Original Article
https://doi.org/10.5115/acb.24.318
eISSN 2093-3673
Anatomy & Cell Biology acb

Protective effect of *Chlorella vulgaris* against experimental hepatic ischemia reperfusion injury downregulating oxidative stress, inflammation, and apoptosis

Amira E. Farage[1], Mona A. Abdel-Kareem[1,2], Medhat Taha[3,4], Sara Abubakr[4], Nora Elshehawy Helal[4], Mahmoud Hendawy[4,5], Hanan A. Elgendy[4,6], Ahmed Abdel-monem Elmetwally[7], Hala Mahfouz[8], Tourki A. S. Baokbah[9], Mohammed R. Rabei[10,11], Mohammad Akbar Hossain[12], Azza I. Helal[13], Mohie Mahmoud Ibrahim[4,14]

[1]Department of Anatomy and Embryology, Faculty of Medicine, Kafrelsheikh University, Kafr El Sheikh, [2]Department of Anatomy and Embryology, Faculty of Medicine, Delta University for Science and Technology, Gamasa, Egypt, [3]Department of Anatomy, Al-Qunfudah Medical College, Umm Al-Qura University, Al-Qunfudhah, Saudi Arabia, [4]Department of Human Anatomy and Embryology, Faculty of Medicine, Mansoura University, Mansoura, Egypt, [5]Department of Human Anatomy and Embryology, Faculty of Medicine, Al-Baha University, Al-Baha, [6]Department of Anatomy, Faculty of Medicine, Northern Border University, Arar, Saudi Arabia, [7]Department of Clinical Pharmacology, Faculty of Medicine, Mansoura University, Mansoura, [8]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Kafrelsheikh University, Kafr El Sheikh, Egypt, [9]Department of Medical Emergency Services, College of Health Sciences in Al-Qunfudah, Umm Al-Qura University, Al-Qunfudhah, Saudi Arabia, [10]Department of Medical Physiology, Faculty of Medicine, Mansoura University, Mansoura, Egypt, [11]Department of Basic Medical Sciences, Faculty of Medicine, Ibn Sina University for Medical Sciences, Amman, Jordan, [12]Department of Pharmacology & Toxicology, Faculty of Medicine in Al-Qunfudah, Umm Al-Qura University, Al-Qunfudhah, Saudi Arabia, [13]Department of Histology and Cell Biology, Faculty of Medicine, Kafrelsheikh University, Kafr El Sheikh, Egypt, [14]Department of Basic Medical and Dental Sciences, Faculty of Dentistry, Zarqa University, Zarqa, Jordan

Abstract: Hepatic ischemia/reperfusion (Hep I/R) is a great health burden during hepatic transplantation surgery. The present work aimed to examine the mitigative effect of *Chlorella vulgaris* against Hep I/R and its underlying protective mechanisms. The animals in the present research were classified into four equal experimental groups (n=6): the sham group, sham+*Chlorella vulgaris* group, Hep I/R group, and Hep I/R+*Chlorella vulgaris* group. Hepatic ischemia results in liver impairment, as evidenced by elevated liver enzyme levels and altered liver histology. It also reduced antioxidant enzyme levels and increased lipid peroxidation. Additionally, the Hep I/R group displayed significant suppression of the nuclear factor erythroid 2-related factor 2 (Nrf2)/haem oxygenase-1 pathway. There was a marked elevation in the expression of inflammatory markers, including nuclear factor kappa beta (NF-κB), tumor necrosis factor-alpha, interleukin-1 beta, interleukin-6, and myloperoxidase, and NOD-like receptor protein 3 (NLRP3) and caspase-1. Furthermore, the levels of apoptotic markers such as caspase-3 and Bax were greater than those in the sham groups. Pretreatment with *Chlorella vulgaris* significantly protected against Hep I/R by reversing these effects. Rats pretreated with *Chlorella vulgaris* exhibited a hepatoprotective effect against Hep I/R through its inhibition of the NF-κB and NLRP3 cascades and Nrf2 stimulation.

Key words: Reperfusion injury, *Chlorella vulgaris*, Nuclear factor kappa beta, Oxidative stress, NOD-like receptor protein 3 inflammasom

Received December 7, 2024; 1st Revised January 14, 2025; 2nd Revised January 27, 2025; Accepted January 27, 2025

Corresponding author:
Medhat Taha
Department of Anatomy, Al-Qunfudah Medical College, Umm Al-Qura University, Al-Qunfudhah 35516, Saudi Arabia
E-mail: medhattaha53@yahoo.com

Introduction

Liver ischemia occurs in many liver pathologies, such as liver resection and liver transplantation, leading to significant mortality and morbidity [1]. The initial stage of hepatic ischemia/reperfusion (Hep I/R) damage is characterized by oxidative stress induced by Kupffer cells, which causes hepatocellular damage and subsequently leads to extensive neutrophil infiltration [2].

The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is considered one of the key factors in protection against Hep I/R-induced oxidative stress injury and the inflammatory response and is crucial for regulating intracellular antioxidant processes [3]. Following I/R, Nrf2 translocates to the nucleus, where it activates various detoxifying genes, including haem oxygenase-1 (HO-1), which plays an important role in preventing oxidative injury [4, 5]. A significant pathway involved in inflammation and apoptosis includes the nuclear factor nuclear factor kappa beta (NF-κB) and NOD-like receptor protein 3 (NLRP3) [6]. NF-κB-mediated secretion of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) is activated [7, 8]. Additionally, stimulation of NF-κB is important for the upregulation of the NLRP3 inflammasome [9]. Inflammasomes, particularly NLRP3, play crucial roles in liver disease by inhibiting inflammation and minimizing neutrophil infiltration during hepatic I/R injury [10, 11].

Chlorella vulgaris is a green alga that is rich in lutein [12, 13]. Often considered a superfood, it contains approximately 18 amino acids, 60% protein, twenty vitamins, and minerals such as calcium, magnesium, potassium, iron and phosphorus [14]. Like *Chlorella*, microalgae also contain various antioxidants, including lutein, carotenoids, astaxanthin, chlorophyll, and phycobiliproteins [15, 16]. Supplementation with *Chlorella sp.* has shown numerous fruitful physiological effects, such as antioxidative [17], antitumor [18], hypocholesterolemic [19], antihypertensive [20], and hypoglycemic and hypolipidemic effects [21, 22]. *Chlorella* has demonstrated hepatoprotective potential against carbon tetrachloride-induced hepatic degeneration in animals [23, 24]. However, no additional studies have extensively examined the hepatoprotective effect of *Chlorella vulgaris* [25]. The aim of this work was to examine the hepatoprotective impact of *Chlorella vulgaris* against hepatitis I/R, explore its histopathological changes, and explore its biochemical and molecular mechanisms.

Materials and Methods

Rats and experimental plan

The study involved 24 adult male albino rats, each weighing nearly 165 grams. The animals were held in metal cages with easy access to water and food. The study was conducted in accordance with the Canadian Council on Animal Care Guidelines. The Ethical Committee of Kafrelsheikh University, Kafr El Sheikh, Egypt, approved the housing and management of the animals (code number: KFS-IACUC/234/2024). The rats were allocated into four groups, each containing six rats. Sham animals: rats underwent median laparotomy without performing liver I/R and were given normal saline. Sham+*Chlorella vulgaris* group: sham group rats treated with *Chlorella vulgaris* (500 mg/kg body weight daily) for 7 days [26]. In the hepatic I/R group, the animals underwent surgery and experienced hepatic ischemia for thirty minutes accompanied by 24 hours of reperfusion. I/R+*Chlorella vulgaris* group: rats were treated with the previous dose and duration before the I/R procedure, with the treatment continuing during the ischemia period at the same daily dose.

Preparation of Chlorella vulgaris methanolic extract

Following the procedure of Qasem et al. [27], the methanolic extract of *Chlorella vulgaris* was prepared as follows: the powder used in the preparation of *Chlorella vulgaris* was purchased from the National Research Center, Cairo, Egypt. The mixture was soaked in 99.5% ethanol at a 1:5 ratio (w/v) in a dark room with frequent shaking at room temperature. The mixture was filtered through Whatman filter paper number one and then concentrated via a rotary evaporator. Finally, the extract was kept at 5°C in a refrigerator until use.

Liver ischemia/reperfusion

I/R was performed as previously described [28]. The rats were anesthetized with thiopental (0.05 g/kg, intraperitoneally) and placed in the supine position. A midline anterior abdominal wall incision was made via a microvascular clamp, and seventy percent hepatic ischemia was induced for half an hour, as confirmed by the pale appearance of the clamped hepatic lobes. After that, the clamp was carefully removed to allow 24 hours of reperfusion.

Sample collections

Following hepatic reperfusion, samples of blood were collected from the abdominal aorta. The blood was centrifuged

https://doi.org/10.5115/acb.24.318

for separation of the serum, which was subsequently used for liver function assays. Following blood collection, the animals were harvested, and their livers were extracted and divided into three parts. First, the samples were homogenized, and the supernatants were stored at –80°C for use in biochemical assays. Second fraction: immersed in ten percent formalin for histological examination. The third fraction was stored at –80°C for molecular measurements.

Assessment of liver function indicators

The serum levels of alanine aminotransferase (ALT) (Cat# EEA001), aspartate aminotransferase (AST) (Cat# EEA003), and alkaline phosphatase (ALP) (Cat# ab83369) were measured via diagnostic kits following the manufacturer's instructions.

Biochemical and ELISAs

The hepatic levels of malonaldehyde (MDA), GPx, superoxide dismutase (SOD), and catalase (CAT) were measured via ELISA kits from Biodiagnostic, following the manufacturer's instructions. Hepatic supernatant levels of HO-1, TNF-α, IL-6, IL-1β, Bax, and caspase-3 were measured via commercial kits (Cat# SL0341Ra, RK00029, SL0411Ra, SL0402Ra, MBS2512405, NBP2-75024, respectively). All procedures were conducted according to the manufacturers' guidelines.

Histopathological and immunohistochemistry investigations

Hepatic samples were fixed in 10% formalin for forty-eight hours. The samples were then washed under running tap water overnight, dehydrated in increasing alcohol concentrations (70%–100%), cleaned with xylene, and embedded in paraffin. The blocks were sliced at a thickness of five μm, stained with hematoxylin and eosin (H&E), and examined via an Olympus light microscope (Olympus) [29]. The Suzuki et al.'s [30] score is a widely used grading system to assess liver damage, particularly in the context of Hep I/R injury. It ranges from 0 to 4, with higher scores indicating more severe damage [30]. We assessed our H&E-stained sections according to this score to evaluate hepatic damage.

For the immunohistochemical procedure, the expression of myloperoxidase (MPO), NF-κB, caspase-1, and Nrf2 was examined as previously described [31]. The following antibodies were used: Cat#. Immunoexpression quantitative analysis was performed via NIS-Elements software (Nikon Instruments Inc.). The optical density in the immunohistochemical areas of each image was calculated by counting positive cells at 400× magnification. A blinded expert observed ten consecutive, nonoverlapping sections per image.

Quantitative real-time PCR

Total hepatic tissue RNA was extracted with TRIzol reagent (Thermo Fisher Scientific). Then, the products were reverse transcribed into cDNA via a reverse-sense first-strand cDNA synthesis kit. quantitative real-time polymerase chain

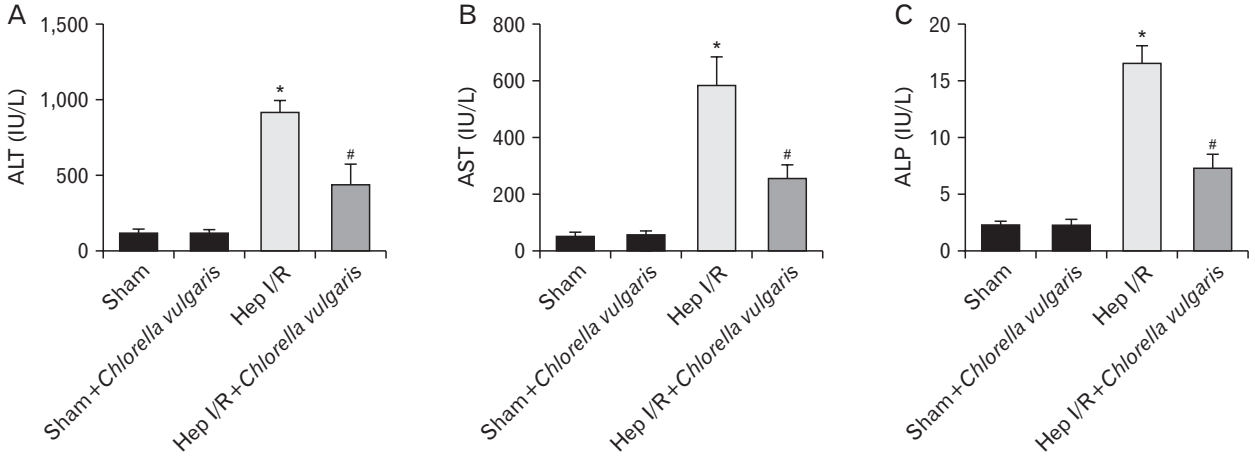

Fig. 1. Effect of *Chlorella vulgaris* intake for 7 days prior to hepatic ischemia/reperfusion (Hep I/R) in liver enzymes, (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), and (C) alkaline phosphatase (ALP). All our results presented mean±SD. **P* <0.05 significant to sham groups, #*P*<0.05 significant to Hep I/R group.

reaction (qRT-PCR) was performed with maximum SYBR green/rox qPCR kits. The specific primers used were as follows: NF-κB, forward 5'TGGGACGACACCTCTACACA 3 and reverse 3'GGAGCTCATCTCATAGTTGTCC 5'; NLRP3, forward 5'CAGACCTCCAAGACCACGACTG 3' and reverse 3'CATCCGCAGCCAATGAACAGAG 5'; and the housekeeping gene β-actin, forward 5'TGACAGGATGCAGAAGGAGA 3' and reverse 3'TAGAGCCACCAATCCACACA 5'. Analysis

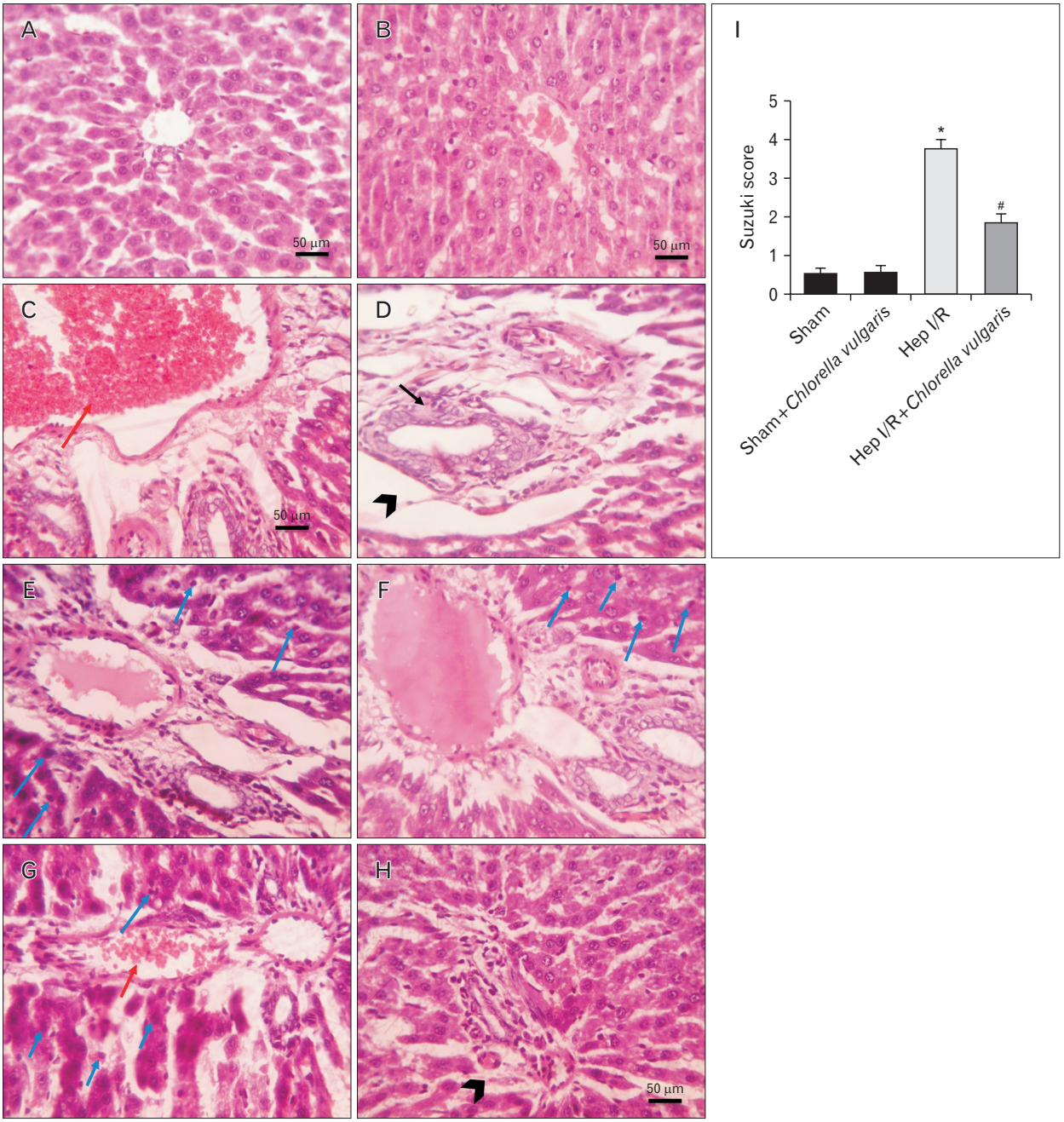

Fig. 2. Histological examination of hepatic tissues revealed. (A, B) H&E-stained hepatic slices show well-organized hepatic parenchyma with normal hepatocytes, sinusoids, central veins, and portal areas in the sham and sham+*Chlorella vulgaris* groups. (C–G) Hepatic sections from the hepatic ischemia/reperfusion (Hep I/R) group exhibit marked extension of portal areas due to vascular congestion (red arrows), edema (arrowhead), and dilated bile ductules (black arrow), pyknotic hepatocytes (blue arrows) along with dilated sinusoids and atrophied hepatic cords. (H) Hepatic sections from the Hep I/R+*Chlorella vulgaris* group show impaired hepatic parenchyma with decreased portal edema (arrowhead). (I) Suzuki score. Magnifications: ×400 (scale bars 50 μm). **P*<0.05 significant to sham groups, #*P*<0.05 significant to Hep I/R group.

https://doi.org/10.5115/acb.24.318

of the melting curve was used for validation of the PCR amplification results. The mRNA expression level was determined through the cycle threshold formula $2^{-\Delta\Delta CT}$, with β-actin used as the reference gene for normalization.

Statistical analysis

Statistical analysis for all the study variables was performed via GraphPad Prism software version 8.0.2 (GraphPad Software), and ANOVA was used to clarify the differences between the experimental groups, followed by Tukey's test. $P<0.05$ was considered significant.

Results

Hepatoprotective effect of Chlorella vulgaris pretreatment on Hep I/R

Compared with the sham conditions, Hep I/R induced liver impairment, as indicated by significant ($P<0.05$) elevations in the levels of the liver enzymes ALT, AST, and ALP (Fig. 1). However, pretreatment with *Chlorella vulgaris* significantly ($P<0.05$) decreased these liver function test enzyme markers compared with those in the Hep I/R group.

Impact of Chlorella vulgaris on hepatic histopathology

H&E examination of the sham groups revealed a classic picture of the hepatic parenchyma with healthy hepatocytes (Fig. 2A, B). In contrast, the Hep I/R group revealed significant hepatic histological deterioration, including atrophied hepatocytes, vascular congestion, marked dilatation in bile ductules and sinusoids, and hepatic pyknosis (Fig. 2C–G). Fortunately, pretreatment with *Chlorella vulgaris* clearly improved the hepatic histological appearance, with minimal distortion in the hepatic parenchyma and a decrease in edema in the portal region (Fig. 2H). Histological damage to hepatic tissues in the Hep I/R group was confirmed by the significant increase in the Suzuki score, which markedly decreased with *Chlorella vulgaris* pretreatment (Fig. 2I).

Chlorella vulgaris pretreatment halted Hep I/R-induced oxidative insult and upregulated the Nrf2/HO-1 pathway

Compared with that in the sham group, Hep I/R significantly ($P<0.05$) increased the level of MDA while simultaneously decreasing the activity of the antioxidant enzymes SOD and CAT in hepatic tissue homogenates (Fig. 3). These effects were reversed by pretreatment with *Chlorella vulgaris*.

Pretreatment with *Chlorella vulgaris* significantly ($P<0.05$) upregulated Nrf2 and HO-1, as evidenced by an increase in the expression of Nrf2 (Fig. 4G–I) and the protein level of HO-1, as measured by ELISA (Fig. 4J), compared with those in the Hep I/R group (Fig. 4E, F).

Chlorella vulgaris pretreatment prevents Hep I/R-induced inflammation and neutrophil infiltration

As shown in Fig. 5, the Hep I/R group presented a noticeable increase in the nuclear immunoexpression and mRNA levels of the inflammatory transcription factor NF-κB, along with elevated ELISA levels of the inflammatory mediators TNF-α, IL-1β, and IL-6. Additionally, as shown in Fig. 6,

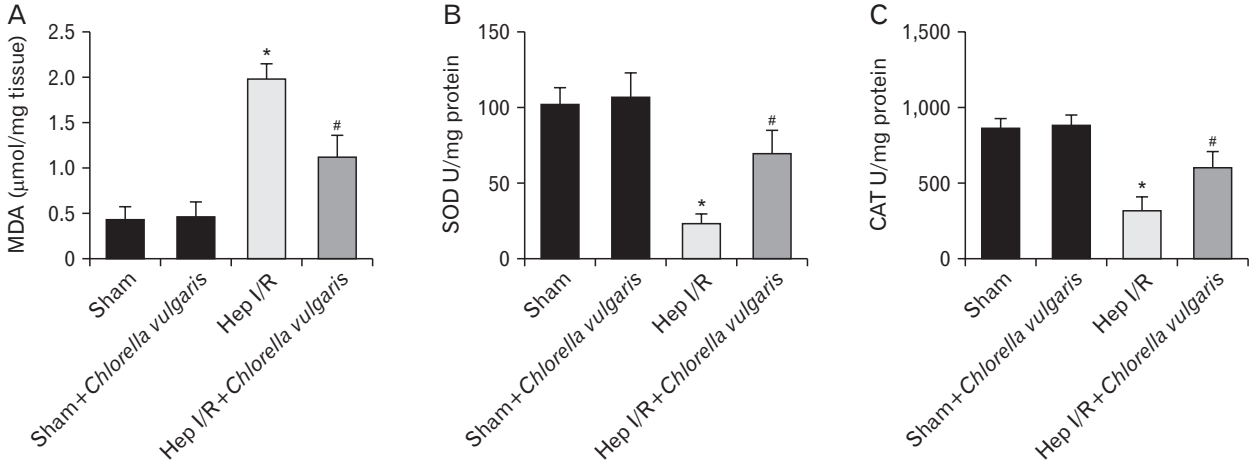

Fig. 3. Impact of *Chlorella vulgaris* pretreatment on (A) malonaldehyde (MDA), (B) superoxide dismutase (SOD), and (C) catalase (CAT). All measurements expressed as a mean±SD. *$P<0.05$ vs. to sham groups, #$P<0.05$ vs. hepatic ischemia/reperfusion (Hep I/R) group.

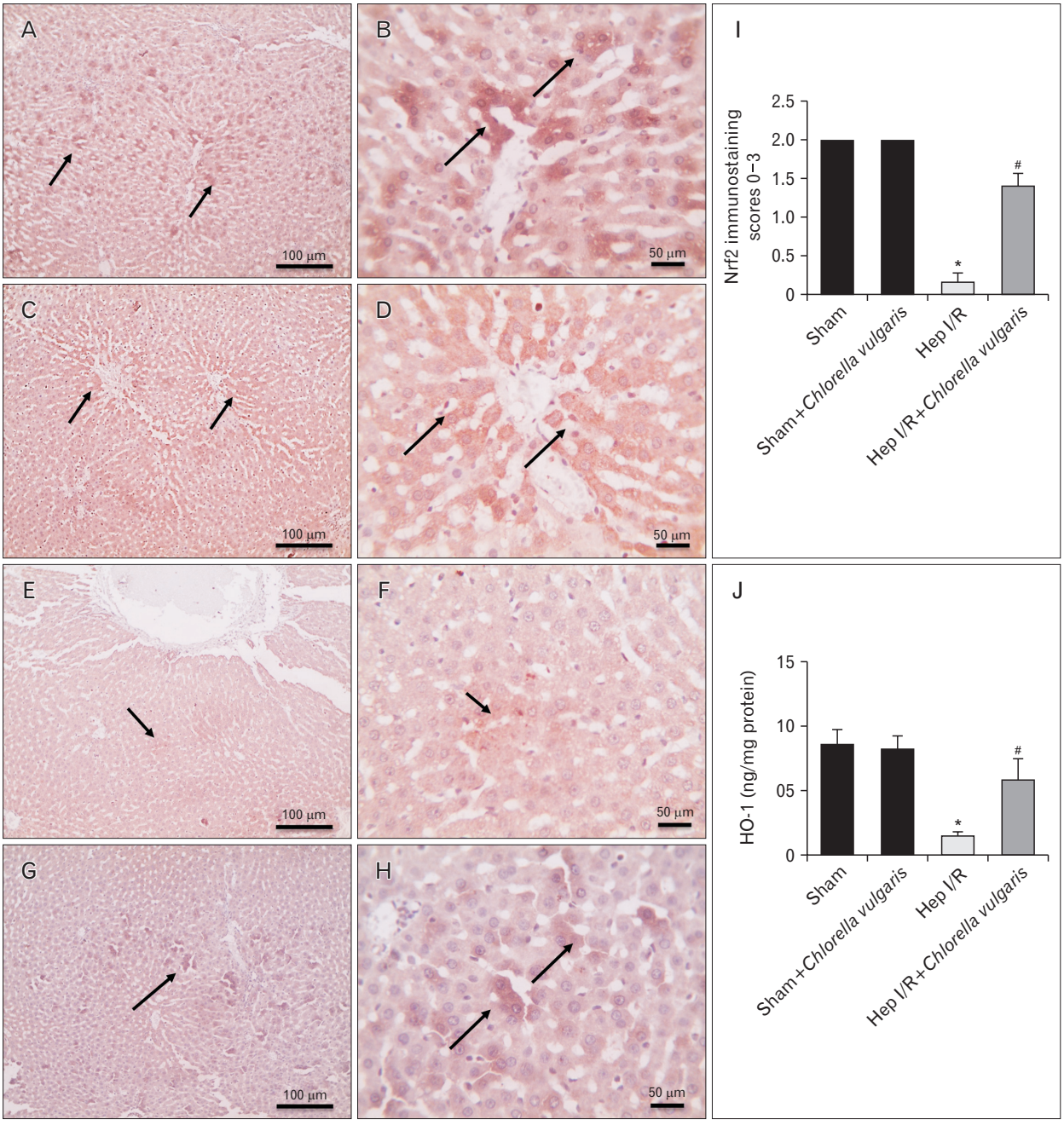

Fig. 4. Nuclear factor erythroid 2-related factor 2 (Nrf2) immunostained hepatic sections depicted marked positive expression in many hepatocytes (black arrows) in the sham and sham+*Chlorella vulgaris* groups (A–D). Liver slices from the hepatic ischemia/reperfusion (Hep I/R) group show mild positive expression in a few hepatocytes (black arrows) (E, F). Hepatic slices from the Hep I/R+*Chlorella vulgaris* group show increased positive expression in hepatocytes (black arrows) (G, H). Magnifications: (A, C, E, G) ×100 (scale bars 100 μm) and (B, D, F, H) ×400 (scale bars 50 μm). (I) Histogram of Nrf2 immunostaining. (J) ELISA level of haem oxygenase-1 (HO-1) in hepatic supernatant. All results are shown as mean±SD. **P*<0.05 vs. sham groups, #*P*<0.05 vs. Hep I/R group.

compared with the sham group, the Hep I/R group presented a marked increase in the immunoexpression of MPO, an indicator of neutrophil infiltration. However, in the Hep I/R+*Chlorella vulgaris* group, there was a significant decrease in the gene and immunoexpression of NF-κB, as well as inflammatory cytokines, and a marked decrease in neutrophil infiltration. Overall, *Chlorella vulgaris* has a strong anti-inflammatory effect on Hep I/R-induced hepatic inflammation.

https://doi.org/10.5115/acb.24.318

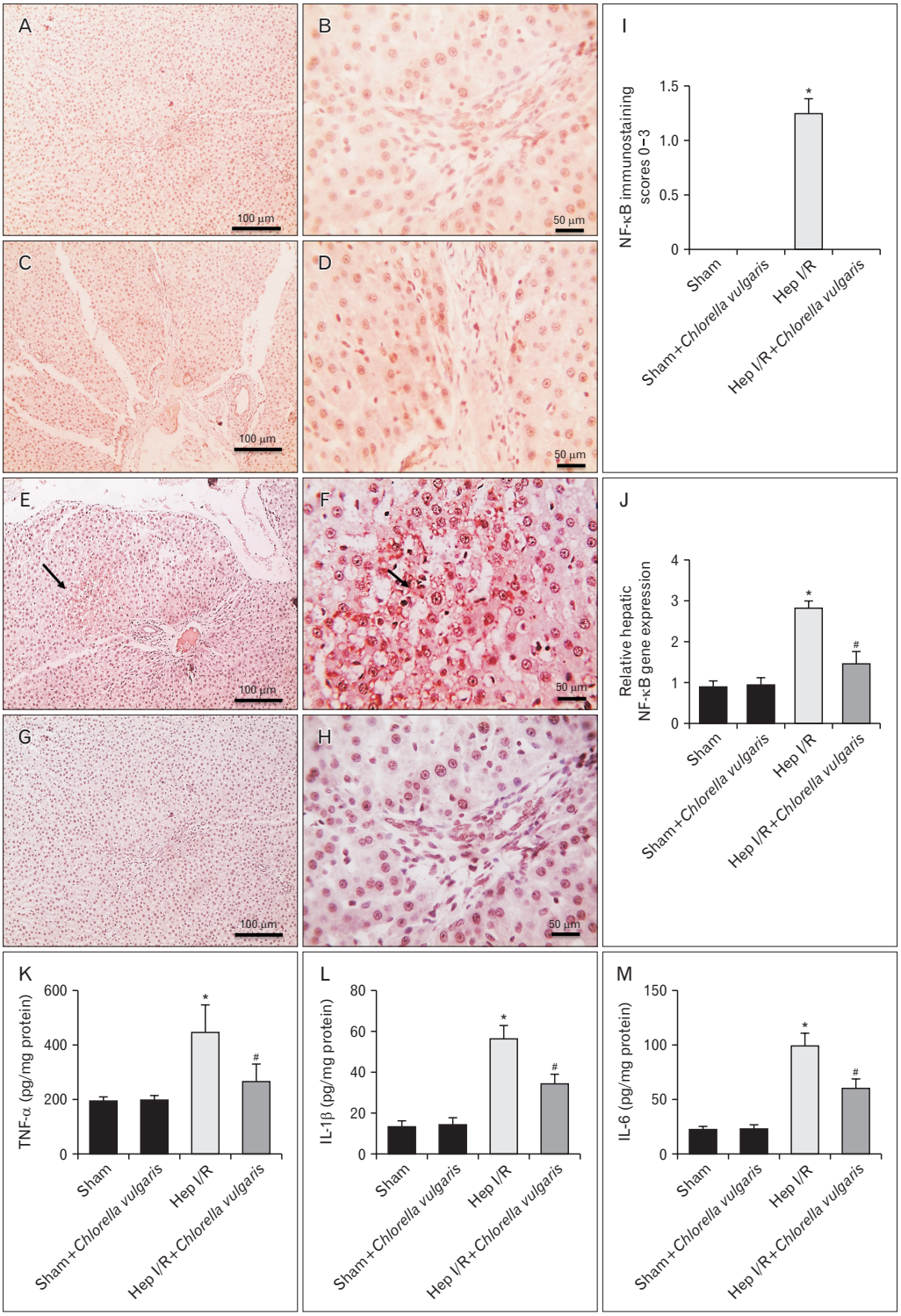

Fig. 5. Immunostained hepatic sections for nuclear factor kappa beta (NF-κB) demonstrated negative expression in hepatocytes in both the sham group and the sham+*Chlorella vulgaris* group (A–D). Hepatic slices from the hepatic ischemia/reperfusion (Hep I/R) group display focal positive expression in some hepatocytes (indicated by black arrows) (E, F). Hep I/R+*Chlorella vulgaris* group liver sections revealed a negative expression in hepatocytes (G, H). Magnifications are (A, C, E, G) ×100 (scale bars 100 μm) and (B, D, F, H) ×400 (scale bars 50 μm). (I) Histogram of NF-κB immunolabelling scores. (J) Gene expression of NF-κB. ELISA levels of (K) tumor necrosis factor-alpha (TNF-α), (L) interleukin-1 beta (IL-1β), and (M) interleukin-6 (IL-6). All values are presented as mean±SD. **P*<0.05 indicates significance to sham groups, #*P*<0.05 indicates significance to the Hep I/R group.

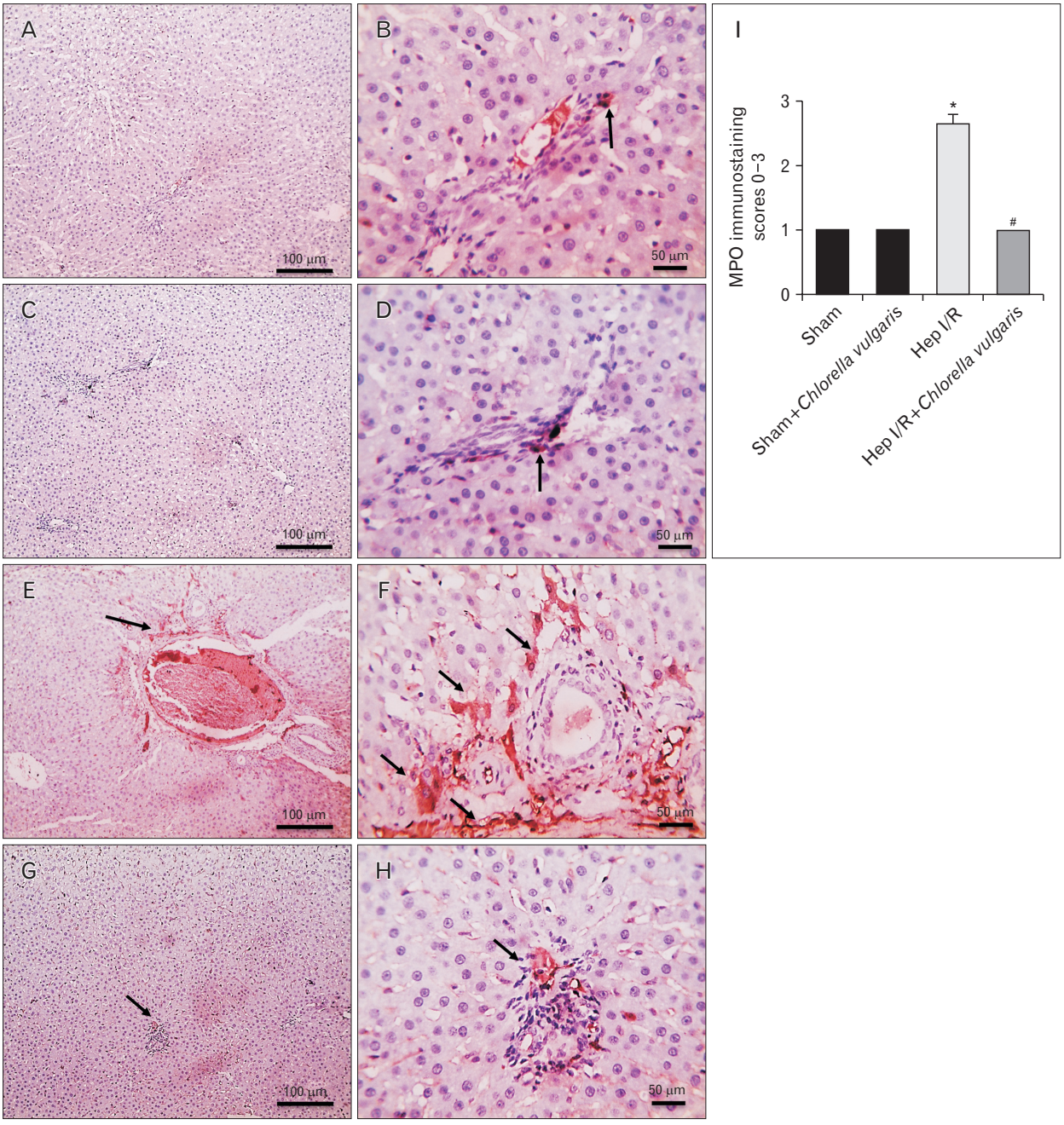

Fig. 6. Microscopic images of myloperoxidase (MPO) immunostain showed a few positively stained MPO neutrophils in the portal areas of both the sham group and the sham+*Chlorella vulgaris* group (A–D). Hepatic sections from the hepatic ischemia/reperfusion (Hep I/R) group display many positively stained neutrophils and periportal hepatocytes (indicated by black arrows) (E, F). Hepatic sections from the Hep I/R+*Chlorella vulgaris* group show few positively stained neutrophils and periportal hepatocytes (black arrows) (G, H). Magnifications are (A, C, E, G) ×100 (scale bars 100 μm) and (B, D, F, H) ×400 (scale bars 50 μm). (I) Histogram of MPO immunostaining labeling. All study results are presented as mean±SD. **P*<0.05 vs. sham rats, #*P*<0.05 vs. Hep I/R group.

Downregulatory effect of Chlorella vulgaris on Hep I/R-induced pyroptosis

As shown in Fig. 7, compared with the sham group, the Hep I/R group presented a noticeable increase in the relative fold change in the expression of NLRP3, as well as the expression of caspase-1, a pyroptotic marker. However, prior treatment with *Chlorella vulgaris* significantly downregulated both NLRP3 and caspase-1 gene expression and im-

https://doi.org/10.5115/acb.24.318

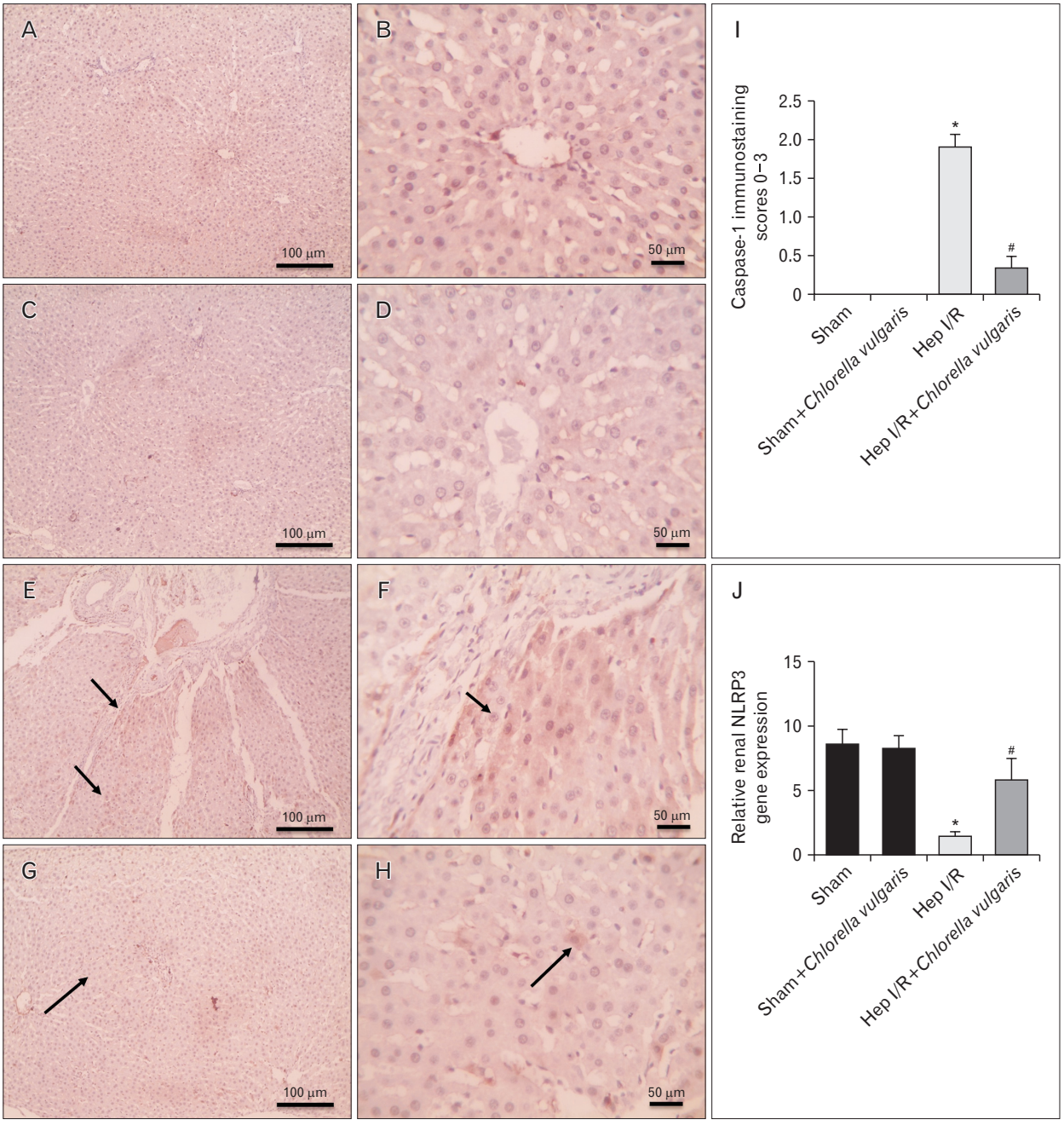

Fig. 7. Caspase-1 immunostained hepatic slides showed a negative expression in hepatocytes in both the sham group and the sham+*Chlorella vulgaris* group (A–D). Hepatic sections from the hepatic ischemia/reperfusion (Hep I/R) group exhibited a marked positive expression in many hepatocytes (indicated by black arrows) (E, F). Hepatic sections from the Hep I/R+*Chlorella vulgaris* group show decreased positive expression in very few hepatocytes (black arrows) (G, H). Magnifications are (A, C, E, G) ×100 (scale bars 100 μm) and (B, D, F, H) ×400 (scale bars 50 μm). (I) Histogram of caspase-1 immunostaining scores. (J) mRNA levels of NOD-like receptor protein 3 (NLRP3) in hepatic tissues. All variables are expressed as mean±SD. **P*<0.05 vs. sham groups, #*P*<0.05 vs. Hep I/R rats.

munostaining. Hence, *Chlorella vulgaris* pretreatment clearly mitigated Hep I/R-induced hepatocyte pyroptosis, a form of inflammatory cell death.

Anti-apoptotic effect of Chlorella vulgaris on Hep I/R-induced hepatic apoptosis

Compared with sham surgery, Hep I/R resulted in a significant ($P<0.05$) increase in the protein levels of the apoptot-

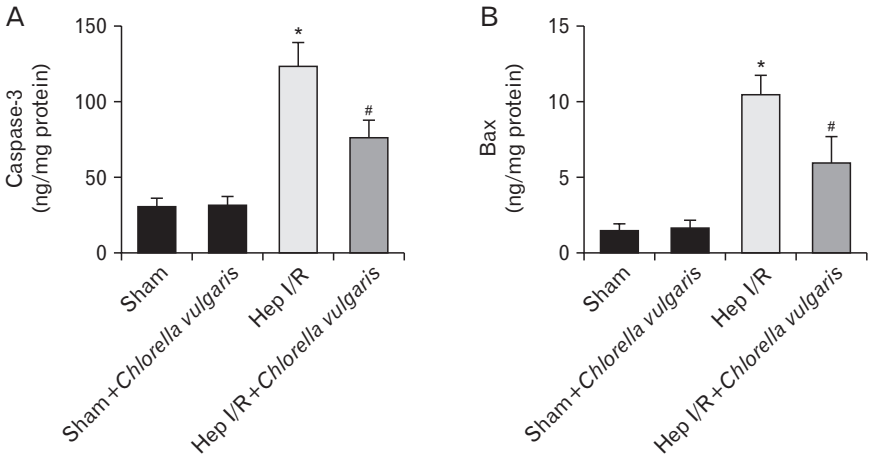

Fig. 8. ELISA level of (A) hepatic caspase-3 and (B) Bax in different group. All our values presented as mean±SD. **P*<0.05 means a significancy to sham groups, #*P*<0.05 means a significancy to hepatic ischemia/reperfusion (Hep I/R) group.

ic markers caspase-3 and Bax (Fig. 8). However, pretreatment with *Chlorella vulgaris* significantly ($P<0.05$) reduced these protein levels, thereby combating hepatic apoptosis induced by ischemia/reperfusion.

Discussion

Hep I/R is considered one of the main causes of mortality after liver surgery and transplantation. Reducing neutrophil infiltration minimizes the inflammatory process, which helps reduce Hep I/R-induced hepatic degeneration [32]. *Chlorella vulgaris*, characterized by its high content of chlorophyll pigments, minerals, and vitamin complexes [33, 34], is rich in antioxidants that can protect cells from the undesirable effects of reactive oxygen species (ROS) accumulation [34]. In this study, the hepatoprotective effect of *Chlorella vulgaris* against a Hep I/R experimental model was investigated. These results suggest several protective mechanisms, including a reduction in liver enzymes and hepatic pathology, mitigation of hepatic oxidative stress through Nrf2/HO-1 upregulation, and a reduction in hepatic inflammation through NF-κB/NLRP3/IL-1β inhibition and neutrophil infiltration. Additionally, there was a reduction in hepatocyte apoptosis.

Hep I/R resulted in a marked elevation in the levels of hepatic enzymes, indicating liver injury, which is consistent with previous findings [35, 36]. These changes were confirmed histopathologically by the shrunken appearance of the hepatic cords and portal edema. Pretreatment with *Chlorella vulgaris* has been shown to have hepatoprotective effects on I/R by reducing liver enzyme levels and improving the appearance of hepatic cords, as supported by previous studies [23, 37, 38].

Oxidative stress plays a pivotal role in I/R pathogenesis via the accumulation of ROS, which deplete defensive oxidative enzymes [39, 40]. The results of this study revealed that hepatic I/R markedly elevated MDA, with decreases in SOD and CAT, which is consistent with previous findings [41, 42]. Moreover, pretreatment with *Chlorella vulgaris* mitigated induced oxidative stress by inhibiting MDA and increasing hepatic SOD and CAT activities, which is in line with the findings of Eissa et al. [43], who reported the hepatoprotective effect of *Chlorella vulgaris* against cadmium-induced oxidative insult through its reduction in MDA, nitric oxide, and increase in the levels of SOD, GPX, and CAT.

Nrf2 is a well-known intrinsic regulator of endogenous antioxidant mechanisms [44]. Under stress conditions, the dissociation process between Keap-1 and Nrf2 occurs, allowing Nrf2 to translocate to the nucleus, where it attaches to antioxidant response elements, aiding in the recruitment of transcription factors [45]. HO-1, a well-known inducible enzyme, is transcribed by Nrf2, which helps to perform its antioxidant defense function [46]. Previous research has documented the inhibition of the Nrf2/HO-1 signaling pathway in the pathology of Hep I/R [47]. In the present work, hepatic I/R markedly downregulated the immunoexpression of Nrf2 and the hepatic protein level of HO-1, which aligns with previous results by Morsy et al. [48]. Conversely, *Chlorella vulgaris* reversed this response, which was supported by a study by Farag et al. [49], who reported the ameliorative effect of *Chlorella vulgaris* against cadmium-induced oxidative insult through its upregulation of the hepatic Nrf2/HO-1 pathway. The antioxidant power of *Chlorella vulgaris* can be attributed to its antioxidant components or its upregulation

https://doi.org/10.5115/acb.24.318

of the Nrf2 transcription factor. These antioxidant characteristics explain its modulatory effect on liver enzymes and hepatic pathology.

The activation of NF-κB plays a vital role in the pathogenesis of Hep I/R through its upregulation of the inflammatory cascade response [50]. NLRP3 is a well-known complex whose activation is triggered during stressful conditions, and it is involved in Hep I/R pathogenesis [28]. NLRP3 suppression helps in the inhibition of neutrophil infiltration in hepatic I/R [11]. Consistent with previous data, our results revealed a significant increase in the NF-κB/NLRP3 pathway through the upregulation of NF-κB and NLRP3 gene expression, as well as the increased expression of myeloperoxidase (a neutrophil infiltration marker), accompanied by a surge in the hepatic levels of inflammatory cytokines. This aligns with the findings of Gendy et al. [28]. Conversely, pretreatment with *Chlorella vulgaris* downregulates the NF-κB transcription factor, resulting in decreased TNF-α, IL-1β, and IL-6 levels in hepatic homogenates. Moreover, it suppresses the mRNA expression of the NLRP3 inflammasome, leading to reduced immunoexpression of caspase-1 and subsequent MPO immunoexpression. These findings are in line with the results of Farag et al. [49], who reported the downregulatory effect of *Chlorella vulgaris* on NF-κB and inflammatory cytokines as a hepatoprotective mechanism against cadmium-induced hepatotoxicity. Additionally, Nakashima et al. [51] reported the ameliorative effect of *Chlorella vulgaris* on mitochondrial ROS formation through its attenuation of NLRP3 activation.

In Hep I/R, the apoptotic process plays a pivotal role in hepatocyte damage [52]. This is evidenced in our work by a significant increase in the protein contents of the proapoptotic markers caspase-3 and Bax. These data are consistent with previous work [28]. Pretreatment with *Chlorella vulgaris* mitigated Hep I/R-induced hepatic apoptosis by decreasing the levels of caspase-3 and Bax, which aligns with the findings of Aboumosalem et al. [53], who reported the inhibitory effect of *Chlorella vulgaris* on colonic cellular apoptosis in an acetic acid-induced colitis experimental model.

In conclusion, our results demonstrate the hepatoprotective effect of pretreatment with *Chlorella vulgaris* against Hep I/R. This protection is evidenced by the modulation of hepatic enzymes and histological improvements. These protective effects can be attributed to the modulation of oxidative markers upstream of the Nrf2/HO-1 signaling pathway. Additionally, *Chlorella vulgaris* attenuated Hep I/R-induced inflammation by inhibiting the NF-κB/NLRP3/IL-1β complex pathway, leading to a reduction in TNF-α, IL-1β, and IL-6 levels and neutrophil infiltration. Furthermore, it prevented hepatocyte apoptosis by decreasing the levels of caspase-3 and Bax. These findings focused on the preventive effect of *Chlorella vulgaris* on Hep I/R injury in patients undergoing liver transplantation.

ORCID

Amira E. Farage: https://orcid.org/0000-0003-1871-4522
Mona A. Abdel-Kareem: https://orcid.org/0000-0001-7889-7741
Medhat Taha: https://orcid.org/0000-0001-8440-5002
Sara Abubakr: https://orcid.org/0000-0002-2666-3721
Nora Elshehawy Helal: https://orcid.org/0000-0001-6630-3256
Mahmoud Hendawy: https://orcid.org/0000-0002-6393-0868
Hanan A. Elgendy: https://orcid.org/0000-0002-2751-5093
Ahmed Abdel-monem Elmetwally: https://orcid.org/0000-0001-7633-8639
Hala Mahfouz: https://orcid.org/0000-0003-4110-3746
Tourki A. S. Baokbah: https://orcid.org/0000-0001-6091-198X
Mohammed R. Rabei: https://orcid.org/0000-0001-9685-7399
Mohammad Akbar Hossain: https://orcid.org/0000-0001-8990-9875
Azza I. Helal: https://orcid.org/0009-0000-2441-8215
Mohie Mahmoud Ibrahim: https://orcid.org/0000-0001-8027-5298

Author Contributions

Conceptualization: MT, MH, AIH. Data acquisition: MAA, HAE, TASB. Data analysis or interpretation: AEF, MT, SA, AAE, HM, NE. Drafting of the manuscript: MT. Critical revision of the manuscript: SA, MRR, MMI. Approval of the final version of the manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Funding

None.

Acknowledgements

The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work.

References

1. Xie T, Li K, Gong X, Jiang R, Huang W, Chen X, Tie H, Zhou Q, Wu S, Wan J, Wang B. Paeoniflorin protects against liver ischemia/reperfusion injury in mice via inhibiting HMGB1-TLR4 signaling pathway. Phytother Res 2018;32:2247-55.
2. Arumugam TV, Okun E, Tang SC, Thundyil J, Taylor SM, Woodruff TM. Toll-like receptors in ischemia-reperfusion injury. Shock 2009;32:4-16.
3. Xu D, Xu M, Jeong S, Qian Y, Wu H, Xia Q, Kong X. The role of Nrf2 in liver disease: novel molecular mechanisms and therapeutic approaches. Front Pharmacol 2018;9:1428.
4. Ahmed SM, Luo L, Namani A, Wang XJ, Tang X. Nrf2 signaling pathway: pivotal roles in inflammation. Biochim Biophys Acta Mol Basis Dis 2017;1863:585-97.
5. Drummond GS, Baum J, Greenberg M, Lewis D, Abraham NG. HO-1 overexpression and underexpression: clinical implications. Arch Biochem Biophys 2019;673:108073.
6. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. Int J Mol Sci 2019;20:3328.
7. Luo M, Yan D, Sun Q, Tao J, Xu L, Sun H, Zhao H. Ginsenoside Rg1 attenuates cardiomyocyte apoptosis and inflammation via the TLR4/NF-kB/NLRP3 pathway. J Cell Biochem 2020;121:2994-3004.
8. Vaure C, Liu Y. A comparative review of toll-like receptor 4 expression and functionality in different animal species. Front Immunol 2014;5:316.
9. Sutterwala FS, Haasken S, Cassel SL. Mechanism of NLRP3 inflammasome activation. Ann N Y Acad Sci 2014;1319:82-95.
10. Szabo G, Csak T. Inflammasomes in liver diseases. J Hepatol 2012;57:642-54.
11. Inoue Y, Shirasuna K, Kimura H, Usui F, Kawashima A, Karasawa T, Tago K, Dezaki K, Nishimura S, Sagara J, Noda T, Iwakura Y, Tsutsui H, Taniguchi S, Yanagisawa K, Yada T, Yasuda Y, Takahashi M. NLRP3 regulates neutrophil functions and contributes to hepatic ischemia-reperfusion injury independently of inflammasomes. J Immunol 2014;192:4342-51.
12. Jeon JY, Kim KE, Im HJ, Im, , Oh ST, Lim SU, Kwon HS, Moon BH, Kim JM, An BK, Kang CW. The production of lutein-enriched eggs with dietary Chlorella. Korean J Food Sci Ani Resour 2012;32:13-7.
13. Buono S, Langellotti AL, Martello A, Rinna F, Fogliano V. Functional ingredients from microalgae. Food Funct 2014;5:1669-85.
14. Bengwayan PT, Laygo JC, Pacio AE, Poyaoan JLZ, Rebugio JF, Yuson ALL. A comparative study on the antioxidant property of Chlorella (*Chlorella sp.*) tablet and glutathione tablet. E-Int Sci Res J 2010;2:25-35.
15. Plaza M, Herrero M, Cifuentes A, Ibáñez E. Innovative natural functional ingredients from microalgae. J Agric Food Chem 2009;57:7159-70.
16. Ahmed F, Fanning K, Netzel M, Turner W, Li Y, Schenk PM. Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters. Food Chem 2014;165:300-6.
17. Ko SC, Kim D, Jeon YJ. Protective effect of a novel antioxidative peptide purified from a marine Chlorella ellipsoidea protein against free radical-induced oxidative stress. Food Chem Toxicol 2012;50:2294-302.
18. Wang X, Zhang X. Separation, antitumor activities, and encapsulation of polypeptide from Chlorella pyrenoidosa. Biotechnol Prog 2013;29:681-7.
19. Cherng JY, Shih MF. Preventing dyslipidemia by Chlorella pyrenoidosa in rats and hamsters after chronic high fat diet treatment. Life Sci 2005;76:3001-13.
20. Sheih IC, Fang TJ, Wu TK. Isolation and characterisation of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide from the algae protein waste. Food Chem 2009;115:279-84.
21. Chovančíková M, Šimek V. Effects of hight-fat and *Chlorella vulgaris* feeding on changes in lipid metabolism in mice. Biol Bratisl 2001;56:661-6.
22. Cherng JY, Shih MF. Improving glycogenesis in Streptozocin (STZ) diabetic mice after administration of green algae Chlorella. Life Sci 2006;78:1181-6.
23. Mohseni R, Alavian SM, Sadeghabadi ZA, Heiat M. Therapeutic effects of Chlorella vulgaris on carbon tetrachloride induced liver fibrosis by targeting Hippo signaling pathway and AMPK/FOXO1 axis. Mol Biol Rep 2021;48:117-26.
24. Kim HK, Li L, Lee HS, Park MO, Bilehal D, Li W, Kim YH. Protective effects of Chlorella vulgaris extract on carbon tetrachloride-induced acute liver injury in mice. Food Sci Biotechnol 2009;18:1186-92.
25. Li L, Li W, Kim YH, Lee YW. Chlorella vulgaris extract ameliorates carbon tetrachloride-induced acute hepatic injury in mice. Exp Toxicol Pathol 2013;65:73-80.
26. Peng HY, Chu YC, Chen SJ, Chou ST. Hepatoprotection of chlorella against carbon tetrachloride-induced oxidative damage in rats. In Vivo 2009;23:747-54.
27. Qasem W, Mohamed EA, Hamed AA, El-Sayed AEKB, Salah El Din RA. Antimicrobial and anticancer activity of some microalgae species. Egypt J Phycol 2016;17:33-49.
28. Gendy A, El-Abhar H, Mohsen AR. Cilostazol hepatoprotective effect against ischemia/reperfusion: involvement of GSK-3β, cyclin D1 and WNT/β-catenin pathway. J Pharmacol Res 2014;4:75.

29. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques E-Book. Elsevier; 2018.
30. Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. Transplantation 1993;55:1265-72.
31. El-Emam SZ, Soubh AA, Al-Mokaddem AK, Abo El-Ella DM. Geraniol activates Nrf-2/HO-1 signaling pathway mediating protection against oxidative stress-induced apoptosis in hepatic ischemia-reperfusion injury. Naunyn Schmiedebergs Arch Pharmacol 2020;393:1849-58.
32. Pawlikowska-Pawlega B, Gruszecki WI, Misiak L, Paduch R, Piersiak T, Zarzyka B, Pawelec J, Gawron A. Modification of membranes by quercetin, a naturally occurring flavonoid, via its incorporation in the polar head group. Biochim Biophys Acta 2007;1768:2195-204.
33. Safi C, Zebib B, Merah O, Pontalier PY, Vaca-Garcia C. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: a review. Renew Sustain Energy Rev 2014; 35:265-78.
34. Mathew B, Sankaranarayanan R, Nair PP, Varghese C, Somanathan T, Amma BP, Amma NS, Nair MK. Evaluation of chemoprevention of oral cancer with Spirulina fusiformis. Nutr Cancer 1995;24:197-202.
35. Ko JS, Gwak MS, Kim GS, Shin YH, Ryu S, Kim JS, Kim SJ, Kim ST. The protective effect of ischemic preconditioning against hepatic ischemic-reperfusion injury under isoflurane anesthesia in rats. Transplant Proc 2013;45:1704-7.
36. Kim D, Choi JW, Han S, Gwak MS, Kim GS, Jeon SY, Ryu S, Hahm TS, Ko JS. Ischemic Preconditioning Protects Against Hepatic Ischemia-Reperfusion Injury Under Propofol Anesthesia in Rats. Transplant Proc 2020;52:2964-9.
37. Elsheikh S, Galal AA, Fadil R. Hepatoprotective impact of Chlorella vulgaris powder on deltamethrin intoxicated rats. Zagazig Vet J 2018;46:17-24.
38. Moradi MN, Behrouj H, Alipoor B, Kheiripour N, Ghasemi H, Ghasemi H. Chlorella vulgaris is an effective supplement in counteracting non-alcoholic fatty liver disease-related complications through modulation of dyslipidemia, insulin resistance, and inflammatory pathways. J Food Biochem 2021;45:e13914.
39. Elias-Miró M, Jiménez-Castro MB, Rodés J, Peralta C. Current knowledge on oxidative stress in hepatic ischemia/reperfusion. Free Radic Res 2013;47:555-68.
40. Li Z, Wang Y, Zhang Y, Wang X, Gao B, Li Y, Li R, Wang J. Protective Effects of Fisetin on Hepatic Ischemia-reperfusion Injury Through Alleviation of Apoptosis and Oxidative Stress. Arch Med Res 2021;52:163-73.
41. Morsy MA, Abdel-Gaber SA, Rifaai RA, Mohammed MM, Nair AB, Abdelzaher WY. Protective mechanisms of telmisartan against hepatic ischemia/reperfusion injury in rats may involve PPARγ-induced TLR4/NF-κB suppression. Biomed Pharmacother 2022;145:112374.
42. Kou X, Zhu J, Xie X, Hao M, Zhao Y. The protective effect of glycyrrhizin on hepatic ischemia-reperfusion injury in rats and possible related signal pathway. Iran J Basic Med Sci 2020;23: 1232-8.
43. Eissa MM, Ahmed MM, Abd Eldaim MA, Mousa AA, Elkirdasy AF, Mohamed MA, Orabi SH. Chlorella vulgaris ameliorates sodium nitrite-induced hepatotoxicity in rats. Environ Sci Pollut Res Int 2021;28:9731-41.
44. Xu G, Wang X, Xiong Y, Ma X, Qu L. Effect of sevoflurane pretreatment in relieving liver ischemia/reperfusion-induced pulmonary and hepatic injury. Acta Cir Bras 2019;34:e201900805.
45. Baird L, Yamamoto M. The molecular mechanisms regulating the KEAP1-NRF2 pathway. Mol Cell Biol 2020;40:e00099-20.
46. Ryter SW. Heme oxygenase-1/carbon monoxide as modulators of autophagy and inflammation. Arch Biochem Biophys 2019; 678:108186.
47. Zhao Y, Kong GY, Pei WM, Zhou B, Zhang QQ, Pan BB. Dexmedetomidine alleviates hepatic injury via the inhibition of oxidative stress and activation of the Nrf2/HO-1 signaling pathway. Eur Cytokine Netw 2019;30:88-97.
48. Morsy MA, Ibrahim YF, Abdel Hafez SMN, Zenhom NM, Nair AB, Venugopala KN, Shinu P, Abdel-Gaber SA. Paeonol attenuates hepatic ischemia/reperfusion injury by modulating the Nrf2/HO-1 and TLR4/MYD88/NF-κB signaling pathways. Antioxidants (Basel) 2022;11:1687.
49. Farag MR, Alagawany M, Mahdy EAA, El-Hady E, Abou-Zeid SM, Mawed SA, Azzam MM, Crescenzo G, Abo-Elmaaty AMA. Benefits of *Chlorella vulgaris* against cadmium chloride-induced hepatic and renal toxicities via restoring the cellular redox homeostasis and modulating Nrf2 and NF-KB pathways in male rats. Biomedicines 2023;11:2414.
50. Zhang S, Feng Z, Gao W, Duan Y, Fan G, Geng X, Wu B, Li K, Liu K, Peng C. Aucubin attenuates liver ischemia-reperfusion injury by inhibiting the HMGB1/TLR-4/NF-κB signaling pathway, oxidative stress, and apoptosis. Front Pharmacol 2020;11: 544124.
51. Nakashima Y, Gotoh K, Mizuguchi S, Setoyama D, Takata Y, Kanno T, Kang D. Attenuating effect of *Chlorella* extract on NLRP3 inflammasome activation by mitochondrial reactive oxygen species. Front Nutr 2021;8:763492.
52. Konishi T, Lentsch AB. Hepatic ischemia/reperfusion: mechanisms of tissue injury, repair, and regeneration. Gene Expr 2017;17:277-87.
53. Aboumosalem H, Mokhbatly AAA, Goda W, Ghazy EW, Abou Elazab MF, Abdelatty A, Elbialy ZI, Assar DH. Chlorella vulgaris effectively attenuates acetic acid-induced colitis in rats by inhibiting NF-κB, and caspase-3, while activating IL-10 expression. Egypt J Vet Sci 2025;56:1761-81.