Gomphrena globosa L. extract alleviates carbon tetrachoride-induced liver injury in mice by activating antioxidant signaling pathways and promoting autophagy

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Research Article

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Abstract

Background Carbon tetrachloride (CCL_4) is highly toxic to animal liver and is a major factor leading to liver injury. *Gomphrena globosa* L. (GgL) is a comestible plant with anti-inflammation and antioxidation properties. This study was aim to investigate the potential therapeutic effects of GgL on liver injury.

Methods and Results A mice model of chronic liver injury was established by intraperitoneal injection of CCl_4 (0.4 mL/kg) for 3 weeks, and the mice were treated intraperitoneally with different concentrations of GgL crude extract (GgCE; 100, 200, 300 mg/kg) or Bifendatatum (Bif; 20 mg/kg) in the last 2 weeks. The results showed that GgCE treatment could alleviate the liver injury induced by CCL_4, improve the pathological changes caused by CCL_4 on the mice liver, and enhance the antioxidant capacity. We also found that GgCE increased the expression of antioxidant stress related proteins, decreased the phosphorylation levels of autophagy related proteins PI3K and mTOR, and decreased the expression of LC3 Ⅱ and P62 proteins.

Conclusions These results suggest that GgCE alleviated CCL_4-induced chronic liver injury in mice by activating antioxidant signaling pathways and promoting autophagy, indicating a potential therapeutic effect of GgCE on liver injury.

1. Introduction

Liver injury has become a common clinical health hazard. Chronic liver injury occurs as a result of abnormal liver function caused by long-term repeated damage to liver cells, and chronic liver dysfunction can lead to liver fibrosis or cirrhosis(1). CCl_4 is a colorless, toxic liquid organic compound. At high concentrations, CCL_4 can cause severe damage to the liver, leading to lipid peroxidation and inflammatory responses in liver tissue, resulting in hepatocyte damage and necrosis, and even liver fibrosis and cirrhosis in severe cases. CCl_4 is widely used to establish models of chronic liver injury in animals(2). CCl_4 stimulation induces liver parenchyma damage, causing aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities are increased(3). However, no effective drugs have been identified for the treatment of CCL_4-induced chronic liver injury.

Many liver diseases are associated with the development and progression of reactive oxygen species (ROS), and it has been found that ROS excess is a direct cause of oxidative stress(4). Formation of ROS is one of the manifestations of CCL_4-induced liver injury in mice(5), and importantly, excessive accumulation of ROS can promote lipid peroxidation and produce lipid peroxidation products, such as malondialdehyde(6). Glutathion (GSH), glutathion peroxidase (GSH-Px), malondialdehyde (MDA) and superoxide dismutase (SOD) are common indicators used to evaluate resistance to oxidative stress(7).

Nrf2, a key factor in the cellular response to oxidative stress, is regulated by Keap1, a binding protein for Nrf2 in the cytoplasm, bound to actin(8). There is evidence that modification of the cysteine residues of Keap1 allows Nrf2 to accumulate in the nucleus and express Nrf2 target genes(9). Nrf2 in hepatocytes

plays an important role in blocking CCL_4-induced liver injury by regulating oxidative and inflammatory responses(10). Conversely, Nrf2 silencing can aggravate the process of CCL_4-induced liver injury in mice(11). Indeed, chemically induced Nrf2 expression has been shown to protect the liver from various hepatotoxic agents(12). Similarly, Nrf2-activated downstream antioxidant proteins have antioxidant effects. Glutamate cysteine ligase (GCL) consists of a catalytic subunit (GCLC) and a modifing subunit (GCLM)(13). As a downstream molecule of Nrf2, it catalyzes the synthesis of glutathione (GSH), which is an important antioxidant and cofactor for glutathione S-transferase binding(14). HO-1 and NQO1 are also downstream molecules of Nrf2 signaling pathway(15). Under normal conditions, Nrf2 is inhibited. However, when free radicals attack the body, Nrf2 enters the nucleus and activates HO-1 and NQO1 to scavenge free radicals and increase the antioxidant capacity of the body to combat liver injury(16). Therefore, activation of Nrf2 and its downstream target protein is an important research direction for the treatment of CCL_4-induced liver injury.

Autophagy is an important phenomenon in the removal of metabolic waste by the body(17). And PI3K/AKT signaling pathway, which can regulate downstream mTOR, inhibits autophagy by activating mTOR is an important upstream signaling pathway of autophagy(18). Numerous studies have shown that autophagy can be inhibited when liver injury occurs. In fact, enhanced autophagy can prevent the progression of a variety of liver diseases, including liver injury(19). When autophagy is enhanced, LC3 Ⅰ can be transformed into LC3 Ⅱ and translocated to the autophagosome membrane, facilitating the formation of double-membrane autophagosome(20). P62 interacts with LC3 and acts as a cargo adapter to transport damaged mitochondria to the autophagosome(21). It is generally believed that accumulation of P62 indicates impaired autophagy, while reduction of P62 indicates enhanced autophagy(22). In conclusion, dysregulation of autophagy is associated with the occurrence of liver injury, and regulation of autophagy homeostasis is considered as a potential therapeutic strategy for liver injury.

Chinese herbal medicine has a long history of application in China, and due to the characteristics of rich resources, small toxic and side effects of Chinese herbal medicine. A large number of studies have proved that Chinese herbal medicine and its effective components can significantly improve liver function, reduce inflammatory response and cell apoptosis, thus alleviating liver injury caused by various factors, and the application prospect is very broad(23). *Gomphrena globosa* L. (GgL) is a comestible plant from *amaranthaceae* families(24). In recent years, many studies have found that GgL contains polysaccharides, volatile oil, flavonoids and their glycosides, saponins and other active components(25). Its monomer has been studied in anti-inflammation and anti-oxidation. However, it has not been reported whether GgL crude extract (GgCE) can protect liver injury by anti-oxidation and promoting autophagy. Our experiment was conducted to investigate the protective effect of GgCE on CCL_4-induced chronic liver injury in mice. It provides a theoretical basis for the treatment of liver injury with comestible plant.

2. Materials And Methods

2.1 Chemicals

CCL_4 was purchased from Tianjin Fengchuan Chemical Reagent Technology Co., LTD (Tianjin, China). Bifendatatum (Bif) was purchased from Wanbond Pharmaceutical Group Limited (Zhejiang, China).

2.2 Plant material and extracts preparation

The GgL was purchased from a medicinal plants distributor (Morais e Costa & C.ª Lda, Portugal). Boil 10 g GgL with 500 mL water for 5 min, use Buchner funnel filtered extract, and lyophilize the filtrate to obtain the crude extract of GgL (GgCE). The active ingredients can be found in Silva's research(26).

2.3 Experimental animals

Male C57BL/6 (20 ± 2 g) mice were offered by the Liaoning Changsheng Biotechnology (produc-tion license: SCXK2010-0001; Liaoning, China). All mice studies conformed to the U.S. National Institutes of Health (NIH), the Guide for the Care and Use of Laboratory Animals, and were authorized by the related ethnical regulations of Jilin University (Number of permit: SY202110003). The animals were housed at a room temperature of 24 ± 1℃ (Relative humidity: 40%-80%) with a 12 h light/dark cycle and had free access to food and water. Mice were randomly divided into 7 groups with 5 mice in each group, including control group, CCl_4 (0.4 mL/kg) group, GgCE (300 mg/kg) group, CCl_4 + GgCE (100 mg/kg) group, CCl_4 + GgCE (200 mg/kg) group, CCl_4 + GgCE (300 mg/kg) group, and CCl_4 + Bifendatatum (Bif; 20 mg/kg) group. CCl_4 was dissolved in olive oil, and Bif was dissolved in saline solution. Mice were intraperitoneally injected with CCL_4 twice a week for 3 weeks to establish a CCL_4-induced chronic liver injury model in mice. Starting from the second week, mice were intragastric with different concentrations of GgCE for the last 2 weeks. After the last intragastric administration, the mice fasted without water, and blood was collected from the eye pit 12 h later. Then the liver of mice was taken for index determination or stored at -80℃.

2.4 Measurement of AST, ALT, ROS and SOD in mice serum

The mice blood was left standing at room temperature for 8 h, then centrifuged at 3000 rpm/min for 10 min. The serum was taken, serum AST and ALT levels were determined. Specific experimental steps were performed according to the instructions of the commercial kit (Nanjing Jiancheng Bioengineeering Institute, Jiangsu, China).

2.5 Measurement of MPO, TP, MDA, GSH, and GSH-Px in mice liver

Mice liver tissues were collected and homogenized according to the kit instructions to detect hepatic ROS (FEIYA BIOTECHNOLOGY, Jiangsu, China) levels, GSH, GSH-Px (Solarbio, Beijing, China) activity, MDA content, myeloperoxidase (MPO), and SOD (Nanjing Jiancheng Bioengineeering Institute, Jiangsu, China) activity. The specific test steps were performed according to the commercial kit instructions.

2.6 Histopathological studies

Mice livers were preserved in 10% formalin solution and embedded in paraffin. They were cut into 5 μm sections and stained with hematoxylin and eosin (H&E). The pathological changes of liver were observed under a light microscope.

2.7 Protein expressions studies by western blot

At 4℃, fresh liver tissue was cleaved with RIPA lysate, phosphatase inhibitor and PMSF, supernatant was collected by centrifugation, and the protein weight was unified to 20 ug by adding PBS and loading buff. It was injected into 12% SDS polyacrylamide gel, electrophoresis and then transferred to a PVDF membrane, sealed with 5% skim milk made with TBST, and then Nrf2, β-actin, LC3 Ⅰ/Ⅱ (from Proteintech (Boston, MA, USA)), Keap1, GCLC, GCLM, P62 (from Abcam (Cambridge, MA, USA)), HO-1, NQO1, p-PI3K, PI3K, p-mTOR and mTOR (from Cell Signal Technology (Beverly, MA, USA)) primary antibody incubated at 4℃ overnight. After washing with TBST 4 times for 5 min each time, the membrane was incubated with HRP (Boster, CA, USA) coupled secondary antibody (1: 5000) at room temperature for 1 h, and washed with TBST 4 times for 5 min each time. Finally, the membranes were visualized by Omni-ECL Pico Light Chemiluminescence Kit (YAMEI, Shanghai, China), and the images were obtained by using the Tanon 4200 Luminous Imaging System (YPHBIO, Beijing, China). Image J software was used for western blot bands quantitative analysis.

2.8 Statistical analysis

All experiments were repeated more than three times. All data are presented as mean ± SD. Data were analyzed using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). Differences between groups were analyzed by Ordinary one-way ANOVA test. $P > 0.05$ indicated no significant difference, $0.01 < P < 0.05$ indicated significant difference, $P < 0.01$ was considered to be a very significant difference.

3. Results

3.1 GgCE alleviated CCL_4-induced liver injury in mice liver

In order to detect the protective effect of GgCE on CCL_4-induced liver injury, AST, ALT, MPO and TP kits were used for detection. The results showed that CCL_4 significantly increased serum AST and ALT levels and hepatic MPO activity compared with control group (Fig. 1). Different concentrations of GgCE (100, 200, 300 mg/kg) could reduce serum AST, ALT levels and hepatic MPO activity in CCL_4-induced chronic liver injury, and there were extremely significant differences compared with CCL_4 group. GgCE could reduce serum AST and ALT levels, but its therapeutic effect was not as good as Bif. However, GgCE (300 mg/kg) was superior to Bif in reducing hepatic MPO activity. CCL_4 could observably reduce hepatic TP content (Fig. 1. D), GgCE treatment group could markedly improve hepatic TP content reduction compared with CCL_4 group. These results demonstrated that GgCE alleviated CCL_4-induced liver injury in mice.

Insert Fig. 1

3.2 GgCE improved CCL_4-induced oxidative damage in mice liver

To further explore the effects of GgCE on CCL_4-induced liver oxidative damage, we detected the changes of serum ROS levels, hepatic MDA content, GSH and GSH-Px activity and serum SOD activity. As shown in Fig. 2, compared with the control group, CCL_4 could notably increase serum ROS levels and hepatic MDA content. GgCE treatment group with different concentrations could reduce the cumulative serum ROS levels and alleviate the increased hepatic MDA content caused by CCL_4. Compared with control group, CCL_4 could also markedly reduce hepatic GSH, GSH-Px activity and serum SOD activity, and signally increased that after treatment with different concentrations of GgCE. These results suggested that GgCE improved CCL_4-induced oxidative damage in mice

Insert Fig. 2

3.3 GgCE improved CCL_4-induced pathological changes in mice liver

For purpose of observe the effect of GgCE on CCL_4-induced liver injury in mice in more detail, we made tissue sections of the large lobe of the liver and stained them with H&E. In Fig. 3, under microscope, the liver lobule structure of the control group was normal. However, in the CCL_4 group, the liver tissue structure was disorganized, the hepatocytes were swollen and degenerated, and the central vein showed obvious bleeding symptoms, with congestion and inflammatory cell infiltration, and the tube wall was blurred and unclear. GgCE could improve liver injury in a dose-dependent manner, especially under the action of high dose (300 mg/mL) GgCE, the liver tissue structure was orderly, the central vein had no bleeding and inflammatory cell infiltration, and the overall state of the liver was close to normal. These results indicated that GgCE improved CCl_4-induced pathological changes in mice liver.

Insert Fig. 3

3.4 GgCE activated antioxidant signaling pathways in CCL_4-induced chronic liver injury

The above results showed that GgCE could resist oxidative damage of mice liver induced by CCL_4. In order to explore the effect of GgCE on CCL_4-induced liver oxidative damage from the mechanism, we used western blot assay to detect the expression of related proteins (Fig. 4). The results showed that the expressions of Nrf2, GCLC, GCLM, HO-1 and NQO1 proteins in mice liver tissue were inhibited by CCL_4, while GgCE treatment activated the expression of Nrf2 protein and downregulated the CCL_4-induced increase in Keap1 protein levels in mice liver. In addition, the expression of GCLC, GCLM, HO-1 and NQO1

proteins, the downstream of Nrf2, was activated. These consequences suggest that GgCE alleviated oxidative damage in CCL_4-induced chronic liver injury by activating antioxidant signaling pathways.

Insert Fig. 4

3.5 GgCE promoted autophagy in CCL_4-induced chronic liver injury

The above study found that GgCE had a protective effect on CCL_4-induced liver injury in mice. In order to investigate whether autophagy is the further reason for GgCE to protect liver injury, we used western blot to detect the expression of related autophagy proteins (Fig. 5). By analyzing the protein content, it was found that CCL_4 promoted the phosphorylation of PI3K and mTOR proteins and promoted the expression of P62, and inhibited the expression of LC3 Ⅱ protein. GgCE treatment could effectively reduce the phosphorylation PI3K and mTOR proteins, inhibit the expression of P62 protein, and activate the expression of LC3 Ⅱ protein. The results showed that GgCE alleviated CCL_4-induced chronic liver injury by promoting autophagy.

Insert Fig. 5

3.6 Potential mechanism of GgCE to alleviate CCL_4-induced chronic liver injury

CCL_4 stimulation in mice caused oxidative damage and impaired autophagic pathway, and GgCE treatment in mice could activate antioxidant signaling pathways against oxidative damage and promote autophagy to alleviate liver injury in mice (Fig. 6).

Insert Fig. 6

4. Discussion

Liver injury seriously affects liver function and endangers health, so we need to find more effective drugs to treat liver injury. From the study of Luís R et al.(26), we can analyze that GgCE contains 24 phenolic compounds, includes free phenolic acids, flavonoid glycosides and their aglycones, and 8 betacyanins. These bioactive components have been studied in the field of antioxidants, but no reports have been seen on the treatment of liver injury, and we can get the content of these components from Luís R's study.

In our study, we investigated whether GgCE has a protective effect on CCL_4-induced liver injury. To demonstrate the therapeutic effect of GgCE, we used biochemical kits, H&E staining and western blot. Our experimental results showed that GgCE alleviated CCL_4-induced chronic liver injury in mice by activating antioxidant signaling pathways and promoting autophagy.

CCL_4 can lead to the occurrence of liver injury, which can cause elevation of ALT and AST, hepatocyte inflammation, necrosis, steatosis and other changes(27). AST and ALT mainly exist in hepatocyte, and when the liver is damaged, transaminases in hepatocyte are transferred in the blood, resulting in an increase in the content of transaminase in the blood. ALT is more sensitive to the damage of liver cells than AST(28). The results of this study showed that injection of CCL_4 directly increased the serum AST and ALT levels in mice, which decreased after GgCE treatment, suggesting that GgCE can resist the effects of CCL_4. MPO activity can be used as a measure of neutrophil count, which tends to increase and exacerbate inflammatory responses in models of liver injury(29). We found that hepatic MPO activity was significantly increased after CCL_4 treatment, indicating that CCL_4 can cause increased inflammatory response in mice liver, and GgCE treatment can significantly downregulate hepatic MPO activity, suggesting that GgCE alleviates inflammatory response in mice liver caused by CCL_4. TP is an important test item for clinical liver biochemical indexes. TP maintains the normal colloid osmotic pressure and PH of blood, and it has the functions of transporting various metabolites, regulating the physiological effects of transported substances, relieving toxicity, immunity and nutrition. TP is usually used to detect the nutritional status of the body, and can also be used to identify and diagnose liver diseases(30). We found that GgCE treatment could significantly increase TP levels in mice liver and improve liver dysfunction caused by CCL_4.

In this study, we found that CCL_4 could induce the increase of serum ROS in mice. It is well known that excessive ROS accumulation can lead to oxidative stress and lipid peroxidation, which can aggravate the liver damage process(31). MDA is a metabolite of lipid peroxidation that accumulates in the body after liver injury. MDA can effectively indicate the degree of liver oxidative damage(32). Similarly, we found that CCL_4 can induce MDA production and aggravate liver oxidative damage. It was found that GSH is an important superoxide anion scavenger that scavenges ROS and protect the liver from oxidative stress, and that loss and depletion of GSH can lead to the accumulation of ROS(33). Unfortunately, we found that CCL_4 inhibited the expression of GSH. The body can protect itself from oxidative damage through enzyme-induced and non-enzyme-induced antioxidant pathways(34). The regulation of GSH-Px and SOD is the main mechanism of enzymatic antioxidant activity *in vivo*. SOD can catalyze the degradation of superoxide free radicals and remove free radicals. GSH-Px catalyzes H_2O_2 reduction to prevent cell damage. However, CCL_4 can reduce the activities of GSH-Px and SOD, and hinder the body's antioxidant reaction(35). The results showed that GgCE can reduce ROS and MDA to normal levels, and increase hepatic GSH, GSH-Px and serum SOD activities, suggesting that GgCE can alleviate CCL_4-induced liver oxidative damage by enhancing the body's ability to resist oxidative stress.

When liver injury occurs, liver tissue is disorganized, the cells are swollen and degenerated, and central veins show significant hemorrhage and inflammatory cell infiltration. Similarly, we found that CCL_4-induced liver injury also had the above-mentioned pathological changes. Interestingly, the overall state of the mice liver was near to normal after the treatment of CCL_4-induced liver injury with GgCE, indicating that GgCE can effectively improve chronic liver injury in mice induced by CCL_4.

We found that CCL_4 not only promoted the increase of ROS, but also inhibited the expression of Nrf2 and its downstream proteins. It was found that Nrf2-ARE signaling pathway is a key pathway of cells to resist oxidative stress(36). Moreover, activating Nrf2 can induce the expression of downstream genes such as *GCLC*, *GCLM*, *HO-1* and *NQO1* to resist oxidative stress injury caused by various stimuli(37, 38). Chun's study found that berberine could alleviate CCL_4-induced liver injury in rats by regulating Nrf2-ARE signaling pathway(39), which is similar to our findings that GgCE could counteract CCL_4-induced oxidative stress injury by activating Nrf2 and other antioxidant proteins.

It is well known that liver injury can inhibit autophagy. In autophagy, mTOR, a protein kinase that regulates cell growth and metabolism, is the upstream negative regulator of autophagy and the best characterized regulator of autophagy(40). Activation of PI3K can increase mTOR activity and lead to down-regulation of autophagy(41). LC3 Ⅱ and P62 are markers of detecting autophagy. LC3 Ⅱ exists only in mature autophagosomes, whereas substrate protein P62 recognizes ubiquitinated protein aggregates and directly bind to LC3 II specific motifs(42). Several studies have shown that in the treatment of CCL_4-induced liver injury by promoting autophagy, LC3 Ⅱ protein content will decrease and P62 protein content will increase(43). We also found that CCL_4 has an inhibitory effect on autophagy. However, these effects were reversed by GgCE. This suggests that GgCE may alleviate CCL_4-induced liver injury by promoting autophagy.

In conclusion, our study suggests that GgCE alleviated CCL_4-induced chronic liver injury in mice, possibly by activating antioxidant signaling pathways and promoting autophagy (Fig. 6.). Currently, the therapeutic effect of GgCE on CCL_4-induced chronic liver injury has not been reported, and our study provides a theoretical basis for the use of GgCE as a hepatoprotective agent.

Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
CCL_4	carbon tetrachloride
GgCE	GgL crude extract
GgL	*Gomphrena globosa* L.
GSH	glutathione
GSH-Px	glutathione peroxidase
MDA	malondialdehyde
MPO	myeloperoxidase
ROS	reactive oxygen species
SOD	superoxide dismutase
TP	total protein

Declarations

Author contributions

Yunfei Wei: Writing- Original draft preparation, Preliminary revision. Wenxi Tan, Haihai Feng and Meiyu Jin: Conceptualization, Supervision. Haiyan Qin: Methodology. Hao Yu and Jiaqi Cheng: Suggestions.

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Conflict of 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.

Ethical approval

All mice studies conformed to the U.S. National Institutes of Health (NIH), the Guide for the Care and Use of Laboratory Animals, and were authorized by the related ethnical regulations of Jilin University (Number of permit: SY202110003).

Informed consent

Not applicable as human participant is not involved in this study

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Figures

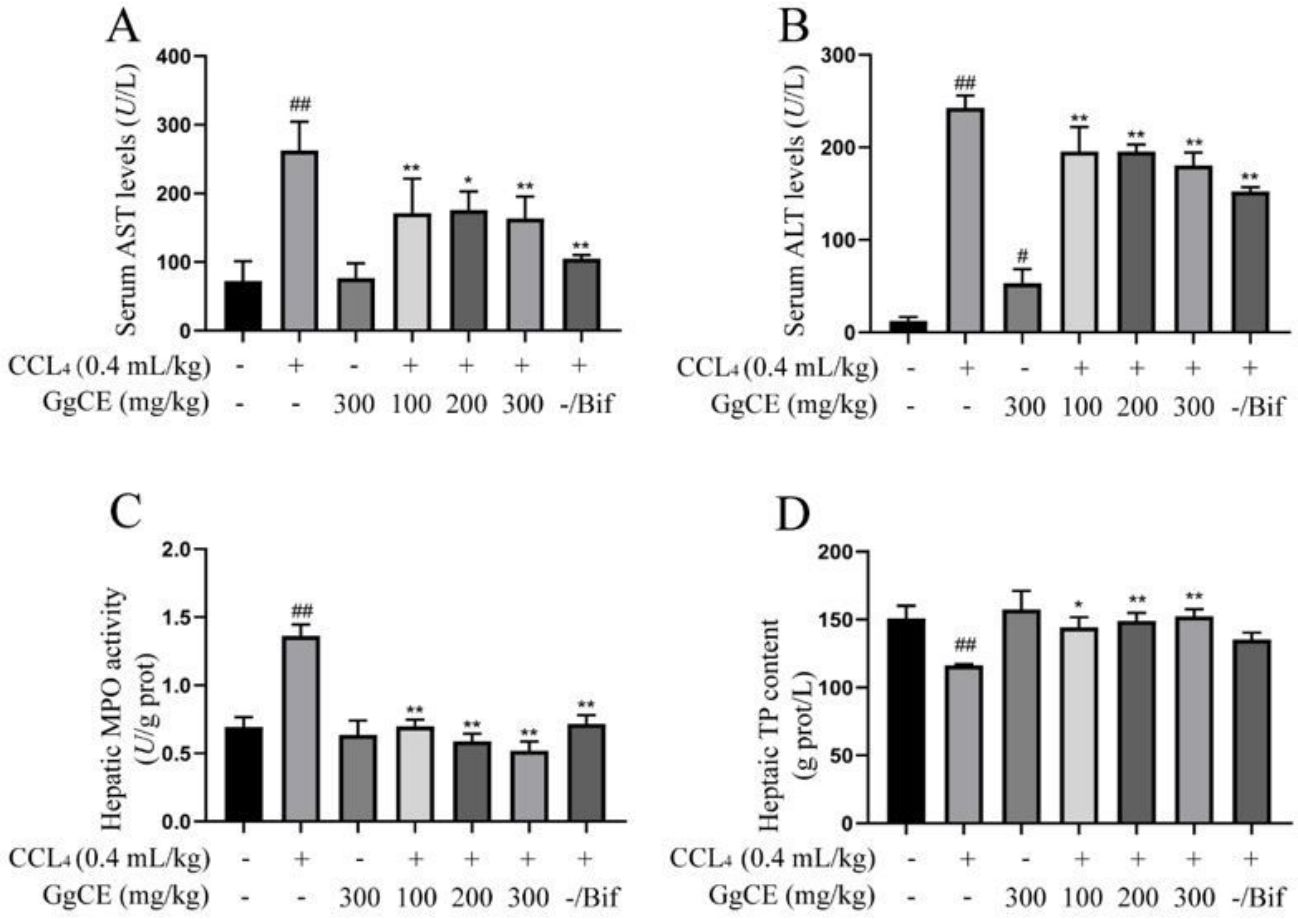

Figure 1

Gomphrena globosa L. crude extract (GgCE) alleviated CCL_4-induced liver injury in mice liver. Chronic liver injury model of mice was established by intraperitoneal injection of CCl_4 (0.4 mL/kg) for 3 weeks, and intragastric treatment of mice with different concentrations of GgCE (100, 200, 300 mg/kg) or Bifendatatum (Bif; 20 mg/kg) for the last 2 weeks. (A) Serum AST levels. (B) Serum ALT levels. (C) Hepatic MPO activity. (D) Hepatic TP content. All data are presented as mean ± SD (three independent experiments). $^{\#\#}P<0.01$, $^{\#}P<0.05$ versus control group; $^{**}P<0.01$, $^{*}P<0.05$ versus CCL_4 group.

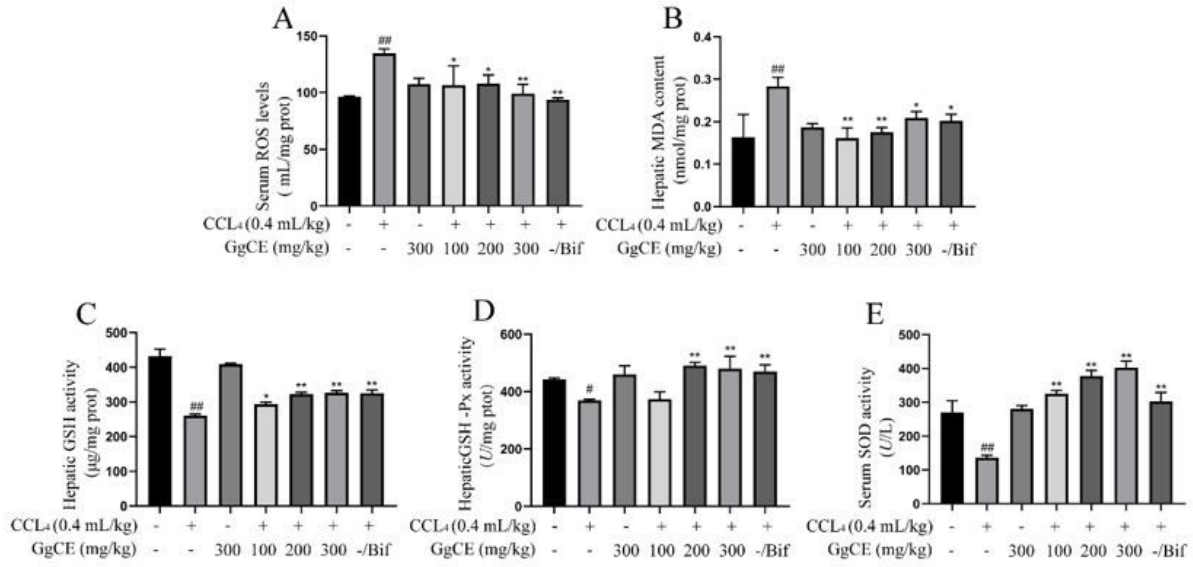

Figure 2

GgCE improved CCL_4-induced oxidative damage in mice liver. Chronic liver injury model of mice was established by intraperitoneal injection of CCl_4 (0.4 mL/kg) for 3 weeks, and intragastric treatment of mice with different concentrations of GgCE (100, 200, 300 mg/kg) or Bif (20 mg/kg) for the last 2 weeks. (A) Serum ROS levels. (B) Hepatic MDA content. (C) Hepatic GSH activity. (D) Hepatic GSH-Px activity. (E) Serum SOD activity. All data are presented as mean ± SD (three independent experiments). $^{\#\#}P<0.01$, $^{\#}P<0.05$ versus control group; $^{**}P<0.01$, $^{*}P<0.05$ versus CCL_4 group.

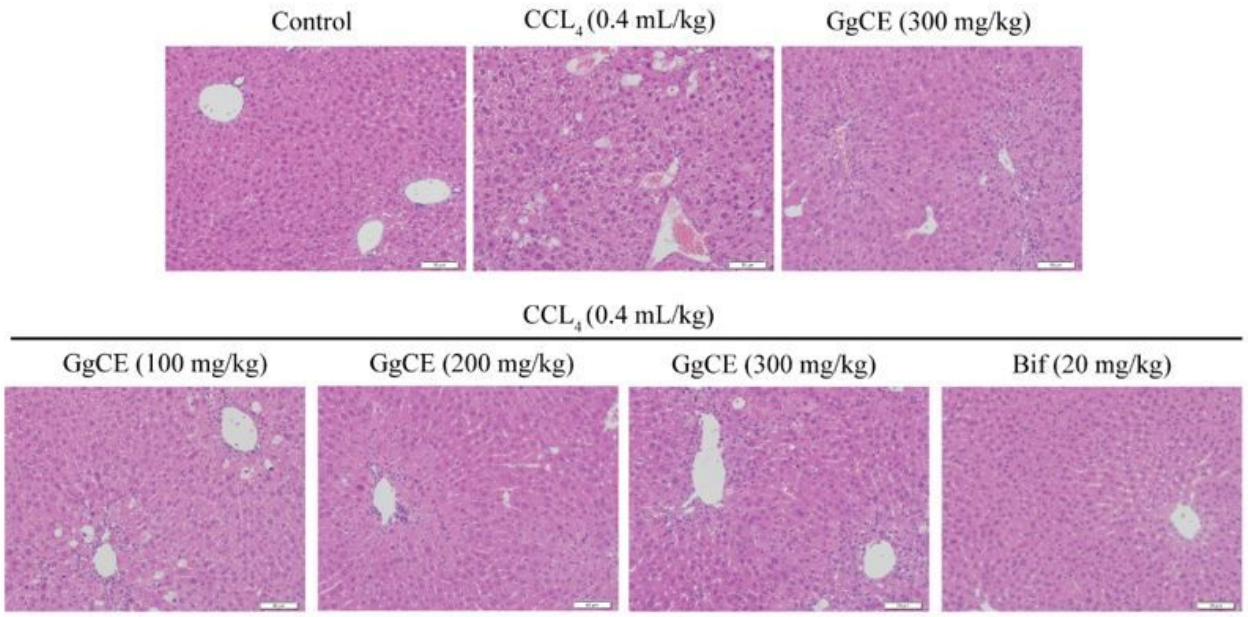

Figure 3

GgCE improved CCL_4-induced pathological changes in mice liver. Chronic liver injury model of mice was established by intraperitoneal injection of CCl_4 (0.4 mL/kg) for 3 weeks, and intragastric treatment of mice with different concentrations of GgCE (100, 200, 300 mg/kg) or Bif (20 mg/kg) for the last 2 weeks. Hematoxylin and eosin (H&E) staining section of mice liver (magnification 200×, scale bar = 50 μm).

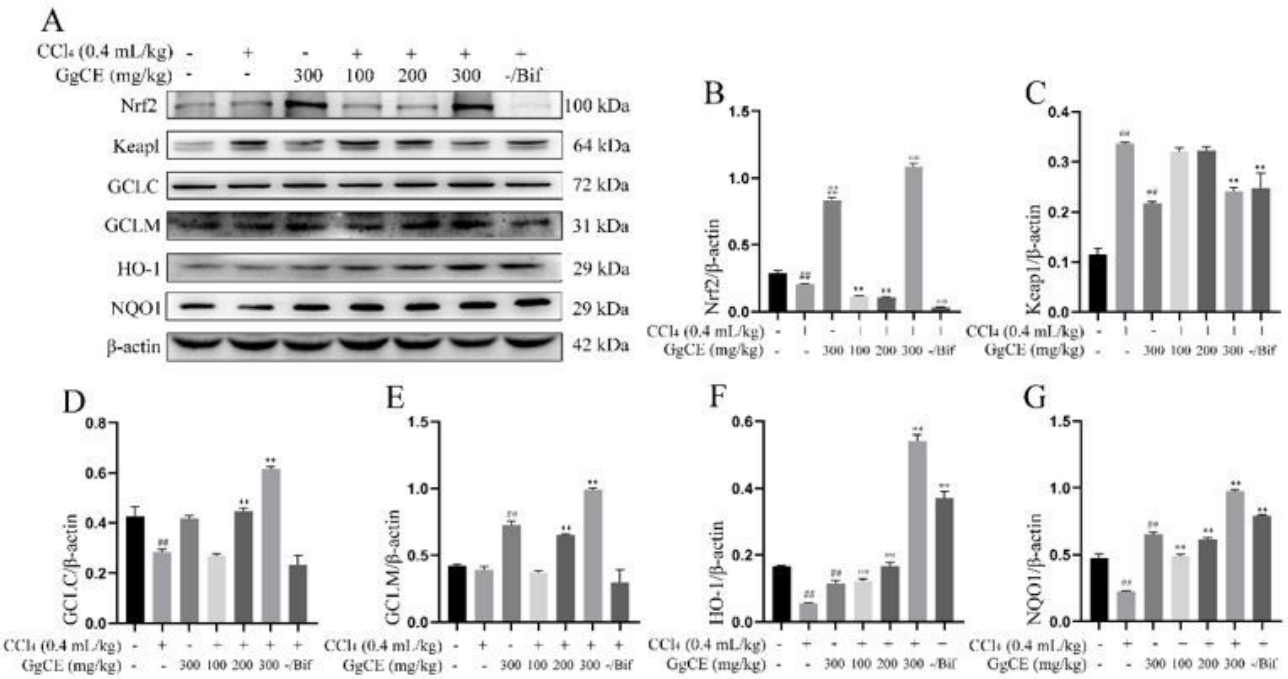

Figure 4

GgCE activated antioxidant signaling pathways in CCL_4-induced chronic liver injury. Chronic liver injury model of mice was established by intraperitoneal injection of CCl_4 (0.4 mL/kg) for 3 weeks, and intragastric treatment of mice with different concentrations of GgCE (100, 200, 300 mg/kg) or Bif (20 mg/kg) for the last 2 weeks. (A) Western blot analysis of Nrf2, Keap1, GCLC, GCLM, HO-1 and NQO1. Expression analysis of (B) Nrf2/β-actin. (C) Keap1/β-actin. (D) GCLC/β-actin. (E) GCLM/β-actin. (F) HO-1/β-actin. (G) NQO1/β-actin. All data are presented as mean ± SD (three independent experiments). $^{\#\#}P<0.01$ versus control group; $^{**}P<0.01$ versus CCL_4 group.

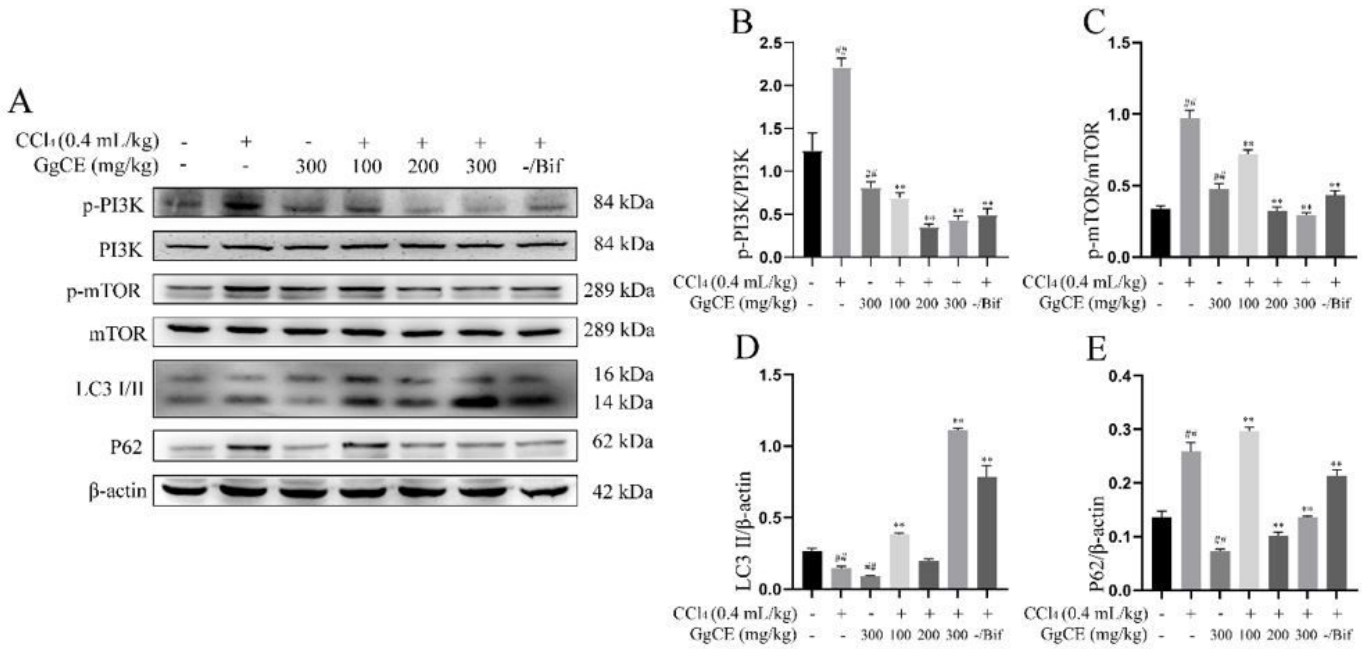

Figure 5

GgCE promoted autophagy in CCL_4-induced chronic liver injury. Chronic liver injury model of mice was established by intraperitoneal injection of CCl_4 (0.4 mL/kg) for 3 weeks, and intragastric treatment of mice with different concentrations of GgCE (100, 200, 300 mg/kg) or Bif (20 mg/kg) for the last 2 weeks. (A) Western blot analysis of p-PI3K, PI3K, p-mTOR, mTOR, LC3 Ⅱ and P62. Expression analysis of (B) p-PI3K/PI3K. (C) p-mTOR/mTOR. (D) LC3 Ⅱ/β-actin. (E) P62/β-actin. All data are presented as mean ±SD (three independent experiments). $^{\#\#}P$<0.01 versus control group; **P<0.01 versus CCL_4 group.

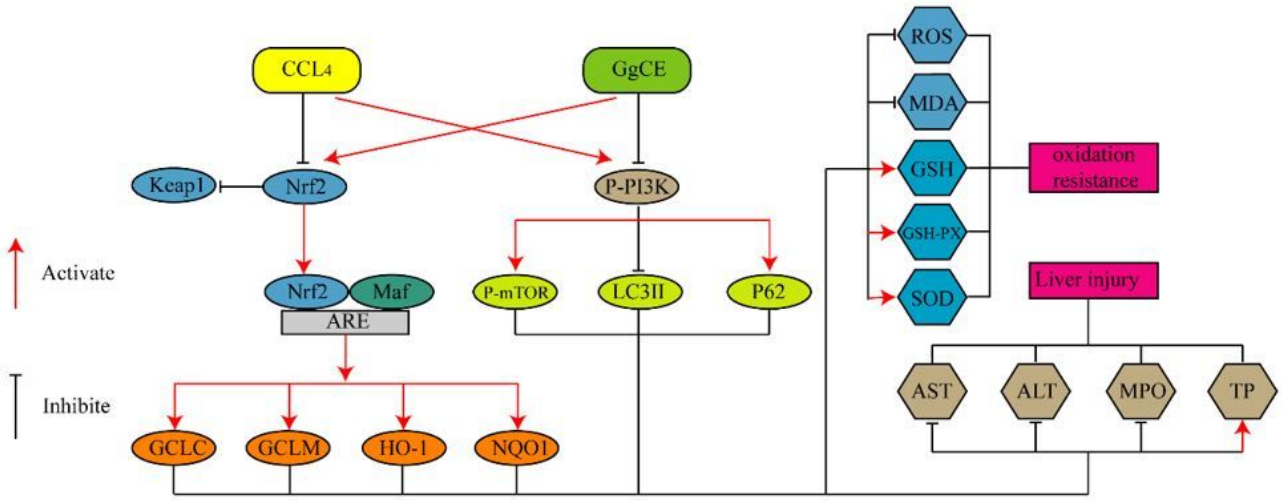

Figure 6

Potential mechanism of GgCE to alleviate CCL_4-induced liver injury. The mechanism of GgCE is opposite to that of CCL_4, GgGE alleviated CCL_4-induced chronic liver injury by activating antioxidant signaling pathways and promoting autophagy.