



## Original Article

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Hepatorenoprotective effects of *Lepidium draba* L. extracts against cyclophosphamide-induced oxidative injuries in rats *via* reducing apoptosis and inflammationYu-Lai You<sup>1#</sup>, Sheng Zheng<sup>2#</sup>, Cheng-Jian Zhao<sup>3</sup>, Ye-Fei Chang<sup>4</sup>, Pei Liu<sup>5</sup>, Xue-Li Zeng<sup>5</sup>, Lian Liu<sup>6✉</sup><sup>1</sup>Department of Hepatobiliary Surgery, Chongqing University Jiangjin Hospital, Chongqing 402260, China<sup>2</sup>Department of Gastroenterology, The Third People's Hospital of Yunnan Province, Kunming, Yunnan 650011, China<sup>3</sup>Hepatobiliary Pancreatic Surgery, Qingdao Municipal Hospital, Qingdao, Shandong 266071, China<sup>4</sup>Department of Laboratory Medicine, The Third People's Hospital of Yunnan Province, Kunming, Yunnan 650011, China<sup>5</sup>Graduate School of Clinical Medicine, Dali University, Dali, Yunnan 671000, China<sup>6</sup>Department of Pharmacy, Shanghai Fengxian District Central Hospital, Shanghai 201406, China

## ABSTRACT

**Objective:** To investigate the protective effects of *Lepidium draba* L. (*L. draba*) on cyclophosphamide (CP)-induced hepatotoxicity and nephrotoxicity in rats.

**Methods:** A total of 36 rats were divided into six groups as follows: the sham control group, the CP group (CP 100 mg/kg *i.p.* on days 1, 7, 14, 21, 28, and 35), the CP groups treated with *L. draba* extract (100, 200 and 400 mg/kg of *L. draba* extract for 28 d), and the *L. draba* extract alone group (400 mg/kg of *L. draba* extract for 28 d). Serum parameters of renal and hepatic function, as well as pro-inflammatory and anti-inflammatory cytokines associated with liver and kidney damage were measured. Moreover, *Bax*, *Bcl-2*, and *caspase-3* gene expression and histopathological changes were assessed.

**Results:** *L. draba* extract alleviated CP-induced hepatotoxicity and nephrotoxicity by decreasing nitric oxide, TBARS, IL-6, TNF- $\alpha$ , and IL-1 $\beta$  levels, as well as increasing superoxide dismutase, catalase and glutathione peroxidase activities, and FRAP, MIF, and TGF- $\beta$  levels. In addition, the extract downregulated the expression of pro-apoptotic genes (*Bax* and *caspase-3*) and mitigated the destruction of glomeruli and renal tubules as well as the degeneration of hepatocytes.

**Conclusions:** *L. draba* extract can protect hepatic and renal structure and function against CP-induced toxicities, and may be used as a therapeutic agent for CP-induced hepatotoxicity and nephrotoxicity.

**KEYWORDS:** *Lepidium draba*; Cyclophosphamide; Oxidative stress; Antioxidant; Apoptosis; Hepatotoxicity; Nephrotoxicity

## 1. Introduction

Antineoplastic agents promote drug-induced apoptosis of cancer cells by enhancing the expression of caspase pathways and activating apoptotic cascade receptors. This process results in the generation of reactive oxygen species (ROS) and electrophilic aldehydes, which can lead to the oxidative degradation of healthy tissues[1]. ROS impairs the physiological function of healthy cells by targeting a variety of biological macromolecules, including proteins, DNA, and polyunsaturated fatty acids. This results in the breakdown of

## Summary

**Question:** Do *Lepidium draba* L. extracts have protective effects on cyclophosphamide-induced hepatotoxicity and nephrotoxicity?

**Findings:** *Lepidium draba* L. extracts could considerably reduce lipid peroxidation, inflammatory cytokines and apoptotic genes in rats with cyclophosphamide-induced injuries.

**Meaning:** *Lepidium draba* L. extracts have antioxidant, anti-inflammatory and anti-apoptotic effects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity.

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double helix chains, the inhibition of DNA precursor synthesis, and cell cycle checkpoint arrest[2,3]. By first activating initiator caspases (caspases 8 and 9) and then effector caspases (caspases 3 and 6), the family of alkylating antineoplastic agents causes cytochrome C release and triggers an apoptotic cascade in normal cells *via* the p53/CD95 signaling pathways[4].

Cyclophosphamide (CP), an alkylating antineoplastic agent, is used directly or through its metabolites (phosphoramidate mustard) to treat a variety of tumors, such as neuroblastoma, lymphoma, leukemia, sarcoma, ovarian cancer, breast cancer, and small cell lung cancer. It also causes crosslinks between DNA chains, which induces apoptosis in cancer cells. The use of this crucial chemotherapeutic medication has been restricted due to extensive adverse effects, which include bone marrow suppression, acute pneumonitis, progressive pulmonary fibrosis, cardiotoxicity, lupus nephritis, bacterial-fungal infections, arthritis, and infertility[5]. Chronic acute renal and hepatic impairment are caused by CP's hepatic metabolism (cytochrome P450 system) and renal excretion, which also alters the physiological structure and function of these organs. Similar research has demonstrated that intraperitoneal injection of 50-100 mg/kg CP results in hepatorenal toxicity, immune system suppression, and hormonal pathway suppression, including hypothalamus-pituitary-gonad, by influencing the apoptotic pathways Bax/Caspase-3, 6, and 7/p53/cytochrome C, and the NF- $\kappa$ B/MAPK pathway, increases ROS, inhibits cell cycle-related enzymes, and suppresses DNA replication in a variety of animal models[6–8]. According to previous studies, CP (at doses <50 mg/kg/*i.p.* injection) inhibited the physiological function of hepatocytes, which raised serum albumin (ALB) and total protein (TP) levels and lowered hepatic enzyme activity [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)][9,10]. CP enhances the inflammatory pathways and causes an increase in lymphocyte infiltration, neutrophilic-macrophage migration, and leukocyte-inflammatory foci regeneration in liver and kidney tissues by raising serum levels of pro-inflammatory cytokines [tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), IL-1 $\beta$ , and cyclooxygenase-2] and lowering anti-inflammatory cytokines [IL-10, macrophage migration inhibitory factor (MIF), and transforming growth factor- $\beta$  (TGF- $\beta$ )] [11–13]. Because medicinal plants contain a lot of natural antioxidants, particularly flavonoids like quercetin, catechin, genistein, daidzein, and kaempferol, they can prevent cancer cells from growing at different phases of their cell cycle without affecting healthy cells[14].

*Lepidium draba* L. (*L. draba*) is a plant of the Brassicaceae family that contains a lot of polyphenolic chemicals, such as flavonoids and isoflavones. Originally from Eastern Europe, the Middle East, and Southeast Asia, this plant can be found growing all over the world. With clustered flowers (3–4 cm) and oval leaves (7–10 cm), this plant is 35–45 cm long[15]. In traditional medicine, the plant's leaves and roots are used to treat digestive issues, menopausal symptoms, menstrual cramps, wound healing, and male infertility.

This herb has been shown in numerous trials to have anti-diabetic, anti-hyperlipidemia, anti-tumor (breast, prostate, and colon), anti-inflammatory, and anti-depressant properties[16]. The presence of significant flavonoid compounds, including quercetin, apigenin, *p*-coumaric acid, catechin, kaempferol, ferulic acid, and hecogenin, has been demonstrated by liquid chromatography electrospray ionization tandem mass spectrometry analysis of the roots and leaves of this plant[17]. In the current study, we aimed to examine the protective effects of *L. draba* on the liver and kidney in rats of CP-induced toxicity by performing biochemical, antioxidant, anti-inflammatory, and anti-apoptotic analyses.

## 2. Materials and methods

### 2.1. Preparation of *L. draba* hydroalcoholic extract

The fresh leaves of *L. draba*, after collection and approval of its identity (voucher number: K001625777), were dried for 72 h at 35 °C and ground (Humboldt soil grinder, H-4199.5F, United States); 520 g of the powder was dissolved in 1 500 mL of 70% ethanol (70 ethanol/30 water, *v/v*). Then, after 72 h, the resulting mixture was filtered through a filter paper (No. 42, Millipore, United States), condensed with an evaporator (Rota vapor R-210, Buchi, Switzerland), and finally dried at 40 °C. Finally, 85 g of the *L. draba* hydroalcoholic extract (an efficiency rate of 16.3%) was stored at 4 °C until use[18].

### 2.2. Animals and experimental design

A total of 36 Wistar rats weighing (180  $\pm$  10) g were used. The animals were provided from the Breeding Center of Laboratory Animals, the Third People's Hospital of Yunnan Province. All rats were kept at a temperature of (21  $\pm$  2) °C, a relative humidity of (55 $\pm$ 5)%, a 12 h:12 h light:dark cycle, and in a propylene cage with free access to water and standard rat pellets. The pellets contained macronutrients including protein (18%–22%, derived from soybean meal), fat (4%–6%, sourced from soybean oil), carbohydrates (50%–60%, from corn), and fiber (4%–6%, from cellulose), with an energy content of approximately 3.0–3.5 kcal/g. The mineral composition included calcium (0.8%–1.2%), phosphorus (0.6%–0.9%), magnesium (0.2%–0.4%), cobalt (0.5–1 ppm), iron (70–150 ppm), zinc (50–100 ppm), copper (6–10 ppm), manganese (50–100 ppm), selenium (0.1–0.3 ppm), and iodine (0.2–0.5 ppm).

After 72 h, the animals were divided into 6 groups (*n*=6/group) as follows (Supplementary Figure): The sham control (SC) group receiving 1 mL of distilled water by gavage daily; the CP control group given 100 mg/kg intraperitoneally (*i.p.*) CP on days 1, 7, 14, 21, 28, and 35; the *L. draba* extract and CP co-treatment groups (100,

200 and 400 *L. draba* + CP) treated with 100, 200 and 400 mg/kg of the extract daily by gavage from the 7th day for 28 d and CP similar to the previous group; the 400 mg/kg *L. draba* group receiving 400 mg/kg of *L. draba* extract daily by gavage from the 7th day for 28 d.

The effective and safe doses used in this study were selected after calculating the LD<sub>50</sub>, the pilot study, and finally in comparison with other studies[19,20]. The *L. draba* extract and CP were dissolved in 1 mL of distilled water (37 °C) and given to rats at a specific time of day.

### 2.3. Acute toxicity test (LD<sub>50</sub>)

LD<sub>50</sub> is the amount of material given all at once that causes the death of 50% of a group of test animals. The LD<sub>50</sub> is one way to measure the short-term poisoning (acute toxicity) of a material. The Lork's two-step method was used to determine the safe dose of the *L. draba* extracts and acute toxicity (LD<sub>50</sub>). For this purpose, 12 Wistar rats in 4 groups (*n* = 3) first received doses of 1, 10, 100, and 1 000 mg/kg/*i.p.*, and after 24-hour monitoring, any toxic symptoms and possible mortality in the groups were evaluated. Tremor and sudden death were mostly seen in examined animals. In the second stage, 3 other Wistar rats in 3 groups (*n* = 1) received doses of 50, 500, and 5 000 mg/kg/*i.p.* *L. draba* extracts were used, and any toxic symptoms and possible mortality were recorded in the groups. Finally, according to the results obtained in two steps and using the following equation, the LD<sub>50</sub> of *L. draba* extracts was calculated.

$$LD_{50} = (D_0 \times D_{100})^{1/2}$$

Where D<sub>100</sub> is the lowest dose at which symptoms of toxicity or death were observed; D<sub>0</sub> is the highest dose at which no signs of toxicity or death were observed.

### 2.4. Total phenolic content (TPC)

The modified Folin-Ciocalteu method was used to measure TPC of *L. draba* extract[21]. In summary, in this method, the *L. draba* extract (50 µL) was first dissolved in Folin-Ciocalteu solution (1 mL), and the resulting mixture was added to sodium carbonate 7.5% (0.8 mL). Then 50, 75, 100, 200, and 400 µg/mL of gallic acid (50 µL) were added and incubated for 2 h at 30 °C. Finally, the absorbance of the final mixture was read at 765 nm with a spectrophotometer (Shimazu, UV-1800, Shimadzu, Japan) and reported in mg gallic acid equivalent (GAE)/g of *L. draba* dried extract[21].

### 2.5. Total flavonoid content (TFC)

The modified rutin method was used to measure TFC of *L. draba* extract[22]. In summary, in this method, *L. draba* extract (50 µL) was first dissolved in 1 mol/L potassium acetate (0.1 mL), 20 mg/mL aluminum trichloride reagent (2.5 mL) and distilled water (2.8 mL),

and the resulting mixture was added with 0.025, 0.037, 0.050 and 0.075 mg/mL of rutin (2.5 mL). Finally, after incubation for 40 min at 25 °C, the absorbance of final mixture was read at 415 nm with a spectrophotometer (Shimazu, UV-1800, Shimadzu, Japan) and reported as mg rutin equivalents (RUE)/1 g dried *L. draba* extract[22].

### 2.6. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The modified DPPH method was used to measure the radical scavenging activity of *L. draba* extract[23]. In brief, 0.025, 0.050, 0.075, 0.100, 0.125, and 0.150 mg/mL of *L. draba* extract (100 µL) was first added to methanol DPPH' radical solution (3.5 mL, 60 µmol/L) and the resulting mixture incubated for 2 h at 25 °C. Finally, the absorbance of the final mixture was read at 515 nm with a spectrophotometer (Shimazu, UV-1800, Shimadzu, Japan) and reported as µmol Trolox equivalent/10 g dried *L. draba* extract.

### 2.7. Ferric reducing antioxidant power (FRAP) assay

The modified FRAP method was used to measure the total antioxidant capacity (TAC) of *L. draba* extract[23]. In brief, in acidic condition (pH 3.6), 10 mg of *L. draba* extract was added with 3 µL of FRAP solution containing a mixture of 2,4,6-tripyridyl-s-triazine (TPTZ) (1 mL, 40 mmol/L), acetate buffer (10 mL, 300 mmol/L) and FeCl<sub>3</sub>•6H<sub>2</sub>O (1 mL, 20 mmol/L) and they were mixed gently. Then 180 µL of distilled water was added to the final mixture and incubated for 15 min at 37 °C. Finally, after centrifugation (15 000 ×*g* for 10 min), the absorption of the mixture was read at 593 nm with a spectrophotometer (Shimazu Multispect 1501, Shimadzu, Japan) and reported as mmol Fe<sup>2+</sup>-TPTZ /1 mg dried *L. draba* extract[23].

### 2.8. Biochemical examinations

To measure serum parameters in the liver and kidney, after pre-anesthesia and anesthesia with an intraperitoneal injection of ketamine 10% (10 mg/kg) and xylazine 2% (40 mg/kg), rats were sacrificed and weighted (on day 36) and blood sampling were performed through cardiac puncture. Serum was separated from blood samples after centrifugation (3 500 ×*g* for 15 min) and stored at -4 °C. Biocompare ELISA commercial kits (Biocompare, San Francisco CA, USA) were used to measure the serum activity of ALT (Cat no. ABIN6730944), AST (Cat no. MBS2019541) and ALP (Cat no. ABIN4947338) and the serum levels of TP (Cat no. orb739146), ALB (Cat no. MBS1601582), creatinine (Cr; Cat no. MBS745220) and blood urea nitrogen (BUN; Cat no. MBS3801103) according to the manufacturer's recommended protocol[24]. The liver and right kidneys were removed and their weights were recorded.

## 2.9. Measurement of serum cytokines

RayBiotech Life ELISA quantitative/colorimetric/sandwich-based commercial kits (RayBiotech Life, GA, USA) were used to measure the serum levels of IL-6 (Cat no. ELR-IL6-1), IL-1 $\beta$  (Cat no. 268-10110-1), TNF- $\alpha$  (Cat no. ELH-TNF $\alpha$ -1), MIF (Cat no. ELH-MIF-1) and TGF- $\beta$  (Cat no. AAH-TGFB-2-2) according to the manufacturer's recommended protocol[25].

## 2.10. Oxidative stress and antioxidant markers in the serum and liver and kidney tissues

Thiobarbituric acid reactive substances (TBARS) measurement method was used to evaluate the lipid peroxidation level. In this method, the liver and kidneys were crushed with a tissue homogenizer, and 100  $\mu$ L of homogenate was added to TBARS solution (50  $\mu$ L phosphoric acid, 50  $\mu$ L thiobarbituric acid 0.02 mol/L, 2  $\mu$ L butylated hydroxytoluene 0.19 mol/L). The final mixture was then incubated for 30 min at 37°C and centrifuged at 12000 rpm for 10 min. Finally, the absorbance of the final mixture was read at 532 nm with a spectrophotometer (Shimazu, UV-1800, Shimadzu, Japan) and reported as nmol/mg[26].

FRAP method was used to evaluate the TAC level. Similar to the method described in section 2.7, 200  $\mu$ L of homogenized liver and kidney tissue was first added to the FRAP solution. The final mixture was then incubated for 15 min at 25°C and centrifuged at 10000 rpm for 15 min. Finally, the absorbance of the final mixture was read at 593 nm with a spectrophotometer (Shimazu, UV-1800, Shimadzu, Japan) and reported as  $\mu$ mol/mg protein[27].

In the present study, the Griess colorimetric method was used to measure nitric oxide level. Briefly, in this method, zinc oxide (ZnSO<sub>2</sub> 6 mg) was added to serum samples (500  $\mu$ L) and centrifuged (10000  $\times g$  for 15 min). The supernatant was then added to the Griess reagent [50  $\mu$ L *N*-(1-naphthyl) ethylenediamine dihydrochloride 5 mmol/L, 50  $\mu$ L sulfanilamide 20 mmol/L, and 100  $\mu$ L vanadium chloride 40 mmol/L] and incubated for 60 min at 37°C. Finally, the absorbance of the final mixture was read at 540 and 630 nm with a spectrophotometer (Shimazu, UV-1800, Shimadzu, Japan) and reported as mmol/mL[23].

A commercial Mybiosource (Mybiosource Inc., San Diego, California, USA) competitive enzyme immunoassay kit (Cat no. MBS744364) was used to measure the serum activity of glutathione peroxidase (GPx). This kit is based on the reaction of a polyclonal anti-GPx antibody and a GPx-HRP conjugate. For this purpose, 100  $\mu$ L of serum samples, standard kit solution, and PBS (as blank control) were added per well. Then the balance solution (10  $\mu$ L) in the kit was added only to the wells containing the sample. Then 50  $\mu$ L of conjugated solution was added to all wells and incubated for 60 min at 37°C. Finally, the absorbance of the final mixture was read

at 450 nm with an ELISA reader (Biotek Instruments, United States) and reported as U/mL[28].

The commercial Mybiosource fluorometric assay kit (Cat no. MBS2567662; Mybiosource Inc., San Diego, California, USA) was used to measure the serum activity of catalase (CAT). For this purpose, 100  $\mu$ L of standard solution (content of 100  $\mu$ mol/L H<sub>2</sub>O<sub>2</sub> solution in distilled water), 25  $\mu$ L of serum samples, and 25  $\mu$ L of chromogen-containing buffer solution were added to each well separately. Then, 25  $\mu$ L of distilled water was added to the wells containing standard solution, and 25  $\mu$ L of chromogen-containing buffer solution was added to the wells containing serum samples. The contents of the wells were gently mixed and incubated for 5 min at 37°C. Finally, the absorbance of the final mixture was read at 535 nm with a fluorescence microplate reader (FLUOstar OPTIMA multidetection microplate reader, BMG LABTECH, Germany) and reported as U/mL[29].

A commercial Mybiosource (Mybiosource Inc., San Diego, California, USA) ELISA kit (Cat no. MBS705758) was used to measure the serum activity of superoxide dismutase (SOD). This kit is based on the reaction of a polyclonal anti-SOD antibody and a GPx-HRP conjugate. For this purpose, 50  $\mu$ L of standard solution and serum sample were poured into each well separately, and then 50  $\mu$ L of HRP-conjugate was added to each well. The contents of the wells were gently mixed for 60 s and incubated for 40 min at 37°C. Then 90  $\mu$ L of TMB substrate was added to each well and incubated again for 20 min at 37°C. Finally, 50  $\mu$ L of stop solution was added to the final mixture, and after 5 minutes of incubation at 37°C, the adsorption of the resulting mixture was read at 450 nm using a microplate reader (Biotek Instruments, United States) and reported as U/mL[30].

## 2.11. qRT-PCR analysis

Norgen Biotek tissue RNA isolation kit (Cat no. 25700, Norgen Biotek, United States) was used to extract RNA from liver and kidney tissues. According to the kit protocol, 15 mg of liver and kidney tissues were first homogenized by adding 300  $\mu$ L of buffer RL, 10  $\mu$ L of  $\beta$ -mercaptoethanol and 600  $\mu$ L RNase-free water. After 10 minutes of incubation at -4°C, 20  $\mu$ L of proteinase K was added to the resulting mixture and incubated for 15 min at 55°C. Then 450  $\mu$ L of 100% ethanol was added to the lysate and the resulting mixture was added to the separating column and centrifuged (7000  $\times g$  for 1 min). Then the column was separated and connected to the collecting tube and the washing solution (450  $\mu$ L) was added to the column twice and centrifuged again (12000  $\times g$  for 5 min). After that, 100  $\mu$ L of enzyme incubation buffer A and 15  $\mu$ L of DNase I were added to the separated mixture and centrifuged (12000  $\times g$  for 5 min). Finally, 60  $\mu$ L of distilled water was added to the pellet and the extracted RNA was stored at -20°C. To evaluate the quality,

purity, and concentration of the extracted RNA, the ratio of 260/280 nm was measured with nano-drop (Thermo Science ND-1000 UV-Vis Spectrophotometer, United States) and using 2% agarose gel electrophoresis[31].

qScriber cDNA synthesis kit (Cat. no. RTK0104, highQu, Kraichtal, Germany) was used to synthesize the cDNA from the extracted RNA. According to the kit protocol, 1 µL of qScriber enzyme blend (random hexamer and oligo primers) was added to 1 000 ng of extracted RNA, 10 µL qScriber reaction mix, and 9 µL of distilled water. The sequences of pro/anti-apoptotic genes [*Bax*: Forward (5'-3'): GCTACAGGGTTTCATCCA, Reverse (3'-5'): ACATCAGCAATCATCTCT; *Bcl-2*: Forward (5'-3'): GGATGACTT CTCTCGTCGCT, Reverse (3'-5'): GACCCACCGAACTCAAAGA; *Caspase-3*: Forward (5'-3'): TACCAGGGCTGCCTTCTCTTG, Reverse (3'-5'): GGATCTCGCTCCTGGAAGATG] were designed using the gene runner software ver. 6.5.52 (Hastings Software, Hudson, United States) and blasted in NCBI database (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>).  $\beta$ -actin [Forward (5'-3'): CCAGCCTTCCTTCTTGGGTA, Reverse (3'-5'): CAATGCCTGGGTACATGGTG] was selected as the housekeeping gene and gene expression was monitored using a Corbett Rotor-Gene 6000 thermo-cycler (Corbett Research, Cambridge, UK). The qPCR mixture consisted of 10 µL of SYBR Green Master Mix, 1 µL of forward/reverse primers, and 1 µL of template cDNA, which was guided in 42 temperature cycles according to the optimum temperature of the primers and the thermo-cycler temperature protocol as follows. First, the pre-synthesis stage (2 min at 55 °C) was performed and at the beginning of each cycle, the denaturation stage (5 min at 95 °C), annealing stage (1 min at 62 °C) and the extension step (2 min at 72 °C) were performed. The temperature rising rate per second was 1 °C from 62 °C to 95 °C after recording the melting curve. Relative expression of the genes was calculated with the help of fold change formula (fold change =  $2^{-\Delta\Delta CT}$ ) and threshold cycle (CT) recorded for each gene after considering CT of the housekeeping gene ( $\beta$ -actin) and control group.  $\Delta\Delta CT = (\text{target CT} - \text{reference CT}) - (\text{target CT} - \text{control CT})$ [32].

## 2.12. Immunohistochemical examination

To evaluate oxidative stress-dependent apoptosis in liver and kidney tissues, immunohistochemistry against p53-positive cells was used. For this purpose, after fixing in 10% formalin (for 72 h) and performing routine tissue process, paraffin-embedded liver and kidney tissues were cut into 5 µm-thick sections and incubated for antigen retrieval process for 24 h at 95 °C. All slides were incubated for 3 min in 3% H<sub>2</sub>O<sub>2</sub> to block endogenous peroxidases. The slides were then incubated in p53 rodent-specific monoclonal antibody (Cat. no. sc-98, Pab 1801, dilution 1:200, Santa Cruz Biotechnology) for 45 min at room temperature, then incubated for 5 min in 5% bovine serum albumin to block unbound antibodies, and finally in chromogen

(3, 3'-diaminobenzidine) for 30 min at 37 °C. Counterstaining of the slides was performed with hematoxylin and 50 fields of view were randomly evaluated with a light microscope (Olympus CH3, Japan) attached to the camera (Moticam Technologies, Japan), and the p53 positive cells/total cells × 100 was calculated at 400× magnification and reported as mean (%) ± SEM[23].

## 2.13. Histopathological assay

After preparing 5 µm sections from paraffin blocks of liver and kidney tissues, hematoxylin and eosin (H&E) staining for liver sections and periodic acid-schiff (PAS) staining for kidney sections were prepared. Tissue sections were evaluated at 100× and 400× magnifications using a light microscope (Olympus CH3, Japan) attached to the camera (Moticam Technologies, Japan).

## 2.14. Statistical analysis

SPSS software ver. 16 (SPSS, IBM, Chicago, USA) was used to analyze the data, and GraphPad Prism ver. 8 software was used to design the graphs. After evaluating the normality of the data using Kolmogorov-Smirnov test, changes in the mean differences of the data in all groups were also performed by one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* analysis.  $P < 0.05$  was considered significant and the data were shown as mean ± standard error of the mean (SEM).

## 2.15. Ethical statement

This study was conducted under the guidelines and standard conditions of keeping laboratory animals and under the supervision of the institutional ethics committee of Chongqing University (Approval No. KY202435, 2023/4/19).

# 3. Results

## 3.1. Acute toxicity test (LD<sub>50</sub>)

After 24 h, the rat treated with 500 mg/kg of the extract died after appearing toxic symptoms. No toxic symptoms such as nausea, anorexia, and severe weight loss were observed in the other groups. According to the formula of Lorke's method, the LD<sub>50</sub> for *L. draba* extract was 2236 mg/kg, which means that doses lower than 2236 mg/kg are safe and can be used for animal studies.

## 3.2. DPPH, FRAP, TFC, and TPC results

*L. draba* extract showed a FRAP level [(8861.6 ± 42.7) mmol Fe(II)/mg] and DPPH radical scavenging activity [(225.6 ± 7.9) µmol Trolox equivalent/10 g]. It also exhibited high levels of TPC

[(109.1 ± 8.9) mg GAE/g] and TFC [(89.9 ± 6.4) mg RUE/g].

### 3.3. Effect of *L. draba* extract on total body, liver, and kidney weights

According to Figure 1, the total body weight of rats on day 36 in the CP group showed a significant decrease ( $P < 0.05$ ) compared with the SC group. Treatment with *L. draba* extract at doses of 200 ( $P < 0.05$ ) and 400 ( $P < 0.05$ ) mg/kg markedly reversed CP-induced decrease. Moreover, CP significantly reduced liver weight ( $P < 0.05$ ), while treatment with 200 ( $P < 0.05$ ) and 400 ( $P < 0.05$ ) mg/kg of *L. draba* extract significantly increased liver weight compared to the CP group. Similar results were observed in kidney weight. Administration of 200 ( $P < 0.05$ ) and 400 ( $P < 0.05$ ) mg/kg *L. draba* extract raised CP-induced reduction in kidney weight. No statistically significant differences ( $P > 0.05$ ) were observed in all three parameters (total body weight, liver weight, and kidney weight) between the 400 mg/kg extract alone group and the SC group (Figure 1).

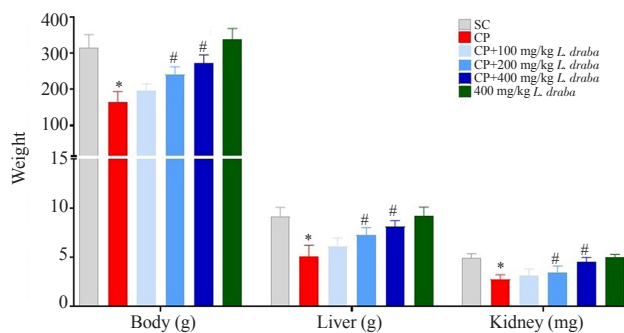


**Figure 1.** Effects of *Lepidium draba* (*L. draba*) extract on total body, liver, and kidney weights of rats with cyclophosphamide (CP)-induced oxidative injury. Data are shown as mean ± SEM ( $n=6$ ). \* $P < 0.05$  compared with the sham control (SC) group; # $P < 0.05$  compared with the CP group.

### 3.4. Biochemical results

As demonstrated in Figure 2, CP significantly increased ( $P < 0.05$ ) the serum levels of liver enzymes (ALT, AST, and ALP) and significantly decreased ( $P < 0.05$ ) TP and ALB compared to the SC group. However, treatment with 200 mg/kg of *L. draba* significantly reduced ( $P < 0.05$ ) liver enzyme activities (ALT and ALP) and significantly increased the serum levels of TP and ALB ( $P < 0.05$ ) compared to the CP group. Furthermore, the serum activities of all three liver enzymes (ALT, AST, and ALP) in the CP group receiving 400 mg/kg of *L. draba* extract were significantly decreased ( $P < 0.05$ ), and the serum levels of TP and ALB were significantly increased ( $P < 0.05$ ) compared to the CP group (Figure 2A).

CP also significantly increased serum levels of BUN ( $P < 0.05$ ) and Cr ( $P < 0.05$ ) compared to the SC group. However, 200 and 400 mg/kg of *L. draba* extract notably decreased BUN and Cr levels ( $P < 0.05$ ) compared to the CP group (Figure 2B). No statistically significant differences ( $P > 0.05$ ) were found in biochemical parameters between the 400 mg/kg extract alone group and the SC group (Figure 2).

### 3.5. Effect of *L. draba* extract on serum antioxidant levels

It was found that CP significantly reduced the activities of GPx ( $P < 0.001$ ), SOD ( $P < 0.001$ ), and CAT ( $P < 0.05$ ). Treatment with 200 and 400 mg/kg of *L. draba* extract significantly increased ( $P < 0.05$ ) the activities of all three antioxidant enzymes (GPx, SOD, and CAT) compared with the CP group. The highest effect on increasing these enzymatic activities [GPx ( $P < 0.05$ ), SOD ( $P < 0.05$ ), and CAT ( $P < 0.05$ )] was observed in the groups receiving CP and 400 mg/kg *L. draba* extract. In addition, NO level was enhanced by CP administration ( $P < 0.001$ ). However, treatment with 200 and 400

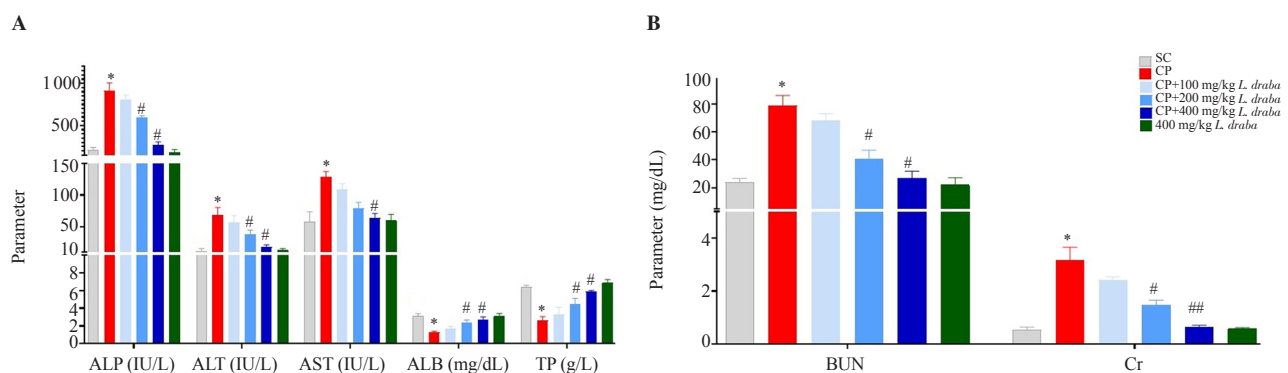


**Figure 2.** Effects of *L. draba* extract on (A) serum activities of ALT, AST, and ALP and serum levels of TP and ALB, as well as (B) Cr and BUN in rats with CP-induced oxidative injury. Data are shown as mean ± SEM ( $n=6$ ). \* $P < 0.05$  compared with the SC group; # $P < 0.05$ , ## $P < 0.001$  compared with the CP group. ALB: albumin, TP: total protein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, BUN: blood urea nitrogen, Cr: creatinine.

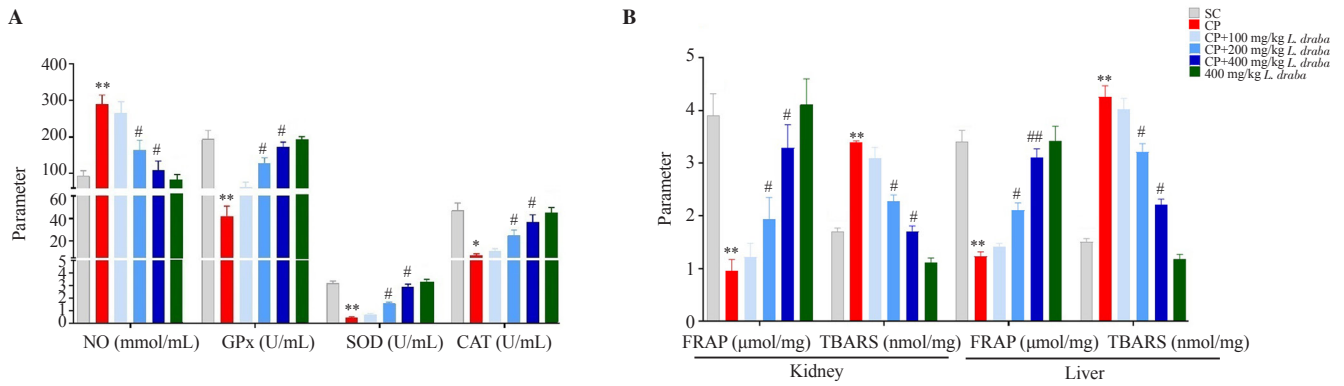


**Figure 3.** Effects of *L. draba* extract on (A) serum level of NO and activities of CAT, GPx, and SOD, and (B) TBARS and FRAP levels in liver and kidney tissues. Data are shown as mean  $\pm$  SEM ( $n=6$ ). \* $P<0.05$ , \*\* $P<0.001$  compared with the SC group; # $P<0.05$ , ## $P<0.001$  compared with the CP group. NO: nitric oxide, GPx: glutathione peroxidase, SOD: superoxide dismutase, CAT: catalase, TBARS: thiobarbituric acid-reactive substances, FRAP: ferric reducing antioxidant power.

mg/kg of *L. draba* extract ( $P < 0.05$ ) caused an obvious drop in its level. No statistically significant differences ( $P > 0.05$ ) were found in serum antioxidant levels between the 400 mg/kg extract alone group and the SC group (Figure 3A).

### 3.6. Effect of *L. draba* extract on liver and kidney TBARS and FRAP levels

FRAP levels in kidney and liver tissues were significantly decreased ( $P < 0.001$ ) in the CP group compared with the SC group, which were reversed by administration of 200 and 400 mg/kg of *L. draba* extract ( $P < 0.05$ ) (Figure 3B). CP also caused a significant increase in the level of TBARS levels in the liver ( $P < 0.001$ ) and kidney ( $P < 0.001$ ) tissues compared to the SC group. Administration of 200 and 400 mg/kg *L. draba* extract pronouncedly decreased the levels of liver and kidney TBARS ( $P < 0.05$ ), indicating its strong antioxidant activity. No statistically significant differences ( $P > 0.05$ ) were found in liver and kidney TBARS and FRAP levels between the 400 mg/kg extract alone group and the SC group (Figure 3).

### 3.7. Effect of *L. draba* extract on inflammation-related cytokines

CP caused a significant increase in serum levels of pro-inflammatory cytokines [IL-1 $\beta$  ( $P < 0.05$ ), IL-6 ( $P < 0.001$ ), and TNF- $\alpha$  ( $P < 0.001$ )] and a significant decrease in anti-inflammatory cytokines [MIF and TGF- $\beta$  ( $P < 0.05$ )]. However, *L. draba* extract induced a dose-dependent reduction in serum levels of pro-inflammatory cytokines, while increasing anti-inflammatory cytokines, with the most significant effect observed at the highest dose. No statistically significant differences ( $P > 0.05$ ) were observed in serum levels of inflammation-related cytokines between the 400 mg/kg extract alone group and the SC group (Figure 4).

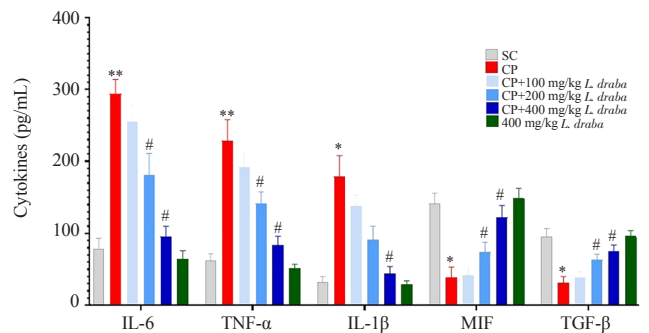


**Figure 4.** Effects of *L. draba* extract on serum levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , MIF, and TGF- $\beta$ . Data are shown as mean  $\pm$  SEM ( $n=6$ ). \* $P<0.05$ , \*\* $P<0.001$  compared with the SC group; # $P<0.05$  compared with the CP group. TNF- $\alpha$ : tumor necrosis factor- $\alpha$ , IL-6: interleukin-6, MIF: macrophage migration inhibitory factor, TGF- $\beta$ : transforming growth factor- $\beta$ .

### 3.8. Effect of *L. draba* extract on *Bax*, *Bcl-2*, and *caspase-3* gene expression

CP caused a significant upregulation in the expression of *Bax* ( $P < 0.05$ ) and *caspase-3* ( $P < 0.001$ ) genes as well as a significant downregulation in *Bcl-2* expression ( $P < 0.001$ ) in liver and kidney tissues compared with the SC group. However, the effect of CP on the expression of *Bax*, *caspase-3*, and *Bcl-2* genes was abrogated by administration of 200 and 400 mg/kg of *L. draba* extract ( $P < 0.05$ ). No statistically significant differences ( $P > 0.05$ ) were found in the expression of apoptotic-related genes in kidney and liver tissues between the group receiving 400 mg/kg extract alone and the SC group (Figure 5).

### 3.9. Immunohistochemical results

Immunohistochemical analysis of liver and kidney tissues showed that CP caused a significant increase ( $P < 0.001$ ) in p53-positive cell percentage compared to the SC group. In contrast, 200 and 400

mg/kg of *L. draba* extract markedly lowered p53-positive cells. No statistically significant differences ( $P > 0.05$ ) were found in the expression of p53 in kidney and liver tissues between the group receiving 400 mg/kg extract alone and the SC group (Figure 6).

### 3.10. Histopathological findings

Histopathological findings on liver tissue showed that CP destroyed the architecture of the liver, causing congestion in the space between hepatocytes [sinusoidal space (S)] and the peri-sinusoidal space (or space of Disse), increased central venule (CV) diameter, and lymphocyte infiltration (LI) into and around the portal triad and hepatic vessels. It also increased eosinophilic granules in hepatocytes

and increased their degeneration (Figure 7). However, *L. draba* extract dose-dependently restored the normal structure of the liver and its physiological function, especially at a dose of 400 mg/kg, by preventing lymphocyte infiltration and hepatic congestion and alleviating the degeneration of hepatocytes (Figure 7).

In addition, CP caused degeneration of glomeruli (DG) and decreased urinary space (US), increased lymphocyte infiltration (LI) in the space between the tubules and around the glomeruli, necrosis of the renal tubules (DT), congestion of renal vessels (C), peritubular and perivascular edema, degeneration of tubular cells along with destruction of the brush border of proximal convoluted tubules, and increased apoptotic cells in the tubular and peritubular space (Figure 7). Histopathological examination of renal tissue in the *L. draba*-

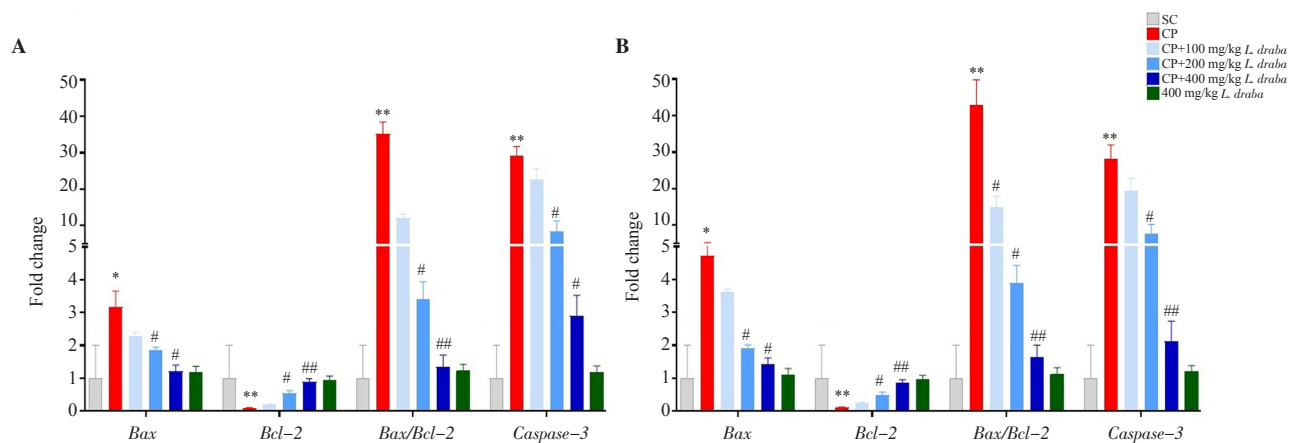


**Figure 5.** Effects of *L. draba* extract on gene expression of *Bax*, *Bcl-2*, and *caspase-3*, as well as *Bax/Bcl-2* ratio in (A) liver and (B) kidney tissues. Data are shown as mean  $\pm$  SEM ( $n=6$ ). \* $P < 0.05$ , \*\* $P < 0.001$  compared with the SC group; # $P < 0.05$ , ## $P < 0.001$  compared with the CP group.

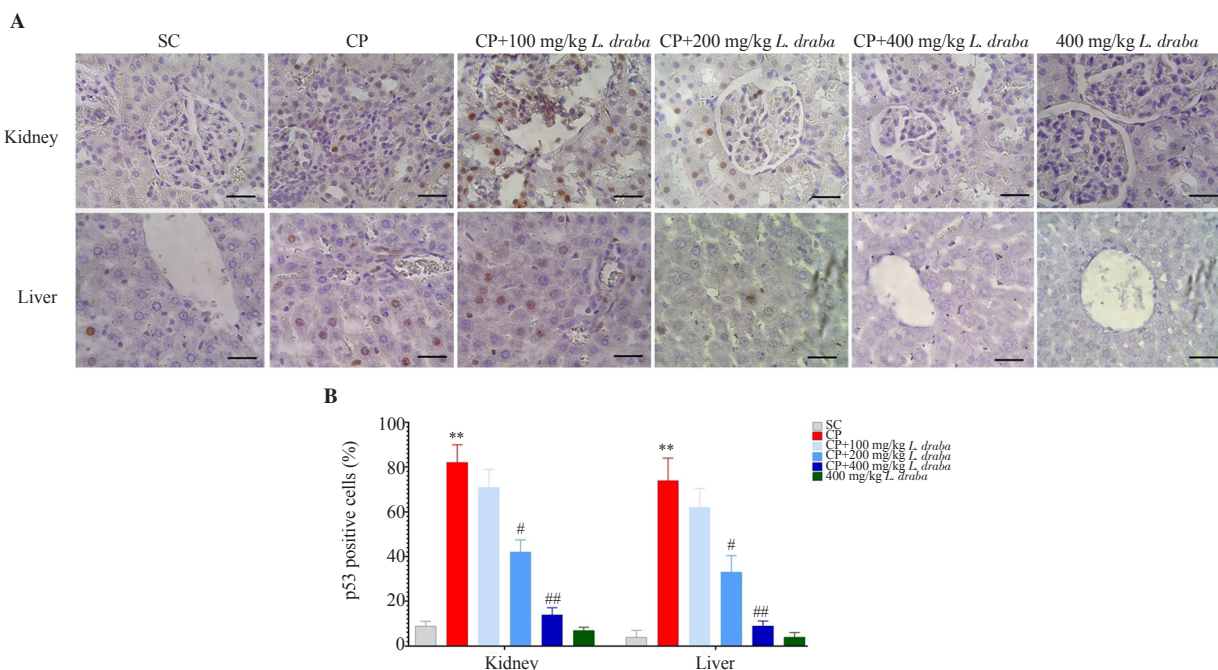


**Figure 6.** Effects of *L. draba* extract on p53-positive cells in (A) kidney and liver tissues (DAB staining 400 $\times$ , scale bar = 25  $\mu$ m). (B) Quantitative results of immunohistochemical examination. Data are shown as mean  $\pm$  SEM ( $n=6$ ). \*\* $P < 0.001$  compared with the SC group; # $P < 0.05$ , ## $P < 0.001$  compared with the CP group. p53-positive cells are stained brown.

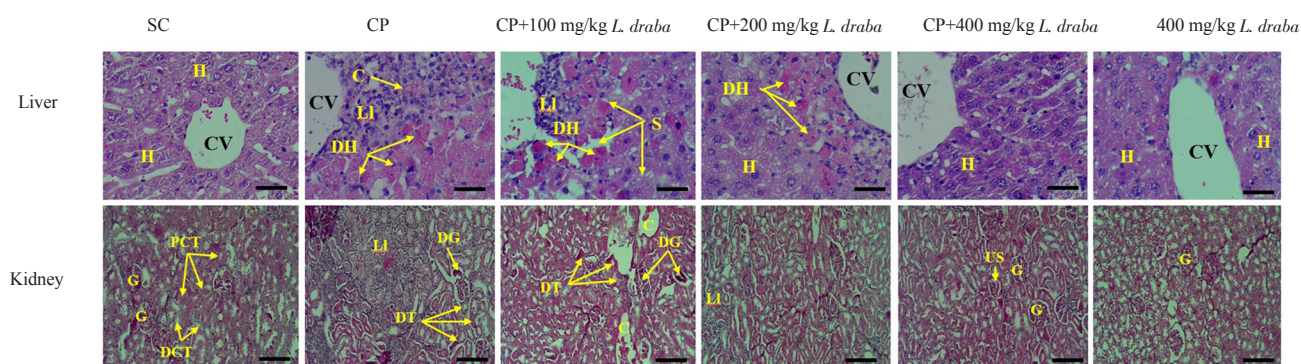


**Figure 7.** Effects of *L. draba* extract on histopathology of liver (H&E  $\times 400$ ; scale bar = 25  $\mu\text{m}$ ) and kidney (PAS  $\times 100$ ; scale bar = 100  $\mu\text{m}$ ) tissues. CV: central venule, LI: lymphatic infiltration, C: hyperemia and congestion, DH: hepatic degeneration, H: normal hepatocytes, S: hepatic sinusoid, G: normal glomerulus, US: urinary space, DG: degenerated glomerulus, DCT: distal convoluted tubule, PCT: proximal convoluted tubule, DT: degenerated tubules.

treated groups showed a dose-dependent effect on mitigating the destruction of glomeruli and renal tubules and ultimately maintained their structure and function. In addition, *L. draba* (especially in the 400 mg/kg *L. draba* + CP group) inhibited tubular and inter-tubular lymphocyte infiltration. After maintaining the function of the tubular/glomerular structure, physiological urinary infiltration occurred, and as a result, tubular/peri-tubular edema and vascular congestion were eliminated.

#### 4. Discussion

In the present study, *L. draba* maintained the structure and function of the liver and kidney against CP-induced oxidative stress through antioxidant, anti-inflammatory, and anti-apoptotic pathways.

CP increased lipid peroxidation (increased TBARS levels) and decreased total antioxidant capacity (decreased FRAP levels) in liver and kidney tissues by inhibiting the activity of free radical scavenging enzymes (GPx, SOD, and CAT) and increasing the production of ROS (NO levels). The results of the present study are consistent with other studies on different models of CP toxicity. A study on CP-induced renal toxicity in mice reported reduced activity of free radical scavenging enzymes (GPx, SOD, and CAT), increased lipid peroxidation (MDA levels), and increased NO levels. Eventually, CP caused dysfunction and structural damage to the kidneys, followed by an increase in Cr and BUN[33]. Another study on the toxic effects of CP (200 mg/kg, *i.p.*) on hepatotoxicity and nephrotoxicity in rats showed that this chemotherapy drug increased liver enzymes (ALT, ALP and AST), urea, Cr, and inflammatory, apoptotic, and autophagy parameters (TNF- $\alpha$ , NF- $\kappa$ B, iNOS, IL-1 $\beta$ , COX-2, IL-6, Bax, and Bcl-2), as well as decreased the activity of antioxidant enzymes (GPx, SOD, and CAT) in the liver and kidney, which caused dysfunction of these organs. In the study of the protective effects of vitamin E and *Panax ginseng* L. extract on

the hepatotoxicity induced by CP (100 mg/kg, *i.p.*), it was found that this chemotherapy drug, along with increased liver enzymes (ALT, ALP, AST) and lipid peroxidation level (MDA), reduced the activity of antioxidant enzymes (GPx, SOD, and CAT) and increased the expression of pro-apoptosis (caspase-3) and pro-inflammatory genes (TNF- $\alpha$  and IL-1 $\beta$ )[34]. In the present study, CP induced impairment in the physiological function of the liver and kidneys by inducing oxidative stress, and finally the levels of liver enzymes (ALT, ALP, and AST), Cr, BUN, and pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) increased and anti-inflammatory cytokines (MIF and TGF- $\beta$ ), ALB, and TP levels decreased compared to the control group. CP triggered apoptosis by enhancing inflammatory pathways (increased expression of pro-inflammatory TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) and the apoptotic pathway of Bax/caspase-3/p53/cytochrome C, and ultimately caused destruction and fibrosis of liver and kidney tissues[35]. Also, at the molecular level, CP increases the expression of caspase-3 and ultimately the release of cytochrome C *via* hepatocyte mitochondria by amplifying the Bax/Bcl-2/caspase-3/p53 (increasing the Bax/Bcl-2 ratio) and Txnip/Trx/NF- $\kappa$ B apoptotic pathways. This event increases cell apoptosis and impairs liver tissue function[36]. In the present study, CP also enhanced the Bax/Bcl-2 ratio and activated caspase-3/p53 apoptotic pathway by increasing ROS levels and ultimately enhancing the percentage of p53-positive cells.

In the present study, LD<sub>50</sub> of *L. draba* extract was 2236 mg/kg, at which rats showed no toxic symptoms or mortality, and all hepato-renal parameters were normal. It has been shown that the LD<sub>50</sub> of this plant extract was 2600 mg/kg, which is in line with our study[19]. *L. draba* (especially 400 mg/kg) maintained hepato-renal physiological activities and normalized the levels of liver enzymes and parameters in the CP groups. It also had free radical scavenging ability and protected the liver and kidney against oxidative damage by reducing NO and TBARS levels and increasing the activity of antioxidant enzymes. Consistent with the present findings, Wang *et*

*al.* showed that *L. draba* reduces lipid peroxidation by increasing the total antioxidant capacity[37].

*L. draba* contains various polyphenolic compounds (flavonoids and isoflavones), especially apigenin,  $\alpha$ -tocopherol, quercetin, caffeoylmalic acid, kaempferol, daidzein, proanthocyanidin derivatives, myricetin, harman, and ferulic acid[17]. TPC and TFC analysis of this plant in the present study also showed a high content of polyphenolic compounds. In addition to their antioxidant properties, a large number of these compounds have high anti-tumor properties. Apigenin, as one of the most important compounds of *L. draba*, suppresses the growth and metastasis of breast, prostate, and colon tumors through PI3K/AKT, Wnt/ $\beta$ -catenin, MAPK/ERK, and NF- $\kappa$ B pathways. This flavonoid compound also inhibits G<sub>2</sub>/M and G<sub>0</sub>/G<sub>1</sub> cell cycle checkpoints in cancer cells without affecting normal cells[38]. Quercetin is another predominant polyphenolic compound in *L. draba* that inhibits inflammatory pathways and suppresses proinflammatory cytokines COX-2, IFN- $\gamma$ , IL-8, IL-6, iNOS, and TNF- $\alpha$ . It also inhibits the proliferation and metastasis of breast, colon, and prostate tumors *via* the PI3K/Akt/mTOR, JNK/JUN, IL-6/STAT3, Bax/Bcl-2/p53, p53/miR-34a/SIRT1, and Notch/AKT/mTOR pathways. In addition, this compound arrests the cell cycle in the G<sub>1</sub> and G<sub>2</sub>/M phases by enhancing the expression of p21, p27, p53, and Chk2 and decreasing the expression of Cdk1 and cyclin B1[39]. In addition to their antitumor effects, some of these compounds, such as kaempferol, protect cells against ROS and metabolites from chemotherapy drugs in various animal and cell line models. This polyphenolic compound protects the liver and kidneys from the toxicity induced by streptozotocin and mercuric chloride, respectively[40,41]. In a study by Wang *et al.* *L. draba* protected testicular cells against CP-induced apoptosis through modulation of ROS-dependent Bax/Bcl2/p53/caspase-3 and Keap1/Nrf2/HO1 pathways[37]. The results of the present study showed that *L. draba* extract (especially at 400 mg/kg) decreased apoptosis of liver and kidney cells by suppressing the Bax/Bcl2/p53/caspase-3 pathway and reducing p53-positive cells. It also inhibited pro-inflammatory cytokines and increased anti-inflammatory cytokines, which is consistent with those found in a previous study[37,42].

Some limitations should be considered regarding the hepatoprotective and renoprotective effects of *L. draba* extracts. Firstly, the research was conducted exclusively in rat models, which may not fully replicate the complex physiological responses observed in humans, potentially limiting the applicability of the findings to clinical settings. Additionally, the study primarily focused on specific pathways related to apoptosis and oxidative stress, leaving other relevant mechanisms that might contribute to the observed protective effects unexplored. Furthermore, variations in the extract preparation methods, dosage, and duration of treatment could influence the efficacy of *L. draba* and result in variability in the outcomes. Lastly, the study did not include a long-term assessment of the effects, which would be essential to evaluating the safety and

sustained benefits of the treatment over time.

In conclusion, the findings of this study exhibit the significant protective effects of *L. draba* L. extract on the liver and kidney following CP administration. Treatment with *L. draba* demonstrated notable improvements in oxidative stress parameters and inflammatory cytokine levels, indicating a reduction in inflammation and cellular damage of the kidney and liver. Moreover, the extract effectively modulated the expression of pro-apoptotic genes, suggesting its role in mitigating apoptotic pathways triggered by toxic effects of chemotherapy agents. These results highlight the potential of *L. draba* as a supportive therapeutic agent to enhance renal and hepatic functionality. However, further clinical investigations are needed to validate these protective benefits in human subjects undergoing similar treatment regimens.

## Conflict of interest statement

The authors declare that they have no conflict of interest.

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## Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

## Authors' contributions

YY contributed in writing draft. SZ and CZ conducted the experimental design and protocol preparation. YC and PL performed statistical analysis and laboratory tests. XZ reviewed the manuscript draft. LL developed the conceptualization and supervised the project.

## References

- [1] Xu B, Ganesan K, Mickymaray S, Alfaiz FA, Thatchinamoorthi R, Al-Aboody M. Immunomodulatory and antineoplastic efficacy of common

- spices and their connection with phenolic antioxidants. *Bioact Comp Health Dis* 2020; **3**(2): 15-31.
- [2] Cauli O. Oxidative stress and cognitive alterations induced by cancer chemotherapy drugs: A scoping review. *Antioxidants* 2021; **10**(7): 1116.
- [3] Shen S, Dean DC, Yu Z, Duan Z. Role of cyclin-dependent kinases (CDKs) in hepatocellular carcinoma: Therapeutic potential of targeting the CDK signaling pathway. *Hepatol Res* 2019; **49**(10):1097-1108.
- [4] Hu L, Chen M, Chen X, Zhao C, Fang Z, Wang H, et al. Chemotherapy-induced pyroptosis is mediated by BAK/BAX-caspase-3-GSDME pathway and inhibited by 2-bromopalmitate *Cell Death Dis* 2021; **11**(4): 1-17.
- [5] Karim S, Shaik RA. Geraniol alleviates cyclophosphamide-induced cardiotoxicity in mice. *Asian Pac J Trop Biomed* 2024; **14**(10): 427-435.
- [6] Kim SH, Lee IC, Baek HS, Shin IS, Moon C, Bae CS, et al. Mechanism for the protective effect of diallyl disulfide against cyclophosphamide acute urotoxicity in rats. *Food Chem Toxicol* 2014; **64**: 110-118.
- [7] Ettaya A, Dhibi S, Samout N, Elfeki A, Hfaiedh N. Hepatoprotective activity of white horehound (*Marrubium vulgare*) extract against cyclophosphamide toxicity in male rat. *Canadian J Physiol Pharmacol* 2016; **94**(4): 441-447.
- [8] Jiang W, Liu J, Li P, Lu Q, Pei X, Sun Y, et al. Magnesium isoglycyrrhizinate shows hepatoprotective effects in a cyclophosphamide-induced model of hepatic injury. *Oncotarget* 2017; **8**(20): 33252-33264.
- [9] Lata S, Singh S, NathTiwari K, Upadhyay R. Evaluation of the antioxidant and hepatoprotective effect of *Phyllanthus fraternus* against a chemotherapeutic drug cyclophosphamide. *Appl Biochem Biotechnol* 2014; **173**(8): 2163-2173.
- [10] Abulyazid I, Abd Elhalim SA, Sharada HM, Aboulthana WM, Abd Elhalim ST. Hepatoprotective effect of carob pods extract (*Ceratonia siliqua* L.) against cyclophosphamide induced alterations in rats. *Int J Current Pharm Rev Res* 2017; **8**(2): 149-162.
- [11] Duléry R, Mohty R, Labopin M, Sestili S, Malard F, Brissot E, et al. Early cardiac toxicity associated with posttransplant cyclophosphamide in allogeneic stem cell transplantation. *Cardio Oncol* 2021; **3**(2): 250-259.
- [12] Gadisa DA, Assefa M, Wang SH, Yimer G. Toxicity profile of doxorubicin-cyclophosphamide and doxorubicin-cyclophosphamide followed by paclitaxel regimen and its associated factors among women with breast cancer in Ethiopia: A prospective cohort study. *J Oncol Pharm Pract* 2020; **26**(8): 1912-1920.
- [13] Alrefaei AE, Alzahrani MA, Alsuhaim SA. Cyclophosphamide related toxicity; a systematic review. *IJMDC* 2022; **6**(5): 740-747.
- [14] Darband SG, Kaviani M, Yousefi B, Sadighparvar S, Pakdel FG, Attari JA, et al. Quercetin: A functional dietary flavonoid with potential chemopreventive properties in colorectal cancer. *J Cell Physiol* 2018; **233**(9): 6544-6560.
- [15] Ali Q, Khalil R, Nadeem M, Azhar MM, Hafeez MM, Malik A. Antibacterial, antioxidant activities and association among plant growth related traits of *Lepidium draba*. *Biologic Clin Sci Res J* 2020; **2020**(11): 1-5.
- [16] Benakashani F, Allafchian A, Jalali SAH. Green synthesis, characterization and antibacterial activity of silver nanoparticles from root extract of *Lepidium draba* weed. *Green Chem Lett Rev* 2017; **10**(4): 324-330.
- [17] Gao XC, Lv JW, Li CN, Zhang NX, Tian LL, Han XY, et al. Screening of the active component promoting Leydig cell proliferation from *Lepidium meyenii* using HPLC-ESI-MS/MS coupled with multivariate statistical analysis. *Molecules* 2019; **24**(11): 2101.
- [18] Razavi R, Molaei R, Moradi M. Biosynthesis of metallic nanoparticles using mulberry fruit (*Morus alba* L.) extract for the preparation of antimicrobial nanocellulose film. *Applied Nanosci* 2020; **10**(2): 465-476.
- [19] Wang Y, Bai L, Li H, Yang W, Li M. Protective effects of *Lepidium draba* L. leaves extract on testis histopathology, oxidative stress indicators, serum reproductive hormones and inflammatory signaling in oxymetholone-treated rat. *Andrologia* 2021; **53**(11): e14239.
- [20] Arena AC, Jorge BC, Silva MC, De Barros AL, Fernandes AAH, Nóbrega RH, et al. *Acrocomia aculeata* oil: Beneficial effects on cyclophosphamide-induced reproductive toxicity in male rats. *Andrologia* 2018; **50**(6): e13028.
- [21] López-Froilán R, Hernández-Ledesma B, Cámara M, Pérez-Rodríguez ML. Evaluation of the antioxidant potential of mixed fruit-based beverages: A new insight on the folin-ciocalteu method. *Food Anal Methods* 2018; **11**(10): 2897-2906.
- [22] Mamou A, Eloutassi N, Alshawwa SZ, Al Kamaly O, Kara M, Bendaoud M, et al. Total phenolic content and antioxidant and antimicrobial activities of *Papaver rhoeas* L. organ extracts growing in taounate region, Morocco. *Molecules* 2022; **27**(3): 854.
- [23] Akbaribazm M, Khazaei MR, Khazaei F, Khazaei M. Doxorubicin and *Trifolium pratense* L.(Red clover) extract synergistically inhibits brain and lung metastases in 4T1 tumor-bearing BALB/c mice. *Food Sci Nutr* 2020; **8**(10): 5557-5570.
- [24] Khaksari M, Esmaili S, Abedloo R, Khastar H. Palmatine ameliorates nephrotoxicity and hepatotoxicity induced by gentamicin in rats. *Arch Physiol Biochem* 2021; **127**(3): 273-278.
- [25] Kawade N, Murai A, Suzuki W, Tokuda Y, Kobayashi M, Horio F. Ascorbic acid deficiency increases hepatic expression of acute phase proteins through the intestine-derived IL-6 and hepatic STAT3 pathway in ODS rats. *J Nutr Biochem* 2019; **70**: 116-124.
- [26] Atli G. How metals directly affect the antioxidant status in the liver and kidney of *Oreochromis niloticus*? An *in vitro* study. *J Trace Elem Med Biol* 2020; **62**. doi: 10.1016/j.jtemb.2020.126567.
- [27] Najafi H, Mohamadi Yarijani Z, Changizi-Ashtiyani S, Mansouri K, Modarresi M, Seyed Madani H, et al. Protective effect of *Malva sylvestris* L. extract in ischemia-reperfusion induced acute kidney and remote liver injury. *PLoS One* 2017; **12**(11). doi: 10.1371/journal.pone.0188270.
- [28] Wang J, Zhu P, Toan S, Li R, Ren J, Zhou H. Pum2-Mff axis fine-tunes mitochondrial quality control in acute ischemic kidney injury. *Cell Biol Toxicol* 2020; **36**(4): 365-378.
- [29] Ibrahim RE, Amer SA, Farroh KY, Al-Gabri NA, Ahmed AI, El-

- Araby DA, et al. The effects of chitosan-vitamin C nanocomposite supplementation on the growth performance, antioxidant status, immune response, and disease resistance of *Nile tilapia* (*Oreochromis niloticus*) fingerlings. *Aquaculture* 2021; **534**. doi: 10.1016/j.aquaculture.2020.736269.
- [30]Kusuyama J, Alves-Wagner AB, Conlin RH, Makarewicz NS, Albertson BG, Prince NB, et al. Placental superoxide dismutase 3 mediates benefits of maternal exercise on offspring health. *Cell Metab* 2021; **33**(5): 939-956.
- [31]Fish L, Zhang S, Johnny XY, Culbertson B, Zhou AY, Goga A, et al. Cancer cells exploit an orphan RNA to drive metastatic progression. *Nat Med* 2018; **24**(11): 1743-1751.
- [32]Cléries R, Galvez J, Espino M, Ribes J, Nunes V, de Heredia ML. Boots Ratio: A web-based statistical analysis of fold-change in qPCR and RT-qPCR data using resampling methods. *Comput Biol Med* 2012; **42**(4): 438-445.
- [33]Goudarzi M, Khodayar MJ, Hosseini Tabatabaei SMT, Ghaznavi H, Fatemi I, Mehrzadi S. Pretreatment with melatonin protects against cyclophosphamide-induced oxidative stress and renal damage in mice. *Fund Clin Pharmacol* 2017; **31**(6): 625-635.
- [34]Abdelfattah-Hassan A, Shalaby SI, Khater SI, El-Shetry ES, Abd El Fadil H, Elsayed SA. Panax ginseng is superior to vitamin E as a hepatoprotector against cyclophosphamide-induced liver damage. *Complement Ther Med* 2019; **46**: 95-102.
- [35]Iqbal A, Syed MA, Ali J, Najmi AK, Haque MM, Haque SE. Nerolidol protects the liver against cyclophosphamide-induced hepatic inflammation, apoptosis, and fibrosis via modulation of Nrf2, NF- $\kappa$ B p65, and caspase-3 signaling molecules in Swiss albino mice. *Biofactors* 2020; **46**(6): 963-973.
- [36]Lixin X, Lijun Y, Songping H. Ganoderic acid A against cyclophosphamide-induced hepatic toxicity in mice. *J Biochem Mol Toxicol* 2019; **33**(4). doi: 10.1002/jbt.22271.
- [37]Wang Y, Bai L, Zhang J, Li H, Yang W, Li M. *Lepidium draba* L. leaves extract ameliorated cyclophosphamide-induced testicular toxicity by modulation of ROS-dependent Keap1/Nrf2/HO1, Bax/Bcl2/p53/caspase-3, and inflammatory signaling pathways. *J Food Biochem* 2021; **45**(12): e13987.
- [38]Yan X, Qi M, Li P, Zhan Y, Shao H. Apigenin in cancer therapy: Anti-cancer effects and mechanisms of action. *Cell Biosci* 2017; **7**(1): 1-16.
- [39]Kashyap D, Mittal S, Sak K, Singhal P, Tuli HS. Molecular mechanisms of action of quercetin in cancer: Recent advances. *Tumor Biol* 2016; **37**(10): 12927-12939.
- [40]Al-Numair KS, Chandramohan G, Veeramani C, Alsaif MA. Ameliorative effect of kaempferol, a flavonoid, on oxidative stress in streptozotocin-induced diabetic rats. *Redox Rep* 2015; **20**(5): 198-209.
- [41]Vijayaprakash S, Langeswaran K, Kumar SG, Revathy R, Balasubramanian MP. Nephro-protective significance of kaempferol on mercuric chloride induced toxicity in Wistar albino rats. *Biomed Aging Pathol* 2012; **3**(3): 119-124.
- [42]Chyad AH. Evaluation of anticancer, analgesic and anti-inflammatory activities of the ethanolic extract of *Lepidium draba* Linn. leaves. *Adv Anim Vet Sci* 2017; **5**(1): 7-13.

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**Hepatorenoprotective effects of *Lepidium draba* L. extracts against cyclophosphamide-induced oxidative injuries in rats *via* reducing apoptosis and inflammation**

**Running title:** *Lepidium draba* L. mitigates cyclophosphamide-induced hepatorenal injury

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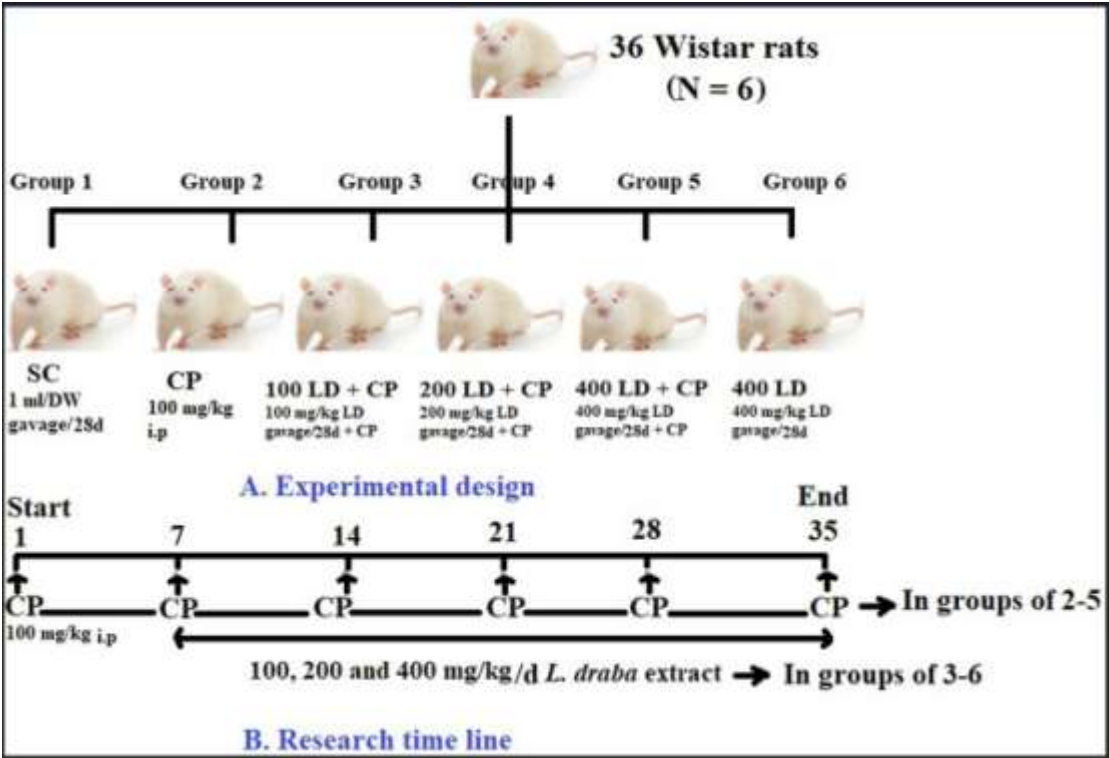
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**Supplementary Figure.** Research timeline and experimental design.