Heliyon 10 (2024) e37010

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Huaier relieves oxaliplatin-induced hepatotoxicity through activation of the PI3K/AKT/Nrf2 signaling pathway in C57BL/6 mice

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ARTICLE INFO

Keywords:
Huaier
Hepatotoxicity
Oxaliplatin
PI3K/AKT/Nrf2

ABSTRACT

Hepatotoxicity caused by the anticancer medication oxaliplatin (OXA) significantly restricts its clinical use and raises the risk of liver damage. Huaier, a fungus found in China, has been demonstrated to have various beneficial effects in adjuvant therapy for cancer. However, the preventive impact of Huaier against OXA-induced hepatotoxicity is still unknown. The potential molecular pathways behind the hepatoprotective activity of Huaier against OXA-induced hepatotoxicity were investigated in the current study Mice were intraperitoneally injected with 10 mg/kg of OXA once a week for six consecutive weeks to establish a liver injury model. Huaier (2 g/kg, 4 g/kg, and 8 g/kg) was administered weekly to mice by gavage for six weeks. Commercial kits were used to determine the contents of glutathione, catalase, superoxide dismutase, and malondialdehyde. Quantitative real-time polymerase chain reaction (qRT-PCR) and Western blotting were used to assess the impact of Huaier therapy on the expression of the PI3K pathway. Huaier exhibited a good protective effect on OXA-induced hepatotoxicity in a dose-dependent manner, which was connected to the suppression of oxidative stress, according to the results of biochemical index detection and histological staining analysis. In addition, Huaier could counteract the OXA-induced suppression of the PI3K/AKT signaling pathway. Moreover, the hepatoprotective effect and PI3K activation of Huaier were eradicated by LY294002. These findings imply that by decreasing oxidative stress, Huaier can minimize OXA-induced liver injury, establishing the groundwork for Huaier to lessen chemotherapy-induced hepatotoxicity in clinical practice.

1. Introduction

In both monotherapy and combination therapy, the novel third-generation platinum compound oxaliplatin (OXA) is effective

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https://doi.org/10.1016/j.heliyon.2024.e37010
Received 23 January 2024; Received in revised form 25 August 2024; Accepted 26 August 2024
Available online 29 August 2024

against advanced ovarian and colorectal malignancies [1]. Although OXA has a high rate of success in curing malignant cancers, its application in clinical tumor therapy is severely constrained by side effects, including peripheral neurotoxicity [2], hypersensitivity reaction [3,4], and hematotoxicity [5]. In addition to that, OXA-induced hepatotoxicity arouses growing concern, which can lead to poor prognosis and increase treatment-related mortality in patients [6] with an incidence rate of 48%–79 % [7–9]. Our previous multicenter clinical study also found that OXA-induced liver dysfunction was a serious and common adverse drug reaction [10] and a comprehensive analysis found that age, chemotherapy regimens, prophylactic use of glucocorticoids, and prophylactic use of antihistamines were associated with the risk of OXA-induced liver injury [11]. Hepatic sinusoidal epithelial cells (HSECs) become swollen, the intercellular space expands, and the hepatic sinusoid wall continues to disintegrate as a result of OXA-induced hepatotoxicity. Clinically side effects include liver nodule regenerative hyperplasia, portal hypertension, thrombocytopenia, and splenomegaly [12]. However, the mechanisms and treatments of OXA-induced hepatotoxicity haven't been clearly illustrated, presenting an obstacle to its clinical application. Therefore, determining the underlying basis of OXA-induced hepatotoxicity and discovering potent hepatoprotective medications are of utmost importance.

Our previous study and other literature reports suggest that the mechanism of hepatotoxicity induced by OXA may be related to oxidative stress [13–15]. In their research, Rubbia-Brandt et al. [14] used microarray analysis to explore the gene expression profile in the livers of patients with OXA-related liver injury. It was found that the expression of pathway-related factors, such as oxidative stress, was up-regulated. Robinson et al. [15] reported that some antioxidant stress-related genes, including *nrf2*, were down-regulated in the process of OXA-induced liver injury. In vitro study has found that platinum drugs, such as OXA, can promote the production of reactive oxidative species (ROS) and deplete glutathione (GSH) in HSECs, with excessive ROS aggravating the damage to HSECs [16]. Other researchers have also reported that inhibiting oxidative stress can play a protective role in OXA-induced hepatotoxicity [17,18].

Trametes robiniophila Murr (Huaier), a widely distributed fungus found in China, contains a variety of organic components and more than 10 minerals [19]. Huaier has a long history of use as a therapeutic herb for treating and preventing diseases. It has been reported that Huaier can inhibit the proliferation and metastasis of tumor cells and can interfere with tumor angiogenesis [20]. Huaier can be used in the adjuvant treatment of cancer, such as breast cancer [21], liver cancer [22] and non-small cell lung cancer [23]. Existing clinical and research studies on Huaier examine the impact on liver cancer cells to investigate its therapeutic effect on liver cancer [24,25], but there is a lack of research relating to other liver injuries, especially drug-induced liver injury. In recent years, the antioxidation effect of Huaier has gradually been noticed [26–29]. This has been preliminarily demonstrated with the crude polysaccharide of Huaier, which is effective at scavenging ROS by DPPH radical scavenging assay [27]. Su et al. [28] identified the components of ethanol extract from Huaier and discovered that it had excellent hypoglycemic and antioxidant properties. For the treatment of diseases, Huaier polysaccharide has been shown to alleviate cisplatin nephrotoxicity in mice by decreasing ROS, which may be explained through its effect on the PI3K/AKT signaling pathway [26]. And the antioxidant activity of Huaier can also help to maintain the intestinal function in sulfate sodium salt-induced ulcerative colitis mice model [29]. However, the protective effect of Huaier against OXA-induced hepatic damage has not been documented.

Therefore, in this study, we evaluated the effect of Huaier extract on OXA-induced hepatotoxicity and explored the potential mechanisms. We demonstrate that Huaier extract inhibits oxidative stress by suppressing the PI3K/AKT pathway. This research will give a solid scientific foundation for the practical use of Huaier in patients undergoing OXA chemotherapy, helping to minimize hepatotoxicity.

2. Materials and methods

2.1. Reagents and antibodies

Oxaliplatin for injection was purchased from Jiangsu Hengrui Pharmaceuticals Co., Ltd. (Lianyungang, Jiangsu, China). Huaier extract, the hot water extract of *Trametes robiniophila Murr.*, was provided by Gaitianli Medicine Co., Ltd. (Qidong, Jiangsu, China). N-acetylcysteine (NAC) was purchased from Minsheng Pharmaceutical Co., Ltd. (Hangzhou, Zhejiang, China). LY294002 was obtained from ApexBio Technology (Huston, USA). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and GSH commercial biochemical detection kits were provided by Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). Haematoxylin and eosin (H&E) dye kits were obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). The BCA Protein Assay Kit was purchased from Servicebio Technology Co., Ltd. (Wuhan, China). Primary antibodies against Phosphatidylinositol 3 Kinase (PI3K), Heme Oxygenase-1 (HO-1), NAD(P)H: Quinone Oxidoreductase 1 (NQO1), Glutamate-Cysteine Ligase, Modifier (GCLM), and Glutamate-Cysteine Ligase, Catalytic (GCLC) were provided by Absin Bioscience Inc. (Shanghai, China), and antibodies against p-AKT, AKT, and β-ACTIN were supplied by Cell Signaling (Boston, MA, USA).

2.2. Quality evaluation and preparation of Huaier extract

In this study, Huaier extract (cat. no: 210124) were provided by Jiangsu Gaitianli Medicine Co., Ltd, which also conducted the component quality analysis. Using anhydrous glucose as the reference substance, the content of the polysaccharide in Huaier was evaluated using anthrone sulfate chromogenic technique at 630 nm [30]. The standard curve was established with absorbance as ordinate and concentration as Abscissa. The internal control standard of polysaccharide content in Huaier extract is within 1.5%–2.8 %. Using bovine serum albumin as the reference substance, the content of the protein in Huaier was determined by using the phenol reagent method at 750 nm [30], and the standard curve was established with absorbance as ordinate and concentration as abscissa.

The internal control standard of protein content in Huaier extract t should not be less than 130.0 mg/g.

2.3. Animals and experimental design

The procedures for all laboratory animals were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals. All animal experiments were reviewed and approved by the Animal Welfare and Ethical Committee of Tongji Hospital (Wuhan, China; approval no.TJH-202101009). This study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). Male, 8-week-old C57BL/6 mice (body weight, 20 ± 2 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The animals were kept in a controlled environment (12-h light–dark cycle with a temperature of 23 ± 2 °C and a relative humidity of 65 ± 5 %) and were provided with standard laboratory food (Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) and water *ad libitum*. All mice had been introduced to the environment at least one week before the formal experiment and no more than 5 mice were placed in one cage.

To investigate the protective effect of the Huaier extract, a total of 63 mice were evenly divided into the (i) control group, (ii) Huaier control group, (iii) OXA model group, (iv) OXA + low-dose Huaier group, (v) OXA + medium-dose Huaier group, (vi) OXA + high-dose Huaier group, and (vii) OXA + NAC group, with nine mice in each group. According to our earlier research, where all mice received weekly administrations of 10 mg/kg OXA for six weeks, the OXA-induced hepatotoxicity model was successfully established [12]. Therefore, groups treated with OXA were intraperitoneally injected with 10 mg/kg OXA per week, and the control group and Huaier control group were intraperitoneally injected with a 5 % glucose solution once a week for six weeks. According to Refs. [31,32], different dose gradients were set in the Huaier treatment groups. Mice in the low-dose, medium-dose, and high-dose Huaier groups were intragastrically administered 2 g/kg, 4 g/kg, and 8 g/kg of Huaier extract, respectively, 30 min before each OXA injection. Lastly, according to the commonly used clinical dose for body surface area conversion, the intraperitoneal injection of a 1.3 g/kg NAC treatment, an antioxidant [33], was applied to the positive treatment group. All treatments lasted six consecutive weeks.

To verify the mechanism of the Huaier extract, a total of 45 male C57BL/6 mice were randomly divided into the (i) control group, (ii) LY294002 control group, (iii) OXA model group, (iv) OXA + Huaier group, and (v) OXA + Huaier + LY294002 group, with nine mice in each group. The OXA-treated groups (groups iii–v) were intraperitoneally injected with 10 mg/kg OXA per week, and the control group and LY294002 control group were intraperitoneally injected with a 5 % glucose solution once a week for six weeks. Huaier-treated groups (groups iv and v) were intragastrically administered 8 g/kg of Huaier extract as we observed the best treatment effect. Lastly, the LY294002, a PI3K pathway inhibitor, treated groups (groups ii and v) were intraperitoneally injected with 30 mg/kg LY294002 per week according to references [34,35]. All treatments lasted six consecutive weeks.

During the experiment period, mice were provided with standard laboratory food and water *ad libitum* and the feeding conditions of mice were consistent with the adaptation period. Every day, the mice's body weights were documented. The mice were starved but allowed access to drink after the final injection. The next day, after injection of sodium pentobarbital (30 mg/kg body weight) to minimize animal suffering, blood samples were taken from the orbital venous plexus using capillary tubes into 1.5 mL Eppendorf tubes. Spleen and liver tissues were also removed and weighed for subsequent analysis (liver index = wet liver weight/body weight × 100 %; spleen index = wet spleen weight/body weight × 100 %). Both liver index and spleen index reflected the enlargement or shrinkage of organs.

2.4. Serum biochemical parameters analysis

The blood samples were placed at 25 °C for 1 h and then centrifuged at 4 °C, 4000 rpm for 5 min to obtain the serum. All the serum samples were stored at −80 °C for further tests. For hepatic function examination, commercial biochemical detection kits were used to determine the serums' levels of AST and ALT in the mice from each group, with the manufacturer's instructions being strictly followed.

2.5. Liver histopathological analysis

Histopathological changes of the liver were observed through H&E staining. In brief, the mouse livers were removed, washed with sterile 0.9 % saline, and fixed in 10 % neutral formaldehyde, then dehydrated in graded ethanol and embedded in paraffin to prepare the section. Each section (4 μm) was routinely stained with H&E dye kits. A liver pathologist blindly examined the histological assessment of OXA-induced hepatotoxicity, focusing on sinusoidal dilatation, coagulative necrosis of hepatocytes, damage to the endothelium of the central vein, and sinusoidal bleeding. Each of these features was graded on a 4-point scale: 0 = absent; 1 = mild; 2 = moderate; 3 = severe. Then, each score is added to get the total score [36]. The histopathological changes of each stained specimen were examined and recorded with an optical microscope (Thermo Fisher Technology Co., Ltd., Waltham, MA, USA).

2.6. Analysis of oxidative stress indexes

Mouse liver tissues were homogenized (1:9 *w/v*) in normal saline and centrifuged at 3500 rpm for 10 min at 4 °C to remove cell debris and nuclei; the resulting supernatant was analyzed. The MDA, SOD, GSH, and CAT levels in the mouse livers of each experimental group were measured according to the kit instructions (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China) to evaluate the oxidative stress-related damage to the liver tissue.

2.7. Western blot analysis

Total protein was extracted from liver tissues (100 mg) derived from the mice in each group using RIPA lysis buffer (1:10, *w/v*). The liver tissues were fully grilled using a Handheld Homogenizer (TIANGEN Biotech Co., Ltd, Beijing, China) and then cleared by centrifugation at 12000×*g* for 5 min at 4 °C. The supernatant was obtained, and the concentrations of protein in each sample were measured with the BCA Protein Assay Kit. The protein was denaturized after being boiled at 95 °C for 6 min. Equal amounts of proteins (40–60 μg) were loaded onto an 8 %–12 % SDS-polyacrylamide gel for electrophoresis, then electrotransferred onto PVDF membranes and blocked with 5 % defatted milk for 1 h at 25 °C. Membranes were incubated with primary antibodies for PI3K (1:1000), Nrf2 (1:1000), HO-1 (1:1000), P-AKT (1:2000), AKT (1:1000), NQO1 (1:1000), GCLC (1:1000), and GCLM (1:1000) overnight at 4 °C and then incubated with the corresponding secondary antibodies (1:2500) for 1 h at 25 °C. ECL luminescence was used to detect the target protein bands. The intensity of each band was quantified by Image Pro Plus 6.0 and normalized to that of β-actin.

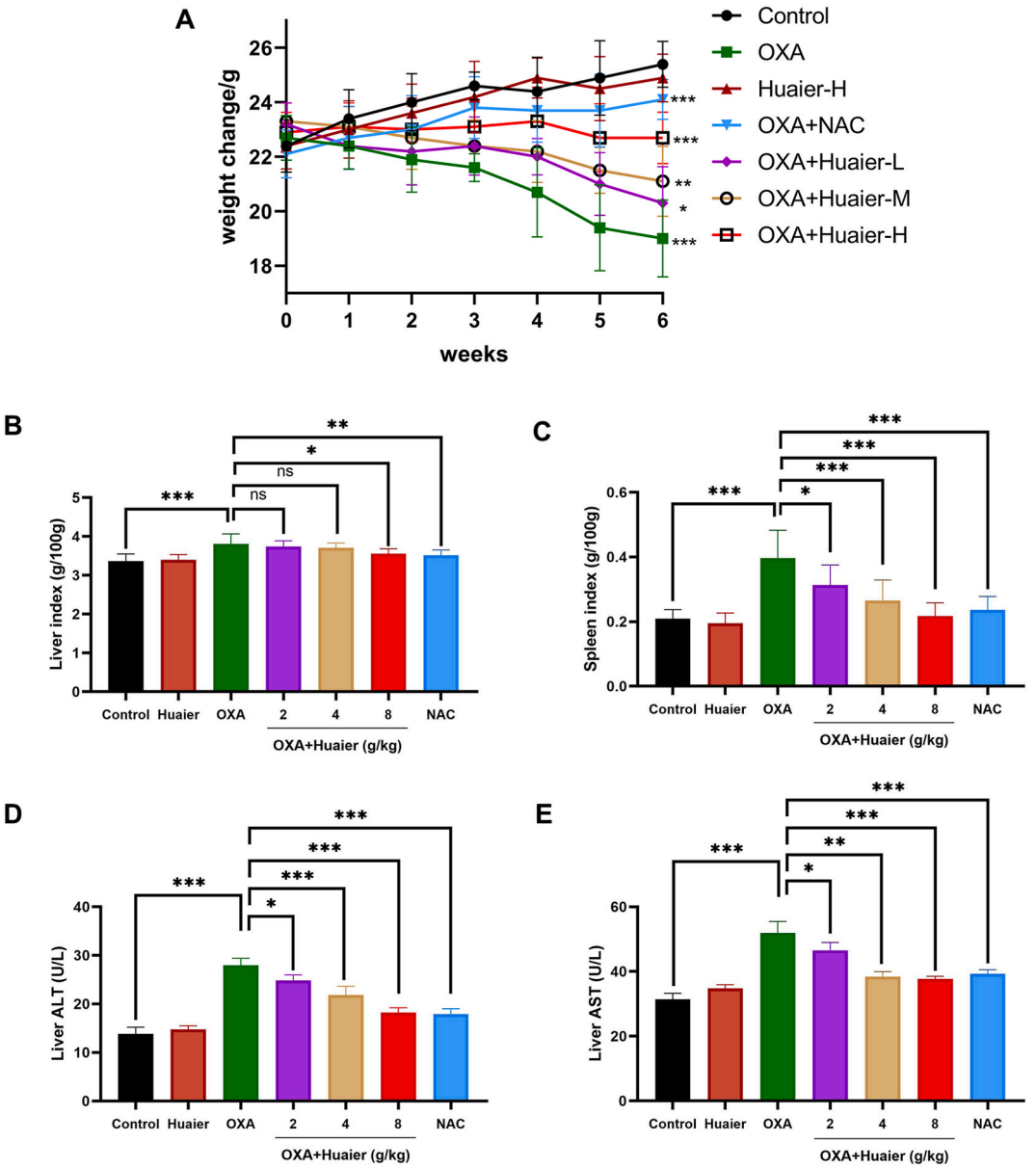

Fig. 1. Effects of Huaier extract on mice with oxaliplatin-induced hepatotoxicity. (A) Body weight; (B) Spleen index; (C) Liver index; (D) Serum alanine aminotransferase (ALT) levels; (E) Serum aspartate aminotransferase (AST) levels. Data shown as $\bar{x} \pm$ SD for each group (n = 9), between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$).

2.8. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Each group of mice liver tissues was grilled with TRIzol reagent (Takara Biotechnology Co., Ltd., Japan) to isolate total RNA. The RNA samples were quantified, and their purity was assessed using a NanoDrop BioChrom apparatus (Harvard Bioscience, Holliston, Massachusetts, USA). A total of 1 μg RNA was isolated and reverse transcribed to cDNA using an iScript cDNA Synthesis Kit (Takara Biotechnology Co., Ltd., Japan) according to the manufacturer's instructions. The concentration of primers was 2 μM in each reaction. The PCR primers are shown in Supplemental Table 1. The PCR cycle parameters were as follows: 95 °C for 5 min followed by 40 cycles at 95 °C for 10 s and 60 °C for 30 s. Results were obtained with Bio-Rad CFX Manager (Bio-Rad Laboratories, Inc, Shanghai, China) and further analyzed by the $2^{-\Delta\Delta Ct}$ method, with the β-actin gene used as the endogenous control [37].

2.9. Statistical analysis

The components of Huaier extract concentrations were calculated according to the corresponding linear regressions. Data was normally distributed. GraphPad Prism 8.0 software was used for the statistical analyses. The data were expressed as the mean and standard deviation ($\overline{x} \pm$ SD). Data between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test, with $p < 0.05$ being considered statistically significant.

3. Results

3.1. The quality of Huaier extract

For the detection of polysaccharide in Huaier extract, the regression equation was y = 28.5781x + 0.0097(R^2 = 0.9995), demonstrating a good correlation between absorbance (y) and concentration (x), being in the quantitative range of 0.0040–0.0200 mg/ml. The polysaccharide content in Huaier extract was 2.0 %. For the detection of protein in Huaier extract, the regression equation was y = 17.3153x + 0.2448(R^2 = 0.9988), which was a good correlation between absorbance (y) and concentration(x), being in the quantitative range of 0.0292–0.0500 mg/ml. The protein content of Huaier extract was 167.4 mg/g, which meets the internal control standard that the average content should not be less than 130.0 mg/g.

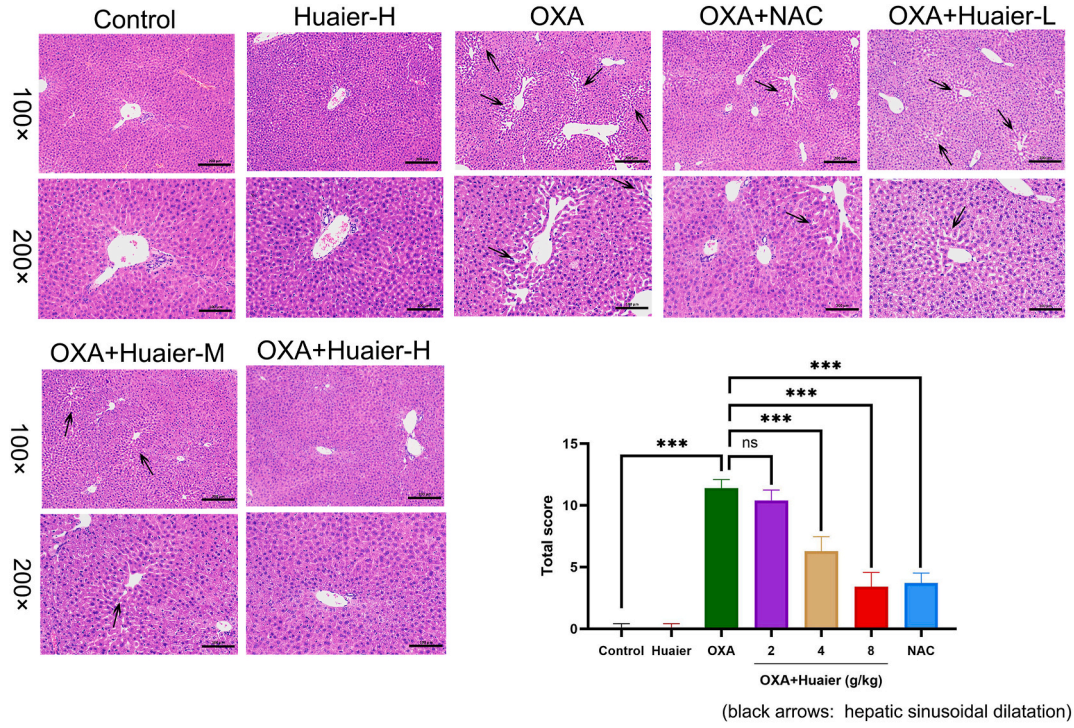

Fig. 2. Effects of Huaier extract on liver pathological damage in mice with oxaliplatin-induced hepatotoxicity. Haematoxylin and eosin (H&E) staining and liver injury score. Black arrows, hepatic sinusoidal dilatation. Data shown as $\overline{x} \pm$ SD for each group (n = 9), between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$). Scale bar: 200 μm (100 ×); 100 μm (200 ×).

3.2. Huaier extract improved OXA-induced hepatotoxicity in mice

3.2.1. Huaier extract enhanced mice weight and reduced organ indexes

First of all, we evaluated the overall state of the mice. As shown in Fig. 1A, the OXA (10 mg/kg) model group's mice displayed 25.20 % weight loss compared with the mice in the control group ($p < 0.001$). Mice administered with low (2 g/kg), medium (4 g/kg), high doses (8 g/kg) of Huaier extract and NAC showed increases in body weight of 6.84 %, 11.05 %, 19.47 %, and 26.80 % respectively, as compared to the OXA group. The mice in the OXA model group had a considerably higher liver index compared to the control group (Fig. 1C). After the treatment of Huaier extract and NAC, the liver index of mice in each group lowered, with the decrease in the high-dose Huaier extract and NAC groups being statistically significant compared to the OXA group (Fig. 1B). Splenomegaly can be a reliable predictor of the occurrence and severity of OXA-induced hepatotoxicity [38]. Depending on the dose, Huaier extract treatment alleviated the spleen enlargement induced by OXA (Fig. 1C). The therapeutic effects of high dose of Huaier extract were similar to that of NAC. Omitting OXA administration, Huaier extract alone had no significant impact on the body weight, liver index, and spleen index of mice compared to the control group.

3.2.2. Huaier extract improved mice biochemical Indicators

To assess whether Huaier extract alleviated OXA-induced liver injury, we performed ALT and AST analysis using commercial kits. As shown in Fig. 1D and E, we observed a significant increase in serum ALT and AST levels in the OXA model group compared with the control group ($p < 0.001$), and the activities of ALT and AST in each dose of the Huaier extract group and the NAC positive group were lower than those in the OXA model group. Also, the administration of Huaier extract alone had no significant effect on the above biochemical indexes (Fig. 1D and E).

3.2.3. Huaier extract improved mice pathological damage

Additionally, histologic sections of the control group (Fig. 2) revealed a physiologically normal segment of the hepatic lobule. The liver sinusoids dilated around the central vein (black arrow) and the enlargement of hepatocyte spaces was clearly observed, and the total liver damage score was dramatically escalated in the OXA model group ($p < 0.001$). While 4 g/kg and 8 g/kg Huaier extract as well as NAC improved the above injuries and reduced the damage score, which indicated that Huaier extract administration could improve these histopathological changes.

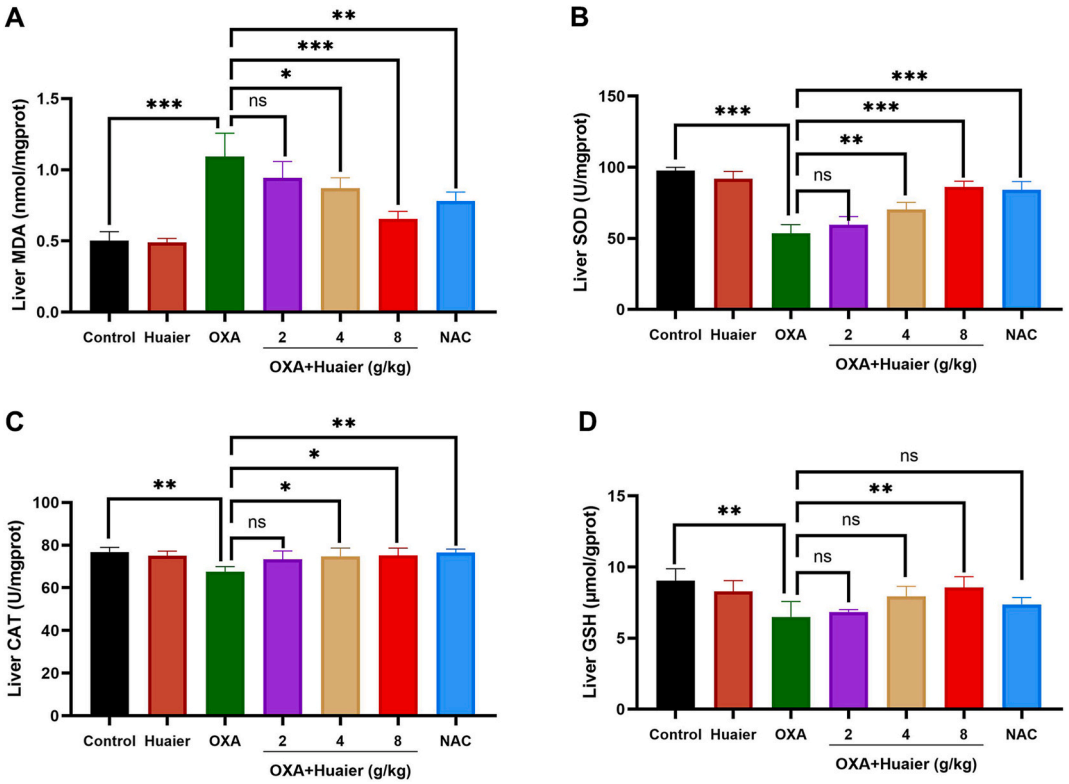

Fig. 3. Effects of Huaier extract on hepatic oxidative stress in mice with oxaliplatin-induced hepatotoxicity. (A) Malondialdehyde (MDA) levels; (B) Superoxide dismutase (SOD) levels; (C) Catalase (CAT) levels; (D) Glutathione (GSH) levels. Data shown as $\bar{x} \pm$ SD for each group (n = 9), between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test (***p < 0.001, **p < 0.01, and *p < 0.05).

3.3. Huaier extract reduced hepatic oxidative stress induced by OXA in mice

Our previous research found that oxidative injury can occur in mice with OXA-induced hepatotoxicity [13]. To investigate the effects of Huaier extract on oxidative stress in mice with OXA-induced hepatotoxicity, the levels of liver GSH (Fig. 3A) SOD (Fig. 3B), CAT (Fig. 3C), MDA (Fig. 3D), and GSH were tested. As shown in Fig. 3, the level of MDA increased significantly for 117.75 % in the OXA model group ($p < 0.001$), while SOD, CAT, and GSH all decreased for 45.17 % ($p < 0.001$), 12.06 % ($p < 0.01$), and 28.05 % ($p < 0.01$) respectively compared with the control group; this suggested the occurrence of oxidative stress. Administration of 4 g/mg Huaier extract increased the level of SOD, CAT, and GSH by 31.27 % ($p < 0.01$), 10.71 % ($p < 0.05$), and 22.05 % respectively, and lowered the MDA content by 20.40 % ($p < 0.05$) compared with the OXA model group. While, the administration of 8 g/kg to mice further alleviated liver oxidative stress, which was manifested in a 60.84 % ($p < 0.001$) increase in SOD, 11.27 % increase in CAT ($p < 0.05$), 31.93 % ($p < 0.01$) increase in GSH, and 40.2 % ($p < 0.001$) decrease in MDA compared with the OXA group.

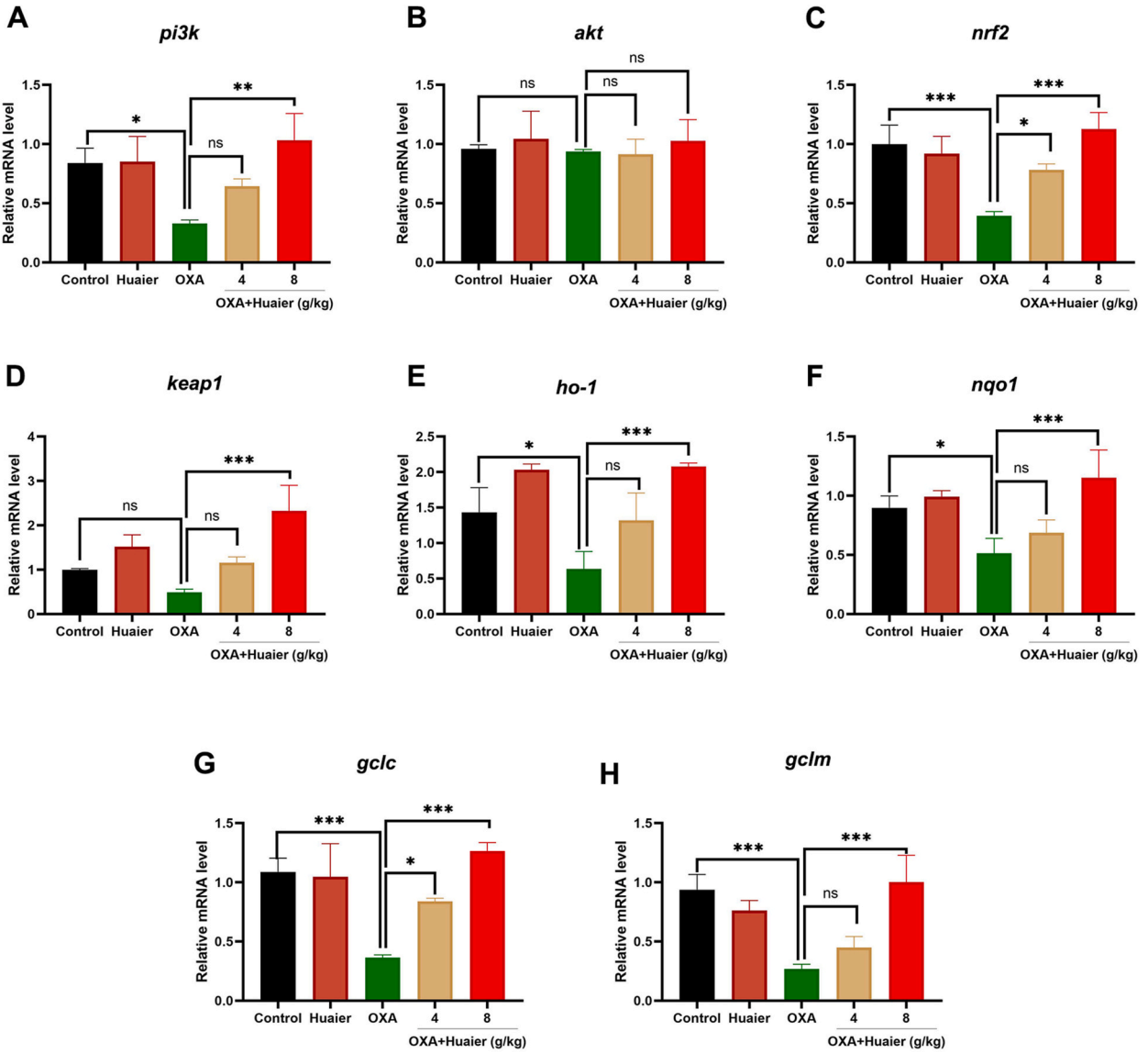

Fig. 4. Effects of Huaier extract on mRNA levels of the PI3K/AKT/Nrf2 pathway in the livers of mice with oxaliplatin-induced hepatotoxicity. Liver PI3K-pathway-related mRNA levels: (A) PI3K; (B) AKT; (C) Nrf2; (D) Keap1; (E) HO-1; (F) NQO1; (G) GCLC; (H) GCLM. Data shown as $\bar{x} \pm$ SD for each group (n = 9), between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$).

3.4. Huaier extract upragulated PI3K/AKT/Nrf2 signaling-pathway-related molecules in OXA-induced hepatotoxicity mice

Combined with the aforementioned research results, it can be concluded that the effect of low-dose Huaier extract on the treatment of liver damage caused by OXA was relatively weak, while medium- and high-dose Huaier extract had significant improvement effects. Reflecting this, medium- and high-dose Huaier extract treatment was adopted to explore the mechanism. Previous studies have proven that the PI3K/AKT/Nrf2 signaling pathway plays an important role in improving redox balance disorder. During oxidative stress, Nrf2 can induce the expression of a variety of protective proteins (HO-1, NQO1, GCLM, and GCLC) to regulate oxidative injury. Thus, the qRT-PCR and Western blot method (Fig. 5A) were applied to measure the PI3K/AKT/Nrf2 signaling-pathway-related molecules during liver injury. It was found that in the OXA model group, the mRNA and protein levels of PI3K (Figs. 4A and 5B), Nrf2 (Figs. 4C and 5E), HO-1 (Figs. 4E and 5F), NQO1(Figs. 4F and 5G), GCLC (Figs. 4G and 5H), and GCLM (Figs. 4H and 5I) in the liver tissue were significantly lower than those of the control group. Moreover, it was found that the mRNA and protein of those molecules were significantly up-regulated in mouse liver pre-treated with Huaier extract, compared with mouse liver treated with OXA alone. We also found that, though the level of AKT of each group had no significant difference in both mRNA (Fig. 4B) and protein levels (Fig. 5C), the p-AKT to AKT ratio (Fig. 5D) decreased in the OXA model group and increased after the action of Huaier extract.

3.5. PI3K inhibitor reversed the ameliorative effect of Huaier extract on hepatic injury induced by OXA

In this part of the study, the optimal dose of 8 g/kg Huaier extract and the PI3K inhibitor LY294002 were applied to verify the role of Huaier-induced PI3K/AKT/Nrf2 signaling pathway activation in mice with OXA-induced hepatotoxicity. In Fig. 6A, Huaier extract treatment increased the body weight of mice with OXA-induced hepatotoxicity, whereas LY294002 weakened this increase by 12.67 % ($p < 0.01$) compared with OXA + Huaier-H group. Furthermore, liver index (Fig. 6B) and spleen index (Fig. 6C) rebounded by 18.17 % ($p < 0.01$) and 69.67 % ($p < 0.001$) respectively, and biochemical indexes (Fig. 6D–E) increased about 40.86 % for ALT and 44.22 % for AST in the OXA + Huaier + LY294002 group, compared with the OXA + Huaier group, indicated that the liver injury was more severe after the administration of the PI3K inhibitor. Furthermore, the PI3K inhibitor reversed the ameliorative effect of Huaier extract on pathological changes in mouse livers (Fig. 7), showing severe hepatic sinusoidal dilatation enlargement.

3.6. PI3K inhibitor reversed the ameliorative effect of Huaier extract on hepatic oxidative stress induced by OXA

In order to confirm whether the PI3K signaling pathway was implicated in oxidative injury in mice, we also assessed the level of oxidative stress. The results in Fig. 8 revealed that the PI3K inhibitor precluded Huaier extract from exerting its antioxidant effects,

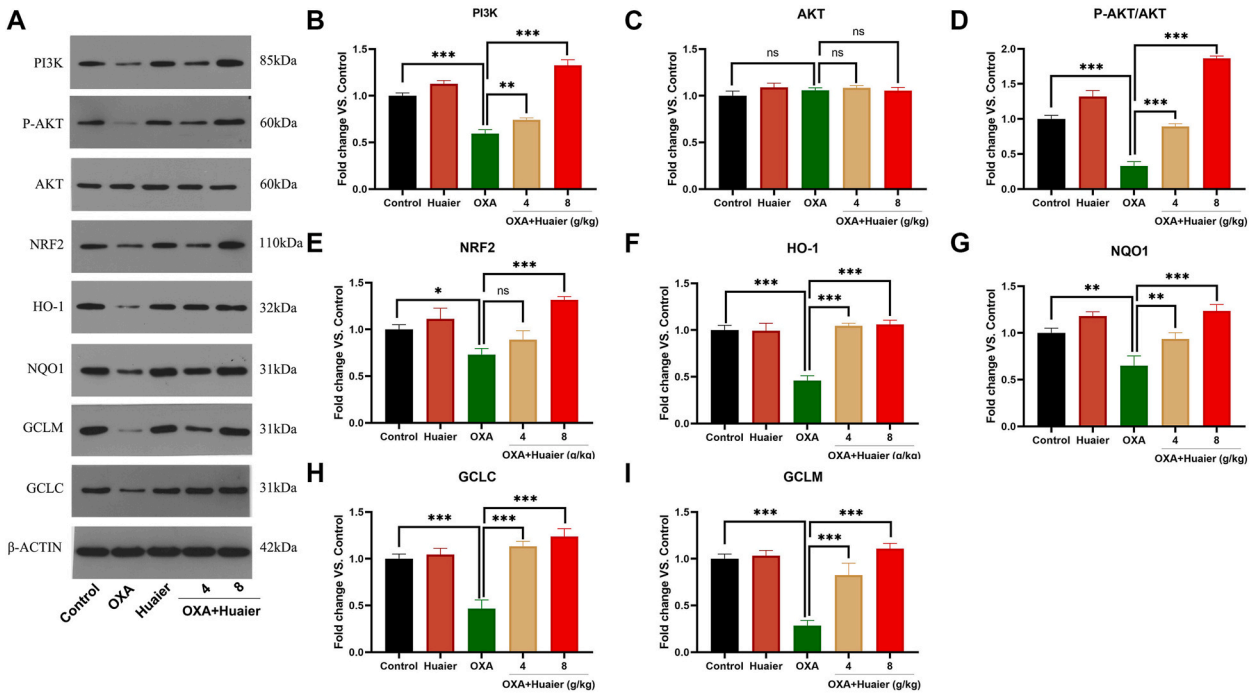

Fig. 5. Effects of Huaier extract on protein levels of the PI3K/AKT/Nrf2 pathway in the livers of mice with oxaliplatin-induced hepatotoxicity. Liver PI3K-pathway-related protein levels: (A) Protein bands of liver PI3K pathway; (B) PI3K; (C) AKT; (D) Nrf2; (E) Keap1; (F) HO-1; (G) NQO1; (H) GCLC; (I) GCLM. Data shown as $\bar{x} \pm SD$ for each group (n = 9), between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$).

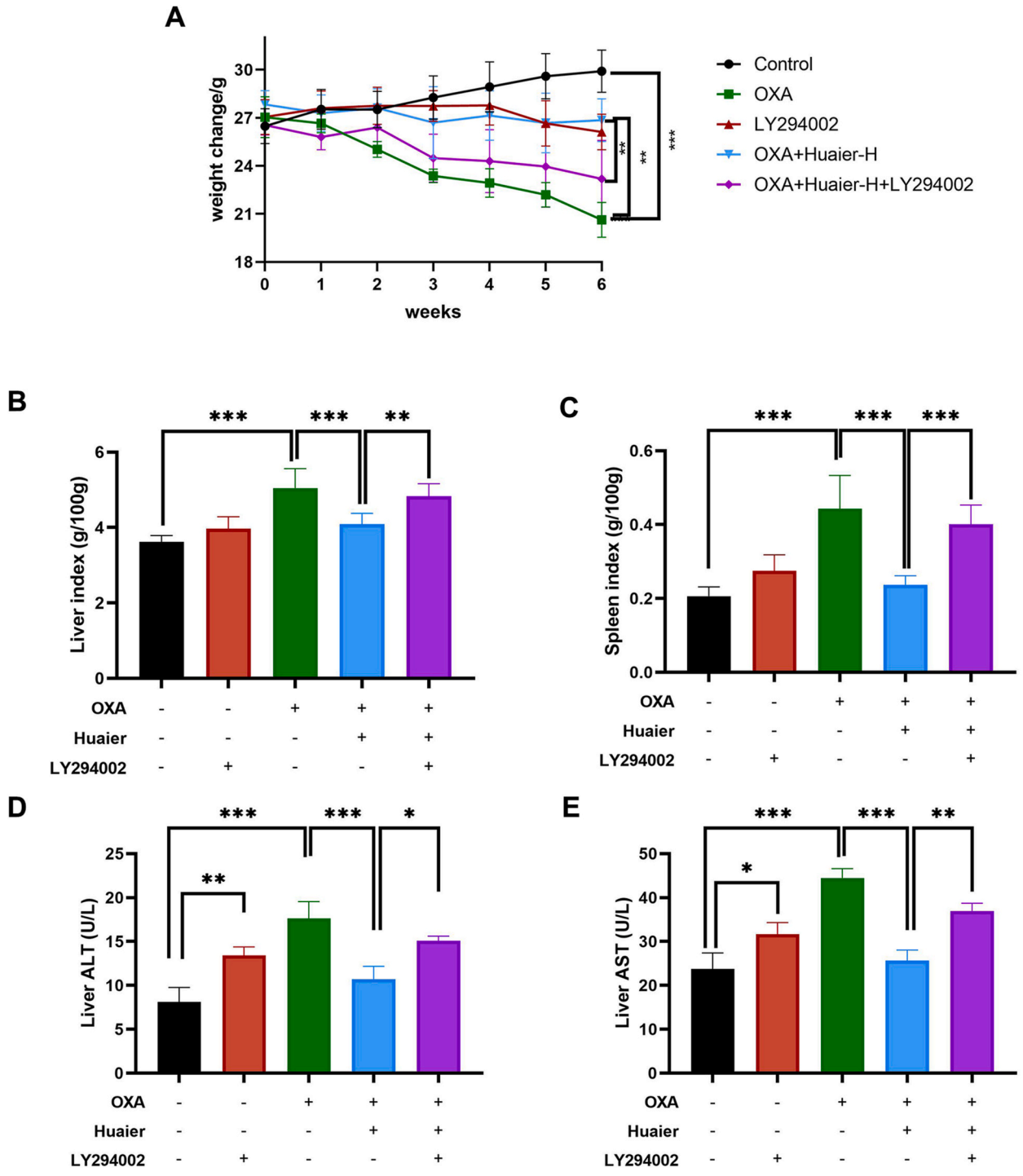

Fig. 6. Effects of Huaier extract and LY294002 on mice with OXA-induced hepatotoxicity. (A) Body weight; (B) Spleen index; (C) Liver index; (D) Serum alanine aminotransferase (ALT) levels; (E) Serum aspartate aminotransferase (AST) levels. Data shown as $\bar{x} \pm$ SD for each group (n = 9), between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$).

indicated by the elevated level of MDA (Fig. 8A) at 45.50 % and decreased levels of SOD (Fig. 8B), CAT (Fig. 8C), and GSH (Fig. 8D) at 14.39 %, 23.51 %, and 25.11 % respectively ($p < 0.05$). Combined with the previous results, the treatment of Huaier extract appeared to relieve OXA-induced hepatotoxicity via the PI3K/AKT/Nrf2 antioxidant pathway. The results of liver oxidative stress indexes indicated that blocking PI3K could reverse the ameliorative effect of Huaier extract in mice with OXA-induced hepatotoxicity. In order

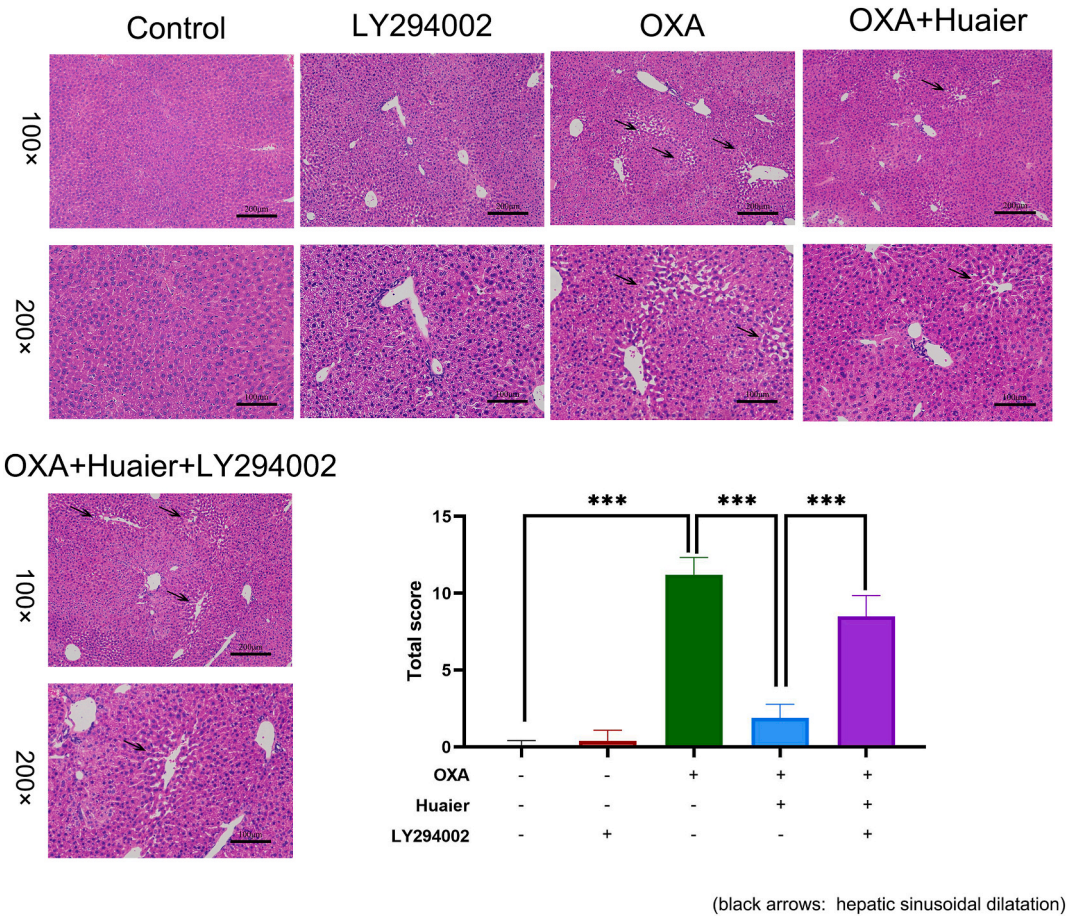

Fig. 7. Effects of Huaier extract and LY294002 on liver pathological damage in mice with oxaliplatin-induced hepatotoxicity. Haematoxylin and eosin (H&E) staining and liver injury score. Black arrows, hepatic sinusoidal dilatation. Data shown as $\bar{x} \pm SD$ for each group (n = 9), between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$). Scale bar: 200 μm (100 ×); 100 μm (200 ×).

to further verify the mechanism of the relevant pathway, we detected the effect of blocking PI3K on the expression of relevant pathway proteins (Fig. 9A). The results indicated that Huaier extract could up-regulate the decrease of PI3K (Fig. 9B), p-AKT/AKT (Fig. 9C–D), and Nrf2 (Fig. 9E) induced by OXA, which were significantly reversed by the PI3K inhibitor. The protective effect of Huaier extract on liver oxidative stress injury brought on by OXA in mice was regulated by the PI3K/P-AKT/Nrf2 signaling pathway, as further evidenced by these studies.

4. Discussion

Huaier is a traditional Chinese medicine that is applied as an adjuvant medicine for cancer therapy. It has been shown that Huaier extract is less toxic to organisms. Intragastric administration of high-dose Huaier extract to mice(> 20000 mg/kg) and rats (> 15000 mg/kg) did not affect the growth of experimental animals. The hemogram, biochemical indices, and growth of the experimental animals were all normal, and there were no signs of drug-induced pathological alterations [39]. The main component is Huaier proteoglycan, and its hydrolysate contains 6 monosaccharides and 18 amino acids [30]. In our study, Jiangsu Qidong Gaitianli Co., Ltd provided and determined the components, mainly polysaccharide and protein of Huaier extract (batch number: 210124). The protein content is 167.4 mg/g and the polysaccharide content is 2.0 %. The "Huaier granule" made from Huaier extract is approved by the State Food and Drug Administration of China. it can be used alone or in combination with other drugs to treat malignant lymphoma, liver cancer, and colon cancer. In this study, we discovered the antioxidant capacity of extract and further demonstrated its application as an adjuvant for clinical chemotherapy.

Oxaliplatin has been successfully employed in colorectal cancer and is the mainstay treatment for metastatic disease. However, Oxaliplatin treatment invariably causes several harmful and adverse effects depending on the dose administered to cancer patients. In addition to common allergic reactions, and neuropathy, recent studies have shown that OXA can also cause liver damage, particularly sinusoidal obstruction syndrome, which is characterized by swollen HSEC cells, expanding intercellular space, and the continued

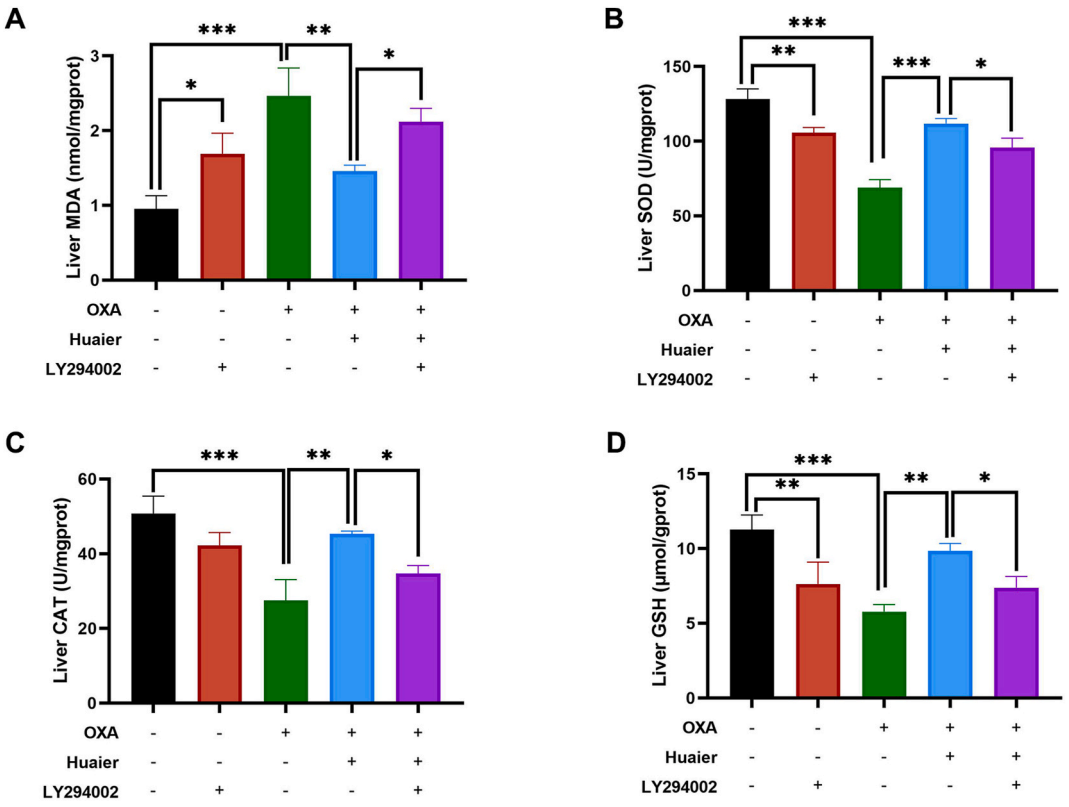

Fig. 8. Effects of Huaier extract and LY294002 on hepatic oxidative stress in mice with oxaliplatin-induced hepatotoxicity. (A) Malondialdehyde (MDA) levels; (B) Superoxide dismutase (SOD) levels; (C) Catalase (CAT) levels; (D) Glutathione (GSH) levels. Data shown as $\bar{x} \pm$ SD for each group (n = 9), between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$).

disintegration of hepatic sinusoid wall. Our current and previous studies demonstrated that oxaliplatin-induced increased oxidative stress in the liver, indicating excessive ROS may play an important role in oxaliplatin-induced liver injury.

In the present study, we examined the processes by which Huaier extract shields the liver against oxaliplatin-based chemotherapy using in vitro models. The decrease in body weight and increases in liver and spleen indexes induced by OXA were mitigated in Huaier-treated groups. In comparison to the OXA model group, serum levels of ALT and AST were considerably lower in the groups treated with Huaier extract. The H&E staining demonstrated OXA-induced hepatic sinusoid dilatation revealing that Huaier extract effectively protected against these pathological changes. Therefore, the findings of our research suggest that Huaier extract could contribute significantly to reducing OXA-induced hepatotoxicity.

Many studies have revealed that oxidative stress is a crucial element in the toxicity caused by platinum medications [40–42], This is also relevant to oxaliplatin-induced hepatotoxicity [43–46]. When excess ROS produced by detrimental stimuli in the body exceeds the liver's clearance ability, this causes an imbalance of the redox state, leading to oxidative stress. Excess ROS can contribute to structural and functional abnormalities in the liver, causing damage to hepatocytic proteins, lipids, and DNA [7]. As an oxidative species, MDA is a peroxidation product that is detrimental to the body [47], while GSH, SOD, and CAT are important antioxidant enzymes that eliminate overexpressed ROS. One of the cellular excretion mechanisms of OXA is that it binds with GSH, which leads to the consumption of intracellular antioxidants [8]. The current study showed that the overproduction of MDA and the depletion of GSH, SOD, and CAT were reversed by Huaier extract, with the high-dose Huaier treatment achieving the best therapeutic effect. A previous study by Fang et al. also demonstrated the protective role of Huaier in cisplatin-induced nephrotoxicity through its antioxidant effects [26]. Xie et al. found that Huaier could reduce the production of ROS and block the invasion of colorectal cancer cells induced by phorbol ester [48]. Accordingly, our results also showed that Huaier extract treatment could relieve OXA-induced liver injury by repressing oxidative stress.

A well-known signaling mechanism that controls oxidative damage, inflammation, and autophagy is the PI3K/AKT pathway [49]. Containing p85, p55, p110, and other subunits, the PI3K complex can activate downstream protein kinase AKT and exert a wide range of biological effects [49]. Numerous studies have shown that the activation of the PI3K/AKT pathway can mediate the positive regulation of intracellular Nrf2 signals and participate in the process of antioxidation in different tissues [50–52]. As a transcription factor, Nrf2 is crucial for regulating oxidative stress and protecting cells. In response to oxidative stress, phosphorylation of Nrf2 causes it to separate from Kelch-like ECH-associated protein 1 and migrate to the nucleus, where it triggers the production of antioxidant enzymes downstream [53]. In addition, the activation of Nrf2 can boost the expression of rate-limiting enzyme-coding genes in

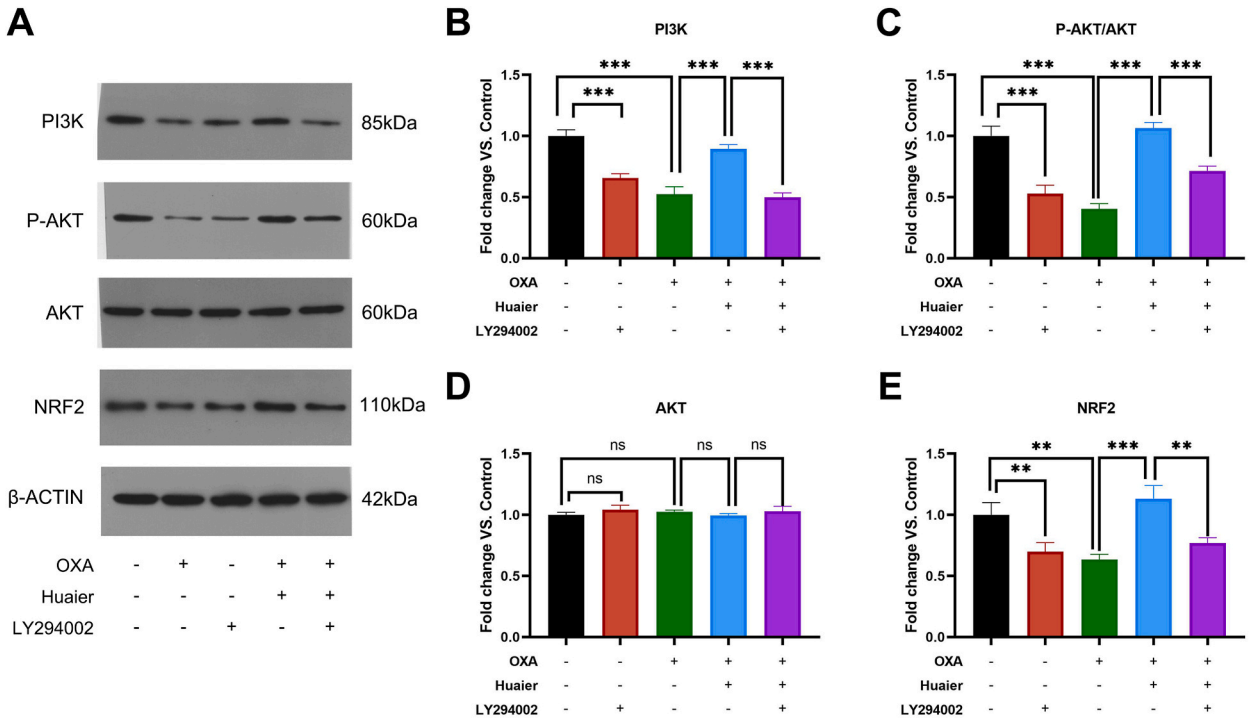

Fig. 9. Effects of Huaier extract and LY294002 on protein levels of the PI3K/AKT/Nrf2 pathway in the livers of mice with oxaliplatin-induced hepatotoxicity. (A) Protein bands of liver PI3K pathway; (B) PI3K; (C) p-AKT/AKT; (D) AKT; (E) Nrf2. Data shown as $\bar{x} \pm$ SD for each group (n = 9), between groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$).

glutamate-cysteine-modified ligase, which can then increase levels of GSH [53]. Huaier polysaccharide was discovered to be capable of decreasing oxidative stress and apoptosis by triggering the PI3K/AKT/mTOR signaling pathway and reducing the nephrotoxicity brought on by cisplatin [26]. In our study, the level of PI3K and phosphorylated AKT was decreased in the OXA model group, with Huaier extract treatment attenuating this impact; correspondingly, compared with the OXA model group, the down-regulation of Nrf2 and its downstream effects was alleviated in the Huaier-treated groups. Moreover, blocking the PI3K pathway by LY294002 can diminish the medicinal impact of Huaier extract on liver injury induced by OXA. Thus, these results suggested that Huaier activates PI3K/Akt signaling to protect against OXA-induced liver injury.

There are still some issues that need to be resolved before Huaier is widely applied in co-treatment with OXA. First, the chemical basis for Huaier extract in alleviating OXA liver injury via antioxidant damage is still unknown. In the future, we will focus on exploring the main components of Huaier extract and exploring the antioxidant active ingredients, which will be more helpful for its clinical application. Second, the mechanisms involved in Huaier extract alleviating OXA-induced hepatotoxicity need to be further verified by in vitro experiments with corresponding hepatocytes.

5. Conclusion

Our study indicates that Huaier extract can ameliorate OXA-induced hepatotoxicity through inhibiting ROS-mediated oxidative stress, with the mechanism for this relating to the PI3K/AKT/Nrf2 signal transduction pathway (Fig. 10). Our results suggest that Huaier has a potential hepatoprotective effect and can be an effective drug to reduce the hepatotoxicity induced by OXA. This research provided a possible theoretical foundation for the clinical use of Huaier as an adjuvant medication for OXA chemotherapy.

Fundings

The present study was supported by the Clinical Toxicology Foundation of the Chinese Society of Toxicology (grant no. CST2020CT107 to CL Zhang), the Research Project of the Drug Clinical Evaluation Professional Committee of China Pharmaceutical Association (grant no. CPA-Z06-CZ-2021-004 to CL Zhang), and the Chen Xiao-ping Foundation for the Development of Science and Technology of Hubei Province (grant no. CXPJJH121003-2122 to XH Ren).

Ethical statement

The present study was approved by the Animal Welfare and Ethical Committee of Tongji Hospital (Wuhan, China; approval no.TJH-

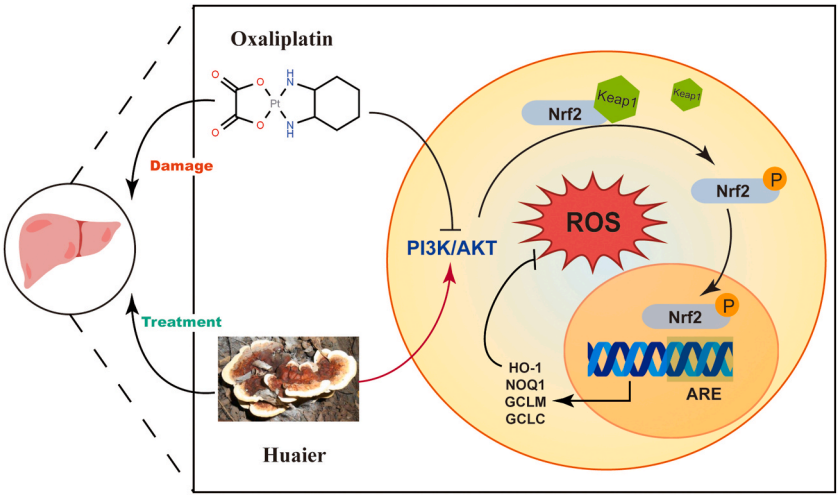

Fig. 10. Schematic representation of the proposed role of Huaier extract in mice with OXA-induced hepatotoxicity. During the use of oxaliplatin, the PI3K/AKT pathway has been in an inhibited state. Huaier extract activates PI3K/AKT to allow the nuclear transfer of Nrf2, which promotes the expression of antioxidant enzymes and the clearance of reactive oxidative species (ROS).

202101009). All procedures conformed to the ARRIVE guidelines.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Xinwei Cheng: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation. **Chen Zhu:** Validation, Methodology, Investigation, Data curation. **Yunzhou Chen:** Writing – review & editing. **Min Li:** Investigation. **Guodong Li:** Investigation. **Yue Zu:** Investigation. **Qianyan Gao:** Investigation. **Tianze Shang:** Investigation. **Dong Liu:** Supervision, Conceptualization. **Chengliang Zhang:** Writing – review & editing, Funding acquisition, Conceptualization. **Xiuhua Ren:** Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37010.

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