of new anti-liver injury drugs.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LW wrote the manuscript. LW, LZ and XH were responsible for the establishment of the animal model. JW, JL and JD performed flow cytometry, PCR and Western blot. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the ethics committee of Lanzhou University Second Hospital.

Consent for publication

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

References

1. Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A: The interaction of hepatic lipid and glucose metabolism in liver diseases. *J. Hepatol* 2012; 56: 952-964.
2. Lee KJ, Choi JH, Hwang YP, Chung YC, Jeong HG: Protective effect of caffeic acid phenethyl ester on tert-butyl hydroperoxide-induced oxidative hepatotoxicity and DNA damage. *Food Chem. Toxicol* 46: 2445-2450, 2008.
3. Prescott, L.F: Hepatotoxicity of mild analgesics. *Br. J. Clin. Pharmacol* 1980;110: 373-379.
4. Ji L, Jiang P, Lu B, Sheng Y, Wang X, Wang Z: Chlorogenic acid, a dietary polyphenol, protects acetaminophen-induced liver injury and its mechanism. *J. Nutr. Biochem.* 2013; 24: 1911-1919.
5. Dahlin DC, Miwa GT, Lu AY, Nelson SD: N-acetyl-p-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen. *Proc Natl Acad Sci* 1984;81: 1327-1331.
6. Saito C, Zwingmann C, Jaeschke H: Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. *Hepatology* 2010;51:246-254.
7. Kanter MZ: Comparison of oral and i.v. acetylcysteine in the treatment of acetaminophen poisoning. *Am J Health Syst Pharm* 2006;63:1821-1827.
8. Saito C, Lemasters JJ, Jaeschke H: c-Jun N-terminal kinase modulates oxidant stress and peroxynitrite formation independent of inducible nitric oxide synthase in acetaminophen hepatotoxicity. *Toxicol Appl Pharmacol* 2010; 246:8-17.
9. Kanter MZ: Comparison of acetylcysteine in the treatment of acetaminophen poisoning. *Am J Health Syst Pharm* 2009; 63:181-187.
10. Whyte AJ, Kehrl T, Brooks DE, Katz KD, Sokolowski D: Safety and effectiveness of acetadote for acetaminophen toxicity. *J Emerg Med* 39:607-611, 2010.
11. Wang AY, Lian LH, Jiang YZ, Wu YL, Nan JX: *Gentiana manshurica* Kitagawa prevents acetaminophen-induced acute hepatic injury in mice via inhibiting JNK/ERK MAPK pathway. *World J. Gastroenterol* 16: 384-391, 2010.
12. Yousef MI, Omar SA, El-Guendi MI, Abdelmegid LA: Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. *Food Chem Toxicol* 2010;48: 3246-3261.
13. Rice-Evans CA, Miller NJ, Bolwell PG., Bramley PM, Pridham JB: The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Res* 1995;22: 375-383.
14. Yazdanparast R, Amin Ardestani A: Suppressive effect of ethyl acetate extract of *Teucrium polium* on

- cellular oxidative damages and apoptosis induced by 2-deoxy-d-ribose: Role of de novo synthesis of glutathione. *Food Chem* 2009;114: 1222–1230.
15. Rigano D, Cardile V, Formisano C, Maldini MT, Piacente S, Bevelacqua J, Russo A, Genista sessilifolia DC, Genista tinctoria L: Inhibit UV light and nitric oxide-induced DNA damage and human melanoma cell growth. *Chem Biol Interac* 2009;180: 211–219.
 16. Wang HB: Cellulase-assisted extraction and antibacterial activity of polysaccharides from the dandelion *Taraxacum officinale*. *Carbohydr. Polym* 2014;103: 140–142.
 17. Schütz K., Carle R, Schieber A: *Taraxacum*—A review on its phytochemical and pharmacological profile. *J. Ethnopharmacol* 2006;1107: 313–323.
 18. Davaatseren M, Hur HJ, Yang HJ, Hwang JT, Park JH, Kim HJ, Kim MJ, Kwon DY, Sung MJ: *Taraxacum officinale* (dandelion) leaf extract alleviates high-fat diet-induced nonalcoholic fatty liver. *Food Chem Toxicol* 2013;8, 30–36.
 19. Zhao Hui, Wang Hongli, Lu Jia, et al. Protective effects of Yiganning granules on paracetamol-induced liver injury in mice. *Zhongnan Pharmacy*, 2017, 015(005):587-590.
 20. Kumari A, Kakkar P: Lupeol prevents acetaminophen-induced in vivo hepatotoxicity by altering the Bax/Bcl-2 and oxidative stress-mediated mitochondrial signaling cascade. *Life Sci* 2012;90:561-570.
 21. Morgan OW, Griffiths C, Majeed A: Interrupted Time-Series Analysis of Regulations to Reduce Paracetamol (Acetaminophen) Poisoning. *PLoS Medicine* 2017;4: e105.
 22. Delmastro MM, Piganelli JD: Oxidative stress and redox modulation potential in type 1 diabetes. *Clin Dev Immunol* 2011;11:593863.
 23. Toyoda Y, Tamai M, Kashikura K: Acetaminophen-induced hepatotoxicity in a liver tissue model consisting of primary hepatocytes assembling around an endothelial cell network. *Drug Metab Dispos* 2012;40:169.
 24. Oz HS, Chen TS: Green-tea Polyphenols Downregulate Cyclooxygenase and Bcl-2 Activity in Acetaminophen-induced Hepatotoxicity. *Dig Dis Sci* 2008;53:2980-2988.
 25. Shine VJ, Latha PG, Suja SNR: Ameliorative effect of alkaloid extract of *Cyclea peltata* (Poir.) Hook f & Thoms roots (ACP) on APAP/CC14 induced liver toxicity in Wistar rats and in vitro free radical scavenging property. *Asian Pac J Trop Biomed* 2014;4: 143-151.
 26. Yi RK, Song JL, Lim YI: Preventive Effect of the Korean Traditional Health Drink (Taemyeongcheong) on Acetaminophen-Induced Hepatic Damage in ICR Mice. *Prev Nutr Food Sci* 2015; 20:52-59.
 27. Zhu R, Wang Y, Zhang L: Oxidative stress and liver disease. *Hepatology Research* 2012; 42:1-10.
 28. Halliwell B, Gutteridge JC: Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. *Lancet* 1984;323:1396-1397.
 29. Halliwell B: Free radicals and antioxidants: updating a personal view. *Nutr Rev* 2012;70: 257-265.
 30. Klionsy DJ: Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol* 2007;8: 931-937.
 31. Reilly TP, Brady JN, Marchick M: A Protective Role for Cyclooxygenase-2 in Drug-Induced Liver Injury in Mice. *Chem Res Toxicol* 2001;14: 1620-1628.
 32. Gunawan BK, Liu ZX, Han D: c-Jun N-Terminal Kinase Plays a Major Role in Murine Acetaminophen Hepatotoxicity. *Gastroenterol* 2006;131: 165-178.
 33. Fan X, Chen P, Jiang Y, Wang Y, Tan H, Zeng H, Wang Y, Qu A, Gonzalez FJ, Huang M, et al: Therapeutic Efficacy of Wuzhi Tablet (*Schisandra sphenanthera* Extract) on Acetaminophen-Induced Hepatotoxicity through a Mechanism Distinct from N-Acetylcysteine. *Drug Metab Dispos* 2015;43: 317-324.
 34. Park CM, Cha YS, Youn HJ, Cho CW, Song YS: Amelioration of oxidative stress by dandelion extract through CYP2E1 suppression against acute liver injury induced by carbon tetrachloride in sprague-dawley rats. *Phytother Res* 2010;24: 1347-1353.
 35. Cai L, Wan D, Yi F, Luan L: Purification, Preliminary Characterization and Hepatoprotective Effects of Polysaccharides from Dandelion Root. *Molecules* 2017; 22: 1409-1425.
 36. Perrone MA, Iellamo F, Donatucci B, Caminiti G, Lombardo M: Oxidative stress, redox state and antioxidant supplementation in physical exercise and professional sports: a brief review. *Acta Med Mediterr* 2020;36: 1245-1251.
 37. Ghamarnia H, Mousabeygi F, Rezvani S. Water Requirement, Crop Coefficients of Peppermint (*Mentha piperita* L.) and Realizing of SIMDualKc Model. *Agrotech Ind Crops* 2021; 1(3): 110-121. doi: 10.22126/atic.2021.6791.1019.
 38. Aryafar S, Sirousmehr A, Najafi S. The Impact of Compost on Seed Yield and Essential Oil of Black Cumin under Drought Stress Conditions. *Agrotech Ind Crops* 2021; 1(3): 139-148. doi: 10.22126/atic.2021.7184.1026.
 39. Almasi, F. Organic Fertilizer Effects on Morphological and Biochemical Traits and Yield in Coriander (*Coriandrum sativum* L.) as an Industrial and Medicinal Plant. *Agrotech Ind Crops* 2021; 1(1): 19-23. doi: 10.22126/etic.2021.6476.1011.
 40. Shaafi, B., Mosavi, S., Abdollahi, M., Sarikhani, H. The Optimized Protocols for Production, Adaptation and Keeping of the Produced Artificial Seeds from Encapsulated Lateral Buds in *Stevia rebaudiana* (Bertoni). *Agrotech Ind Crops* 2021; 1(1): 24-35. doi: 10.22126/etic.2021.6246.1004