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Geraniol alleviates liver ischemia/reperfusion injury by modulating the MAPK/NF-κB signaling pathway

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Abstract

This study investigates the anti-inflammatory effects of Geraniol (GER) in mitigating Liver Ischemia/Reperfusion Injury (LIRI) by targeting the Mitogen-Activated Protein Kinase (MAPK)/Nuclear Factor-kappa B (NF-κB) signaling pathway. Forty-nine male Wistar rats underwent 1 hour of liver ischemia, followed by 60 minutes and 6 hours of reperfusion. GER was administered at 50 and 100 mg/kg doses immediately before reperfusion. Liver function was evaluated by measuring serum aminotransferase levels. Oxidative stress markers in liver homogenates were assessed. Inflammatory factors were analyzed using Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Enzyme-Linked Immunosorbent Assay (ELISA), and NF-κB and phosphorylated Mitogen-Activated Protein Kinases (p-MAPK) concentrations in liver tissue were measured using immunohistochemistry. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. This study shows that GER effectively mitigates LIRI through its anti-inflammatory properties, significantly improving liver function, reducing oxidative stress markers, and suppressing NF κB and p-MAPK activity. Histopathological analysis confirmed that GER significantly attenuated liver damage. These findings suggest that GER exerts its protective effects by modulating the NF-κB and MAPK signaling pathways.

Introduction

Liver Ischemia/Reperfusion Injury (LIRI) represents a complex pathophysiological process with far-reaching clinical consequences. It occurs when hepatic tissue is exposed to prolonged ischemia followed by reperfusion, as seen in hepatectomy, liver transplantation, and trauma.[1] LIRI arises when insufficient oxygen supply forces hepatocytes to shift from aerobic to anaerobic metabolism, a transition that exacerbates cellular injury and significantly influences patient prognosis. Strategies to mitigate LIRI and enhance hepatic resilience are therefore critical for improving clinical outcomes.[2] Herbs, herbal extracts, and isolated natural secondary metabolites are widely employed to treat various clinical conditions, particularly those associated with oxidative stress.[3,4] Geraniol (2E)-3,7-dimethylocta-(2,6-dien-1-ol) (GER) is a monocyclic monoterpene alcohol, illustrated in Figure 1, obtained from the essential oils of several fragrant botanicals, including rose (*Rosa damascena* Mill) and lemongrass (*Cymbopogon citratus*).[5,6] It is also approved by the Food and Drug Administration (FDA) for use as a flavoring agent and artificial additive for human consumption.[7] Research has shown that GER has several pharmacological effects, including neuroprotective, antioxidant,[8] analgesic,[9] anti-inflammatory, and anti-tumor.[10] GER has demonstrated potential as a therapeutic agent for various pathological conditions, such as ulcerative colitis,[11] non-alcoholic steatohepatitis,[12] high blood lipids,[13] and heart complications of diabetes.[5]

LIRI is characterized by a complex interplay among mediators, as well as specific cellular and vascular components, along with inflammatory factors that are prominently expressed during Ischemia/Reperfusion (I/R) insult. These interconnected processes lead to hepatocellular dysfunction, commonly reflected by significant elevations in serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels in the I/R group.[14] This increase in liver enzymes is largely attributed to Reactive Oxygen Species (ROS) production by Kupffer cells, which induces lipid peroxidation of hepatocyte membranes.[15] In one study, GER attenuated colitis through its antioxidant, anti-inflammatory, and immunosuppressive properties, possibly by modulating the Wingless-related integration site (Wnt)/Glycogen Synthase Kinase 3 beta (GSK-3β)/Beta-catenin (β-catenin), p38 Mitogen-Activated Protein Kinase (p38 MAPK), the nuclear factor kappa B (NF-κB), and Peroxisome Proliferator-Activated Receptor (PPARγ) signaling pathways.[11]

During the early phase of reperfusion, occurring within the first two hours, ROS are rapidly generated and inflammatory responses are initiated. Experimental evidence highlights the pivotal role of Kupffer cells, specialized hepatic macrophages, in producing these early ROS.[16] These cells also release key proinflammatory cytokines, including Tumor Necrosis Factor-alpha (TNF-α), Interleukin-8 (IL-8), and Interleukin-6 (IL-6), thereby contributing to the inflammatory microenvironment.[17] As reperfusion progresses to the late phase, approximately six hours after its onset, neutrophils become the dominant mediators of injury. Once recruited to the ischemic site, neutrophils release additional ROS, cytokines, cyclooxygenase-2 (COX-2), Myeloperoxidase (MPO), and various inflammatory mediators, collectively amplifying hepatocellular damage.[18] This late-phase exacerbation represents a critical stage in the progression of liver I/R injury.

NF-κB plays a well-established role in regulating key cellular processes, particularly inflammation and immune responses. It orchestrates the expression of numerous proinflammatory cytokines and chemokines throughout the injury process. Beyond its inflammatory functions, NF-κB also contributes to cell survival and regeneration, illustrating its dual role in both pathological and reparative signaling.[19] The MAPK signaling pathway likewise plays a vital role in coordinating cellular processes such as inflammation, apoptosis, and autophagy.[20] Its activation is essential for understanding the mechanisms underlying cell death and liver disease progression. Moreover, MAPK functions as both an upstream activator and regulator of the NF-κB pathway, underscoring their interconnected roles.[21] Therefore, investigating the relationship between MAPK and NF-κB is crucial for understanding LIRI pathogenesis. Given the emerging evidence supporting the anti-inflammatory and antioxidant activities of GER, it is increasingly being explored as a potential therapeutic agent for inflammatory disorders. With an improved understanding of LIRI mechanisms, various strategies, including antioxidant-based therapies have been developed for its prevention and treatment.[22] Accordingly, in this study, we investigated the protective effects of GER against hepatic LIRI through the MAPK/NF-κB signaling pathway in a rat model. By elucidating the role of GER in liver LIRI, we aim to provide new insights into its pharmacological actions and potential therapeutic value in liver disease.

Materials and Methods

Study approval

All experimental procedures involving animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the National Institutes of Health (NIH) guidelines. The study protocol was reviewed and approved by the Ethics Committee for Animal Care at Kerman University of Medical Sciences (Approval ID: IR.KMU.AH.REC.2023.129; approval date: 22 February 2024, Kerman, Iran). All efforts were made to minimize animal suffering and to reduce the number of animals used.

For this experimental study, 49 male Wistar rats weighing 200–250 g were housed under controlled environmental conditions at a temperature of 25 ± 2 °C, with a 12-hour light/12-hour dark photoperiod throughout the study period. Rats were provided with unrestricted access to drinking water and specialized rat feed. Animals were randomly divided into seven groups of seven. In each cage, seven rats were kept.

Geraniol (Sigma-Aldrich, Cat. No. 163333) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

To acclimate the animals to the experimental environment, the study began one week after their arrival. The rats were assigned to the seven groups as follows: a Sham group (Group 1), which underwent laparotomy without ischemia or reperfusion and received intraperitoneal normal saline; a LIRI-1h control group (Group 2), subjected to 1 hour of ischemia followed by 1 hour of reperfusion and treated with normal saline; a LIRI-6h control group (Group 3), subjected to 1 hour of ischemia followed by 6 hours of reperfusion and treated with normal saline; a LIRI + GER 50 mg/kg – 1h reperfusion group (Group 4), which received GER (50 mg/kg) immediately before reperfusion; a LIRI + GER 100 mg/kg – 1h reperfusion group (Group 5), treated similarly with GER (100 mg/kg); a LIRI + GER 50 mg/kg – 6h reperfusion group (Group 6); and a LIRI + GER 100 mg/kg – 6h reperfusion group (Group 7), with GER administered intraperitoneally immediately before reperfusion.

Experimental model of LIRI

The LIRI model was constructed as previously described.[23] To perform surgery, rats were anesthetized with intraperitoneal injection of ketamine hydrochloride 90 mg/kg and xylazine 15 mg/kg. After shaving the hair and disinfecting the abdominal skin area, an incision was made in the midline of the abdomen. After revealing the liver umbilicus, the common hepatic artery and the portal vein were identified and blocked by vascular traumatic bread clips for one hour. Immediately after vascular occlusion, the color of the lobes changed from dark brown to pale brown. After removing the clamps and the obstruction, reperfusion was performed for 1 and 6 hours. The temperature in the rectum was kept at 37°C during the operation with a heating pad. Blood and liver samples were taken 1 and 6 h after reperfusion and kept at -80°C until used. Rats were sacrificed at 1 and 6 hours after reperfusion.

Serum biochemical analysis

The blood samples underwent a cooling process lasting for a duration of 5 hours at a temperature of 4°C. Subsequently, they were subjected to centrifugation at a speed of 4600 revolutions per minute for a span of 10 minutes. The liquid above the sediment was preserved at a freezing temperature of -80°C until further examination of serum ALT and AST levels.[24] The concentrations were determined using colorimetric assay kits according to the manufacturer's instructions (Pars Azmoon Co., Tehran, Iran).

Measuring enzymes related to oxidative stress

Frozen liver tissue was homogenized in lysis buffer (150 mM NaCl, 10 mM Tris, 1% Triton X-100, pH 7.4) containing a protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA). The homogenate was then centrifuged at 13,000 rpm for 10 minutes, and the supernatant was collected for further analysis. Liver homogenates were analyzed using Ultraviolet–visible (UV–VIS) spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan). Lipid peroxidation was assessed by measuring Thiobarbituric Acid Reactive Substances (TBARS), and Malondialdehyde (MDA) levels were calculated. MDA values were expressed as nmol/mg protein, with the pink chromogen measured at 532 nm. Liver Nitric Oxide (NO) levels were determined using the Griess reagent method, and the resulting pink color was measured at 520 nm. NO levels were expressed as μmol/mg protein.[25]

Measurement of MPO

Liver samples were homogenized and resuspended in 1% hexadecyltrimethylammonium bromide. After heating at 60°C for 2 hours, the samples were centrifuged, and the supernatant was incubated with 3,3',5,5'-tetramethylbenzidine and H_2O_2. The change in optical density was measured at 650 nm. The results of MPO activity were expressed as ng protein/mL.[26]

Gene expression analysis using real-time polymerase chain reaction

Trisol reagent (GeneAll Biotechnology, Seoul, South Korea) was used to extract total RNA. RNA concentration and purity were measured at 260 nm using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). The mRNA assay of IL-8, phosphorylated Mitogen-Activated Protein Kinase (p-MAPK), and NF-κB was done using Reverse Transcription Polymerase Chain Reaction (RT-PCR) using cDNA synthesized. The reverse transcription kit (Qiagen, Hilden, Germany), a three-step program, was used to amplify cDNA. For internal control, the Glyceraldehyde-3-Phosphate Dehydrogenase (*GAPDH*) gene was used. Temperature and temporal cycle conditions include an initial denaturation temperature of 95°C for 10 minutes, then for 40 cycles, 95°C for 15 seconds, and at the end of primer coupling and elongation at 60°C for 60 seconds. The forward primer for rat (*Rattus norvegicus*) genes IL-8 was:5´-TGGTAAGGATGGGAAACGAG-3′; IL-8 reverse, 5´-TGCAGAGTGCAGGTATGGAA-3′; and NF-κB forward, 5´-CAGGCTGTGCAGTCAGAGAA-3´; NF-κB reverse, 5´-CTGCTTCCCATAACCAAGGA-3´; p-MAPK forward, 5´-GAGAGTGCATTGGAACACGA-3´; p-MAPK reverse, 5´-CCAAGCTTGAGAAGGAAGCA-3´; GAPDH forward, 5´-AAACCCATCACCATCTTCCA -3´; GAPDH reverse 5´-GGAGGTTGCACCCATATCAA -3´. Using the 2-ΔΔCT formula, the obtained data from Real-Time PCR was analyzed. Real-time PCR was performed on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA).

Enzyme-linked immunosorbent assay

The concentrations of the pro-inflammatory proteins IL-8 (Cat. No. RAB0319, Sigma-Aldrich, St. Louis, MO, USA), NF-κB (Cat. No. RK08775, ZellBio GmbH, Lonsee, Germany) and MAPK (Cat. No. MBS267271, MyBioSource, San Diego, CA, USA) in liver tissue were determined using commercially available ELISA kits according to the manufacturers' instructions. The analytical sensitivity of the IL-8 ELISA kit was 2 pg/mL, while the sensitivities of the NF-κB and MAPK ELISA kits were 0.1 ng/mL and 0.05 ng/mL, respectively. The absorbance of the samples was assessed at a wavelength of 450 nm using an AQ8 ELISA reader (BioTek Instruments, Winooski, VT, USA).

Criteria for scoring

Liver tissue samples were evaluated under a light microscope ((Model BX51, Olympus Corporation, Tokyo, Japan) at magnifications of 20× and 40×. The primary parameters used for scoring included inflammation, edema, necrosis, and congestion. Each tissue sample was scored based on the severity of these parameters as follows: Score 1: Minimal, Score 2: Mild (less than 30%): Minimal tissue damage or low cellular congestion. Score 3: Moderate (30-60%): Moderate tissue damage, with medium levels of congestion or blood accumulation. Score 4: Severe (>60%): Severe tissue damage, with congestion affecting more than 60% of the examined area.

To assess the percentage of congestion, the visible areas of each tissue section were divided into specific regions, and the percentage of congested regions relative to the total area examined was calculated. This percentage was determined by observing the accumulation of blood or cells within blood vessels and sinusoids. Severe congestion was reported when more than 60% of the sample area showed significant blood or cell accumulation.

Liver histopathology assay

Liver tissue samples were fixed in 10% neutral buffered formalin, processed through graded ethanol solutions, cleared in xylene, and embedded in paraffin using standard histological procedures. Paraffin-embedded tissues were sectioned at a thickness of 5 μm and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

The histological severity of ischemia-reperfusion injury was graded using Suzuki's criteria.[27] The severity of injury was rated from zero to 4 (0: absence of injury, 1: minimal damage, 2: mild injury, 3: moderate injury, 4: severe injury) (Table 1). The observations with a magnification of 200× and 400× in 5 microscopic fields of each incision were performed randomly using an optical microscope (Nikon Corporation, Tokyo, Japan).

Immunohistochemical analysis

The paraffin-embedded sections were subjected to a preheating process lasting 2 hours at 60°C, followed by immersion in xylene for half an hour, subsequent dehydration using a gradient of alcohol, and then immersion in a sodium citrate buffer for antigen retrieval for 10 minutes at a temperature of 95°C. The tissue sections were initially treated with 3% hydrogen peroxide for 10 minutes at room temperature in the dark to inhibit endogenous peroxidase activity. Subsequently, the sections were washed three times with Phosphate-Buffered Saline (PBS). Following this, 5% bovine serum albumin was introduced to prevent nonspecific protein binding, and the sections were then incubated overnight at 4°C with antibodies targeting NF-κB (Cell Signaling Technology, Danvers, MA, USA; dilution 1:200) and p-MAPK (Cell Signaling Technology, Danvers, MA, USA; dilution 1:200). This was followed by the application of a secondary antibody (dilution, 1:50) for 1 hour at 37°C. Finally, a 3,3'-Diaminobenzidine (DAB) kit was used to visualize the bound antibodies using a light microscope (Model Eclipse E200, Nikon Corporation, Tokyo, Japan).

Statistical analysis

One-way Analysis of Variance (ANOVA) was used for statistical analysis. If there was a significant difference, the Tukey test was used to determine the level of significance difference between groups. In case of abnormality, the distribution of data was done from its nonparametric equivalent (Kruskal-Wallis and post hoc Mann-Whitney). Also,

$p < 0.05$ was considered as a significant level and the data in all charts are shown as mean ± Standard Error of the Mean (SEM).

Results

Effects of GER on liver function and damage assessment

Serum ALT and AST levels were measured to assess liver function and hepatocellular injury. LIRI induced a marked increase in both enzymes at 1 h and 6 h, confirming successful model induction. GER treatment (50 and 100 mg/kg) significantly attenuated these elevations.

At 1 h, ALT increased from 211.7 U/L in the sham group to 890.3 U/L in LIRI (4.2-fold increase; p<0.001). GER reduced ALT to 760.7 U/L (GER-50; 14.6% reduction vs. LIRI; p=0.04) and 727.0 U/L (GER-100; 18.3% reduction; p=0.010). At 6 h, ALT rose from 215.7 U/L in sham to 1.778 U/L in LIRI (8.2-fold increase; p<0.001). GER treatment decreased ALT to 1.486 U/L (GER-50; 16.5% reduction; p<0.001) and 1.398 U/L (GER-100; 21.4% reduction; $p < 0.001$) (Figure 2A).

AST levels exhibited an even sharper elevation. At 1 h, AST increased from 337.3 U/L (control) to 1,972 U/L in LIRI (5.8-fold increase; p<0.001). GER treatment reduced AST to 1,716 U/L (GER-50; 13.0% reduction; p=0.006) and 1,639 U/L (GER-100; 16.9% reduction; p<0.001). At 6 h, AST surged from 345.0 U/L (control) to 3,467 U/L in LIRI (10.0-fold increase; p<0.001). GER treatment lowered AST to 2,965 U/L (GER-50; 14.5% reduction; p<0.001) and 1,806 U/L (GER-100; 47.9% reduction; p<0.001) (Figure 2B).

To further assess tissue injury, liver morphology was examined by H&E staining (Figure 2C). After 1 h of reperfusion, the sham group showed normal hepatic architecture with preserved hepatocytes, eosinophilic cytoplasm, and intact nuclei. In contrast, the LIRI group displayed pronounced sinusoidal congestion, vacuolization, hepatocellular necrosis, and endothelial disruption. After 6 h of reperfusion, these lesions were more severe, with increased hyperemia and necrosis around central venules. GER treatment (50 and 100 mg/kg) effectively attenuated these histopathological abnormalities, as seen in Figure 2C, showing better-preserved hepatic structure, reduced necrosis, and decreased sinusoidal dilatation.

Histological analysis using Suzuki scores corroborated the biochemical findings. At 1 h, LIRI increased the score from 0.12 (sham) to 3.21. GER treatment reduced scores to

2.81 (GER-50; 0.40 difference vs. LIRI; $p=0.041$) and 2.67 (GER-100; 0.54 difference; $p=0.0049$). At 6 h, LIRI increased scores from 0.25 (sham) to 3.84. GER treatment lowered scores to 3.32 (GER-50; 0.52 difference vs. LIRI; $p=0.0066$) and 3.18 (GER-100; 0.66 difference; $p=0.0009$). Differences between GER-50 and GER-100 were not significant at either time point ($p>0.7$) (Figure 2D).

Effects of GER on oxidative stress

To evaluate the impact of GER on oxidative stress and neutrophil infiltration following LIRI, liver MDA, NO levels, and MPO activity were measured. LIRI significantly increased all three markers at 1 h and 6 h, indicating oxidative damage and inflammatory response, while GER treatment attenuated these changes.

At 1 h, MDA levels increased from 1.953 nmol/mg protein (sham) to 5.377 nmol/mg in LIRI (2.75-fold increase; $p<0.001$). GER reduced MDA to 4.830 nmol/mg (GER-50; 9.7% reduction vs. LIRI; $p=0.03$) and 4.533 nmol/mg (GER-100; 15.7% reduction; $p=0.001$). At 6 h, MDA rose from 1.950 nmol/mg (sham) to 5.893 nmol/mg in LIRI (3.02-fold increase; $p<0.001$). GER decreased MDA to 3.263 nmol/mg (GER-50; 44.6% reduction; $p<0.001$) and 2.790 nmol/mg (GER-100; 52.6% reduction; $p<0.001$) (Figure 3A).

At 1 h, NO increased from 1.027 Umol/mg protein (sham) to 2.310 Umol/mg protein in LIRI (2.25-fold increase; $p<0.001$). GER treatment reduced NO to 1.930 Umol/mg protein (GER-50; 16.5% reduction; $p=0.03$) and 1.920 Umol/mg protein (GER-100; 16.9% reduction; $p=0.03$). At 6 h, NO levels rose from 1.007 Umol/mg protein to 2.863 Umol/mg protein in LIRI (2.84-fold increase; $p<0.001$). GER decreased NO to 2.367 Umol/mg protein (GER-50; 17.3% reduction; $p=0.004$) and 1.880 Umol/mg protein (GER-100; 34.3% reduction; $p<0.001$) (Figure 3B).

Effects of GER on neutrophil infiltration

MPO activity, a marker of neutrophil infiltration, increased from 0.6433 ng/mL (sham) to 9.503 ng/mL in LIRI at 1 h (14.8-fold increase; $p<0.001$). GER reduced MPO to 8.480 ng/mL (GER-50; 10.8% reduction vs. LIRI; $p=0.03$) and 8.117 ng/mL (GER-100; 14.6% reduction; $p<0.001$). At 6 h, MPO activity rose from 0.6700 ng/mL to 9.710 ng/mL in LIRI (14.5-fold increase; $p<0.001$). GER treatment decreased MPO to 8.680

ng/mL (GER-50; 10.6% reduction; p=0.03) and 8.023 ng/mL (GER-100; 17.4% reduction; p<0.001) (Figure 3C).

Effects of GER on LIRI-induced inflammatory cytokine

Hepatic IL-8 expression at both the mRNA and protein levels was significantly upregulated following LIRI, reflecting the inflammatory response. GER treatment (50 and 100 mg/kg) markedly attenuated this increase at 1 h and 6 h, indicating its anti-inflammatory effects.

At 1 h, IL-8 mRNA increased from 0.653 (sham) to 2.545 (LIRI) (3.90-fold increase; p<0.001). GER reduced expression to 1.867 (GER-50; 26.6% reduction vs. LIRI; p=0.03) and 1.882 (GER-100; 26.1% reduction; p=0.04). At 6 h, IL-8 mRNA rose from 1.223 (sham) to 5.752 (LIRI) (4.70-fold increase; p<0.001). GER decreased expression to 4.143 (GER-50; 27.9% reduction; p<0.001) and 3.390 (GER-100; 41.1% reduction; p<0.001). Differences between GER-50 and GER-100 were significant at 6 h (p=0.02) (Figure 4, aB and bB).

Similarly, IL-8 protein levels measured by ELISA mirrored the mRNA results (Figure 4, aA).

At 1 h, protein increased from baseline 0.65 pg/mg protein (sham) to 2.55 pg/mg protein (LIRI; 3.92-fold increase; p<0.001) and was attenuated by GER to 1.87 pg/mg protein (GER-50; 26.7% reduction; p=0.03) and 1.88 pg/mg protein (GER-100; 26.3% reduction; p=0.04). At 6 h, protein levels rose from 1.22 pg/mg protein (sham) to 5.75 pg/mg protein (LIRI; 4.71-fold increase; p<0.001) and were reduced by GER to 4.14 pg/mg protein (GER-50; 27.9% reduction; p<0.001) and 3.39 pg/mg protein (GER-100; 41.1% reduction; p<0.001) (Figure 4,A).

Effects of GER on MAPK/NF-κB signaling pathway in LIRI rats

NF-κB mRNA was significantly elevated after LIRI at both 1 h and 6 h. At 1 h, NF-κB increased from 1.177 (sham) to 3.585 (LIRI; 3.05-fold increase; p<0.001). GER reduced expression to 3.165 (GER-50; 11.7% reduction; p=0.50) and 2.941 (GER-100; 17.9% reduction; p=0.17). At 6 h, NF-κB mRNA rose from 1.202 (sham) to 5.901 (LIRI; 4.91-fold increase; p<0.001). GER decreased expression to 4.723 (GER-50; 20.0% reduction; p=0.005) and 3.870 (GER-100; 34.4% reduction; p<0.001), with a significant difference between GER-50 and GER-100 (p=0.05) (Figure 4, aB).

NF-κB protein followed a similar pattern. At 1 h, protein increased from 1.350 (sham) to 5.467 pg/mg protein (LIRI; 4.05-fold increase; $p<0.001$). GER lowered levels to 4.547 (GER-50; 16.8% reduction; $p=0.03$) and 4.257 pg/mg protein (GER-100; 22.2% reduction; $p=0.005$). At 6 h, protein levels rose from 1.533 (sham) to 6.850 pg/mg protein (LIRI; 4.47-fold increase; $p<0.001$) and were reduced by GER to 5.923 (GER-50; 13.5% reduction; $p=0.03$) and 4.487 pg/mg protein (GER-100; 34.5% reduction; $p<0.001$) (Figure 4, bB).

p-MAPK mRNA was significantly upregulated after LIRI. At 1 h, MAPK mRNA increased from 1.469 (sham) to 3.592 (LIRI; 2.45-fold increase; $p<0.001$) and was decreased by GER to 2.747 (GER-50; 23.5% reduction; $p=0.04$) and 2.292 (GER-100; 36.2% reduction; $p=0.001$). At 6 h, expression increased from 1.466 (sham) to 4.894 (LIRI; 3.34-fold increase; $p<0.001$) and was reduced to 4.096 (GER-50; 16.3% reduction; $p=0.05$) and 3.186 (GER-100; 34.9% reduction; $p<0.001$), with a significant difference between GER-50 and GER-100 ($p=0.02$) (Figure 4, aC).

MAPK protein levels mirrored mRNA results. At 1 h, protein increased from 1.253 (sham) to 6.503 pg/mg protein (LIRI; 5.19-fold increase; $p<0.001$) and was reduced by GER to 5.557 (GER-50; 14.5% reduction; $p=0.02$) and 5.167 pg/mg protein (GER-100; 20.5% reduction; $p=0.001$). At 6 h, MAPK protein increased from 1.343 (sham) to 7.860 pg/mg protein (LIRI; 5.85-fold increase; $p<0.001$) and was attenuated by GER to 6.937 (GER-50; 11.7% reduction; $p=0.02$) and 5.557 pg/mg protein (GER-100; 29.3% reduction; $p<0.001$), with significant differences between GER-50 and GER-100 ($p<0.001$) (Figure 4, bC).

Overall, these results suggest that the protective effects of GER against LIRI involve suppression of the MAPK/NF-κB signaling cascade.

Protective effects of GER following liver injury

Immunohistochemical staining showed that liver cells did not show any changes in the sham group after 6 hrs. In LIRI group, positive immunohistochemical staining was seen in hepatocytes, which indicates necrosis and inflammation (Figure 5A). The GER 50 treatment group after 6 hours of immunohistochemical staining positive p-MAPK in sinusoidal networks was less observed ($p< 0.05$). After 6 h of reperfusion, GER treatment at a dose of 100 mg/kg significantly decreased the expression of p-MAPK compared with the LIRI group ($p < 0.01$). The positive immunohistochemical staining of NF-κB also increased in the LIRI group 6 h after reperfusion around the sinusoids and hepatocytes, while the number of NF-κB-stained cells decreased in the group treated with GER ($p < 0.001$) (Figure 5B).

Discussion

This study investigated the anti-inflammatory effects of geraniol in mitigating LIRI by examining the involvement of the MAPK/NF-κB signaling pathway. Compared with the sham group, rats in the LIRI group exhibited higher serum levels of ALT and AST, increased liver MPO activity, and lower levels of NO and MDA at both 1 and 6 h after reperfusion. GER-treated rats demonstrated a notable reduction in serum ALT, AST, and liver MPO levels, along with a significant increase in NO and MDA levels compared to the LIRI group, highlighting the protective effects of GER on the liver. Furthermore, GER treatment resulted in a decrease in the infiltration of neutrophils. Moreover, GER inhibited NF-κB mRNA and protein expression while reducing p-MAPK mRNA and protein expression levels. The evidence demonstrates that GER's ability to protect against LIRI is due to its anti-inflammatory mechanisms, achieved through the regulation of MAPK/NF-κB.

GER is an acyclic monoterpene derived from plants' mevalonate pathway. However, it is not naturally produced in mammals.[28,29]

GER is widely recognized for its potent antioxidant and anti-inflammatory properties, which play a crucial role in counteracting oxidative stress and alleviating liver inflammation commonly observed in hepatic disorders. Furthermore, it has been observed that GER effectively promotes the regeneration of hepatocytes, a pivotal mechanism in the restitution of hepatic injury. Additionally, extensive research has conclusively shown that GER enhances liver function by triggering a range of significant signaling pathways.[30] These results indicate that further research is necessary to comprehensively understand how GER functions in preventing and treating liver injury, particularly in cases of liver ischemia injury.

The marked elevation of serum ALT and AST levels in the LIRI group indicates significant hepatocellular injury, consistent with the findings reported by Zengin *et al*.[31] This suggests that GER can reduce liver ischemia injury successfully. GER demonstrated its protective role for the liver by improving the levels of ALT and AST, as well as repairing the cell damage in the liver tissue structure, and this is consistent with the recent evidence.[24]

Notably, A more significant difference was seen in the 100 mg/kg-dose group compared to the control group, which shows that GER in this particular dose has a greater role in maintaining liver function. Histological analysis uncovered pronounced pathological alterations in the livers of rats exposed to ischemia, encompassing sinusoidal edema, necrotic occurrences in the centrilobular area, and damage to the central vein endothelium. The observations reported in this study are consistent with the distinguishing attributes of liver injury caused by ischemia-reperfusion. Administering 100 mg/kg GER following liver ischemia-reperfusion significantly mitigated these histological alterations. These findings imply that GER can decrease the biochemical indicators of liver injury and preserve the structural integrity of the liver tissue. These defensive effects are crucial as they have the potential to halt the advancement of liver damage and prevent further complications. Excessive ROS generation during the reperfusion phase is a significant contributor to ischemia-reperfusion injury.[32]

The outcomes presented herein demonstrate the manifestation of hepatic lipid peroxidation caused by the release of ROS during liver ischemia-reperfusion injury, together with a decline in the protective antioxidant defense mechanism. The remarkable antioxidative attribute possessed by GER leads to a substantial reduction in MDA and NO levels, thereby consistent with the findings of the investigation conducted by El Azab *et al*. study, where it was shown that GER reduces the concentration of MDA and NO and restores structural damage to brain tissue caused by a high-fat diet.[33]

Compared with the sham group, NO levels were significantly increased in the LIRI group. However, treatment with GER significantly reduced the production of NO, as shown in Figure 4.

The decrease in NO levels observed in the LIRI+GER group may contribute to the hepatoprotective effect. Conversely, the suppression of iNOS (Inducible Nitric Oxide Synthase) induction and NO production in hepatocyte cultures is considered an indication of hepatoprotection.[34]

The crucial involvement of neutrophils in liver injury occurs through their activation and infiltration into the hepatic vasculature. Previous studies have highlighted the pivotal role of neutrophils in liver damage during ischemia-reperfusion and alcohol-induced hepatitis.[35] In the context of LIRI, research has demonstrated a strong correlation between disease progression and the increased accumulation of neutrophils.[36]

Increased levels of MPO in tissues suggest the existence of invading neutrophils. Nevertheless, our investigation demonstrated that treatment for GER led to a decline in MPO function. This implies that GER might exhibit an inhibitory impact on neutrophil invasion, potentially enhancing defense against ischemia/reperfusion damage.
Several cytokines, such as IL-8, IL-1β, and IL-6, were found to occur at increased concentrations in the circulatory system during the first 24 hours after liver ischemia. This results in an inflammatory reaction in the liver's blood vessels, which triggers the influx of neutrophils and monocytes.[22] In a variety of inflammatory states, such as liver damage, there is a notable increase in levels of IL-6, IL-4, and IL-8, which serve as markers for considerable inflammation.[37]
In the current investigation, it was observed that ischemia significantly increased the levels of IL-8 mRNA and protein expressions in the liver. Nevertheless, administration of GER at 50 or 100 mg/kg dosages notably decreased the levels of hepatic IL-8. These findings strongly indicate that GER's protective effects may be due to its ability to suppress pro-inflammatory cytokines, thereby enhancing its anti-inflammatory properties.[38-40]
Through this process, GER efficiently reduces the generation of these molecules that cause inflammation, consequently decreasing the inflammatory sequence.
During hepatic ischemia-reperfusion injury, the rapid release of inflammatory mediators initiates the activation of transcription factors that orchestrate downstream inflammatory signaling.[41] Among these, NF-κB is considered a central regulator due to its rapid activation following Inhibitor kappa B alpha (IκBα) degradation.[42] Previous studies have consistently demonstrated that NF-κB contributes to both inflammation and apoptosis during hepatic I/R injury.[43]
Importantly, NF-κB exhibits a dual role in hepatic I/R: while excessive NF-κB activation drives inflammation and tissue injury, controlled NF-κB signaling is also required for hepatocyte survival, regeneration, and anti-apoptotic gene expression. This complexity underscores the necessity of interpreting our findings within a balanced mechanistic framework. In our study, ischemia markedly increased NF-κB expression, reflecting its canonical proinflammatory activation. GER treatment significantly attenuated this upregulation. Given NF-κB's dual function, our results suggest that GER may selectively inhibit the pathological, overactivated component of NF-κB signaling while preserving its physiological, pro-survival functions. This is supported by the parallel reduction in IL-8 mRNA and protein levels, indicating targeted suppression of

the NF-κB inflammatory axis rather than a global transcriptional blockade. In contrast, controlled or moderate NF-κB signaling in hepatocytes induces anti-apoptotic and cytoprotective genes, thereby supporting cell survival and liver regeneration without overt inflammation or fibrosis.[44]

Therefore, the changes we observed in NF-κB expression in our study can be interpreted within this conceptual framework: depending on the context, timing, cell type, and extent of activation, NF-κB may drive either pathological inflammation and cell death, or protective, anti-apoptotic and regenerative mechanisms in hepatocytes. Such mechanistic insight supports the rationale for interventions (e.g. with GER or other protective agents) that modulate NF-κB, potentially attenuating inflammatory damage while preserving or enhancing hepatocyte survival and regeneration.

Younis *et al.* demonstrated that GER attenuates methotrexate-induced acute kidney injury by decreasing renal p38 MAPK and NF-κB activation.[45] Hasan *et al*. showed that GER protects the liver by reducing caspase-3/9, COX-2, NF-κB, PCNA (Proliferating Cell Nuclear Antigen), iNOS, and VEGF (Vascular Endothelial Growth Factor) expression and limiting DNA fragmentation, suggesting its potential against 2-AAF (2-Acetylaminofluorene)-induced oxidative stress, inflammation, hyperproliferation, and apoptosis.[46]

MAPK signaling pathway includes three key members: ERK (Extracellular signal-Regulated Kinases), JNK (c-Jun N-terminal Kinase), and p38. They can be activated by various stimuli, such as inflammatory cytokines and ROS. When these MAPKs are activated, they trigger the activation of transcription factors like NF-κB, which then affect gene expression and cellular responses.[47] Our study aimed to investigate how GER influences the activation of MAPKs and the NF-κB pathway in a rat model of LIR injury. Given the complexity and severity of LIRI, the necessity of studying GER's effects becomes evident. GER's ability to suppress key inflammatory pathways and reduce cytokine production positions it as a promising candidate for developing new treatments aimed at reducing liver injury and improving clinical outcomes in patients undergoing hepatic ischemia-reperfusion. Future research should focus on further elucidating these mechanisms and exploring the clinical applications of GER in preventing or treating hepatic fibrosis and other liver-related conditions.

Despite the promising protective effects of GER observed in our study, several limitations should be considered when interpreting these results. First, the experiments were conducted exclusively in a rodent model, and species-specific differences in liver metabolism, immune responses, and signaling pathways, including NF-κB regulation, may limit the direct translation of these findings to humans. Second, the study focused on acute I/R injury, and the efficacy of GER in chronic liver injury, prolonged inflammatory conditions, or fibrotic disease remains untested. Third, pharmacokinetic and pharmacodynamic parameters, including absorption, distribution, metabolism, and excretion of GER, may differ substantially between rodents and humans, potentially affecting both efficacy and safety. Fourth, while the study demonstrates modulation of key molecular targets such as NF-κB, caspases, and inflammatory mediators, the precise mechanistic pathways remain incompletely defined, and additional studies are needed to clarify the interactions between GER and other cellular signaling cascades. Fifth, the experimental design, including sample size and duration of follow-up, may not fully capture long-term outcomes or delayed effects of treatment. Finally, environmental, genetic, and comorbid factors present in human populations are not represented in preclinical models, highlighting the necessity of further studies using chronic, human-relevant models, as well as eventual clinical trials, to establish the therapeutic potential of GER definitively. Therefore, while our results provide initial evidence of hepatoprotective activity, the translation to clinical application should be approached with caution, and conclusions regarding therapeutic efficacy in humans remain preliminary.

Conclusions

Our results demonstrate that LIR injury led to the activation of p-MAPK in the liver, but treatment with GER mitigated this response. Importantly, the suppression of MAPK activity by GER was associated with reduced NF-κB signaling and decreased levels of pro-inflammatory cytokines such as IL-8. These findings suggest that GER exerts its anti-inflammatory effects by interfering with the MAPK/NF-κB signaling axis.

References

1. Yu C, Chen P, Miao L, Di G. The role of the NLRP3 inflammasome and programmed cell death in acute liver injury. Int J Mol Sci 2023;24:3067.

2. Machado IF, Palmeira CM, Rolo AP. Preservation of mitochondrial health in liver ischemia/reperfusion injury. Biomed 2023;11:948.

3. Jain C, Khatana S, Vijayvergia R. Bioactivity of secondary metabolites of various plants: a review. I Pharm Sci Res 2019;10:494-504.

4. Shabani M, Erfani S, Abdolmaleki A, et al. Alpha-pinene modulates inflammatory response and protects against brain ischemia via inducible nitric oxide synthase-nuclear factor–kappa B-cyclooxygenase-2 pathway. Mol Bio Rep 2023;50:6505-16.

5. El-Bassossy HM, Ghaleb H, Elberry AA, et al. Geraniol alleviates diabetic cardiac complications: effect on cardiac ischemia and oxidative stress. Biomed Pharmacother 2017;88:1025-30.

6. Lei ZN, Teng QX, Tian Q, et al. Signaling pathways and therapeutic interventions in gastric cancer. Signal Transduct Target Ther 2022;7:358.

7. de Menezes-Filho JE, Gondim AN, Cruz JS, et al. Geraniol blocks calcium and potassium channels in the mammalian myocardium: useful effects to treat arrhythmias. Basic Clin Pharmacol Toxicol 2014;115:534-44.

8. Rekha KR, Inmozhi Sivakamasundari R. Geraniol protects against the protein and oxidative stress induced by rotenone in an *in vitro* model of Parkinson's disease. Neurochem Res 2018;43:1947-62.

9. La Rocca V, da Fonsêca DV, Silva-Alves KS, et al. Geraniol induces antinociceptive effect in mice evaluated in behavioural and electrophysiological models. Basic Clin Pharmacol Toxicol 2017;120:22-9.

10. Cho M, So I, Chun JN, Jeon JH. The antitumor effects of geraniol: Modulation of cancer hallmark pathways. Int J Oncol 2016;48:1772-82.

11. Soubh AA, Abdallah DM, El-Abhar HS. Geraniol ameliorates TNBS-induced colitis: Involvement of Wnt/β-catenin, p38MAPK, NFκB, and PPARγ signaling pathways. Life Sci 2015;136:142-50.

12. Chen J, Fan X, Zhou L, Gao X. Treatment with geraniol ameliorates methionine-choline-deficient diet-induced non-alcoholic steatohepatitis in rats. J Gastroenterol Hepatol 2016;31:1357-65.

13. Galle M, Kladniew BR, Castro MA, et al. M. Modulation by geraniol of gene expression involved in lipid metabolism leading to a reduction of serum-cholesterol and triglyceride levels. Phytomed 2015;22:696-704.

14. Moustafa D, Rabie M, Attia AS, Saleh AS. Does sirolimus attenuate the damage induced by partial warm ischemia/reperfusion injury in rat livers? Egyptian J Basic Clin Pharmacol 2012;2:41-52.
15. Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver *in vivo*. FASEB J 1990;4:3355-59.
16. Dong X, Liu J, Xu Y, Cao H. Role of macrophages in experimental liver injury and repair in mice. Exp Ther Med 2019;17:3835-47.
17. Du Y, Qian B, Gao L. Aloin preconditioning attenuates hepatic ischemia/reperfusion injury via inhibiting TLR4/MyD88/NF-κB signal pathway *in vivo* and *in vitro*. Oxid Med Cell Longev 2019;2019:3765898.
18. Fuertes Agudo M. Role of cyclooxygenase-2 in ischemia-reperfusion injury in the liver. Doctoral dissertation, Universitat Politècnica de València 2019.
19. Yu H, Lin L, Zhang Z, et al. Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. Signal Transduct Target Ther 2020;5:209.
20. Que J, López JM. Understanding MAPK signaling pathways in apoptosis. Int J Mol Sci 2020;21:2346.
21. Zhang Y, Cardell LO, Edvinsson L, Xu CB. MAPK/NF-κB-dependent upregulation of kinin receptors mediates airway hyperreactivity: a new perspective for the treatment. Pharmacol Res 2013;71:9-18.
22. Malik MNH, Tahir MN, Alsahli TG, et al. Geraniol suppresses oxidative stress, inflammation, and interstitial collagenase to protect against inflammatory arthritis. ACS Omega 2023;8:37128-139.
23. Wu HH. Heat shock protein 70 (HSP70) reduces hepatic inflammatory and oxidative damage in a rat model of liver ischemia/reperfusion injury with hyperbaric oxygen preconditioning. Med Sci Monitor 2018;24:8096.
24. Hasan SK, Sultana S. Geraniol attenuates 2-acetylaminofluorene induced oxidative stress, inflammation and apoptosis in the liver of Wistar rats. Toxicol Mech Methods 2015;25:559-73.
25. Khoshnazar M, Parvardeh S, Bigdeli MR, et al. Alpha-pinene exerts neuroprotective effects via anti-inflammatory and anti-apoptotic mechanisms in a rat model of focal cerebral ischemia-reperfusion. J Stroke Cerebrovasc Dis 2020;29:104977.
26. Cimen O. The effect of Liv-52 on liver ischemia reperfusion damage in rats. BMC Pharmacol Toxicol 2020;21:1-9.

27. Suzuki S, Toledo-Pereyra LH, Rodriguez FJ. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. Transplant 1993;55:1265-72.
28. Lei Y, Fu P, Jun X, Cheng P. Pharmacological properties of geraniol–a review. Planta Medica 2019;85:48-55.
29. Khoshnazar SM, Zeinali N, Mohagheghi M, Shafieipour S. Safety and efficacy of geraniol in gastrointestinal system. Rev Bras Farmacogn 2024;34:121832.
30. Ozkaya A, Sahin Z, Gorgulu AO, et al. Geraniol attenuates hydrogen peroxide-induced liver fatty acid alterations in male rats. J Int Ethnopharmacol 2017;6:29-35.
31. Zengin A, Erikçi A, Telli G, et al. Anti-inflammatory effects of oral and intraperitoneal administration of cerium oxide nanoparticles on experimental hepatic ischemia-reperfusion injury. Turk J Surg 2022;38:255-65.
32. Khoshnazar M, Bigdeli MR, Parvardeh S, et al. Attenuating effect of α-pinene on neurobehavioural deficit, oxidative damage and inflammatory response following focal ischaemic stroke in rat. J Pharm Pharmacol 2019;71:1725-33.
33. El Azab EF, Abdulmalek S. Amelioration of age-related multiple neuronal impairments and inflammation in high-fat diet-fed rats: The prospective multitargets of geraniol. Oxid Med Cell Longev Published online 2022:4812993.
34. Nakatake R, Hishikawa H, Kotsuka M, et al. The proton pump inhibitor lansoprazole has hepatoprotective effects in *in vitro* and *in vivo* rat models of acute liver injury. Digestive Dis Sci 2019;64:2854-66.
35. Jaeschke H. Mechanisms of Liver Injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions. Am J Physiol Gastrointest Liver Physiol 2006;290:G1083-8.
36. Mendes-Braz M, Elias-Miró M, Jiménez-Castro MB, et al. The current state of knowledge of hepatic ischemia-reperfusion injury based on its study in experimental models. J Biomed Biotechnol 2012;2012:298657
37. Kaufmann B, Leszczynska A, Reca A, et al. NLRP3 activation in neutrophils induces lethal autoinflammation, liver inflammation, and fibrosis. EMBO Rep 2024;25:455.
38. Shafieipour S, Khajehpour H, Dabiri S, et al. Effect of geraniol on cyclooxygenase-2 and inducible nitric oxide synthase levels in a rat hepatic ischemia/reperfusion model. Nutrire 2025;50:25.

39. Khoshnazar SM, Mohagheghi M, Rahimi S, et al. Geraniol modulates inflammatory and antioxidant pathways to mitigate intestinal ischemia–reperfusion injury in male rats. Naunyn Schmiedebergs Arch Pharmacol 2025;398:8713-27.
40. Khoshnazar SM, Delavarian F, Rahimi S, et al. The protective effect of geraniol against hepatic ischemia–reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis in rat model. Int J Inflamm 2025;2025:5571327.
41. Abu-Amara M, Yang SY, Tapuria N, et al. Liver ischemia/reperfusion injury: processes in inflammatory networks, a review. Liver Transplant 2010;16:1016-32.
42. Konishi T, Lentsch AB. Hepatic ischemia/reperfusion: mechanisms of tissue injury, repair, and regeneration. Gene Exp 2017;17:277.
43. Gao Y, Li ZT, Jin L, et al. Melatonin attenuates hepatic ischemia-reperfusion injury in rats by inhibiting NF-κB signaling pathway. Hepatobiliary Pancreat Dis Int 2021;20:551-60.
44. Badeli H, Shahrokhi N, Khoshnazar M, et al. Aqueous date fruit efficiency as preventing traumatic brain deterioration and improving pathological parameters after traumatic brain injury in male rats. Cell J 2016;18:416-24.
45. Younis NS, Elsewedy HS, Shehata TM, Mohamed ME. Geraniol averts methotrexate-induced acute kidney injury via Keap1/Nrf2/HO-1 and MAPK/NF-κB pathways. Curr Issues Mol Biol 2021;43:1741-55.
46. Hasan SK, Sultana S. Geraniol attenuates 2-acetylaminofluorene induced oxidative stress, inflammation and apoptosis in the liver of wistar rats. Toxicol Mech Methods 2015;25:559-73.
47. AlAsmari AF, Ali N, Alharbi M, et al. Geraniol ameliorates doxorubicin-mediated kidney injury through alteration of antioxidant status, inflammation, and apoptosis: potential roles of NF-κB and Nrf2/Ho-1. Nutrients 2022;14:1620.

Table 1. Suzuki's criteria of hepatic ischemia/reperfusion injury.

score	**Congestion (%)**	**Vacuolization (%)**	**Necrosis (%)**
0	None	None	None
1	Minimal (10)	Minimal (10)	Single cell necrosis
2	Mild (11~30)	Mild (11~30)	Mild (<30)
3	Moderate (31~60)	Moderate (31~60)	Moderate (<60)
4	Severe (>60)	Severe (>60)	Severe (>60)

Geraniol
(C10H18O)

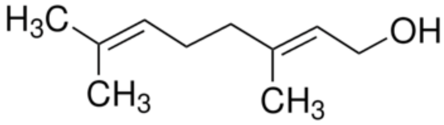


Figure 1. Geraniol (2E)-3,7-Dimethylocta- (2,6-dien-1-ol) chemical structure.

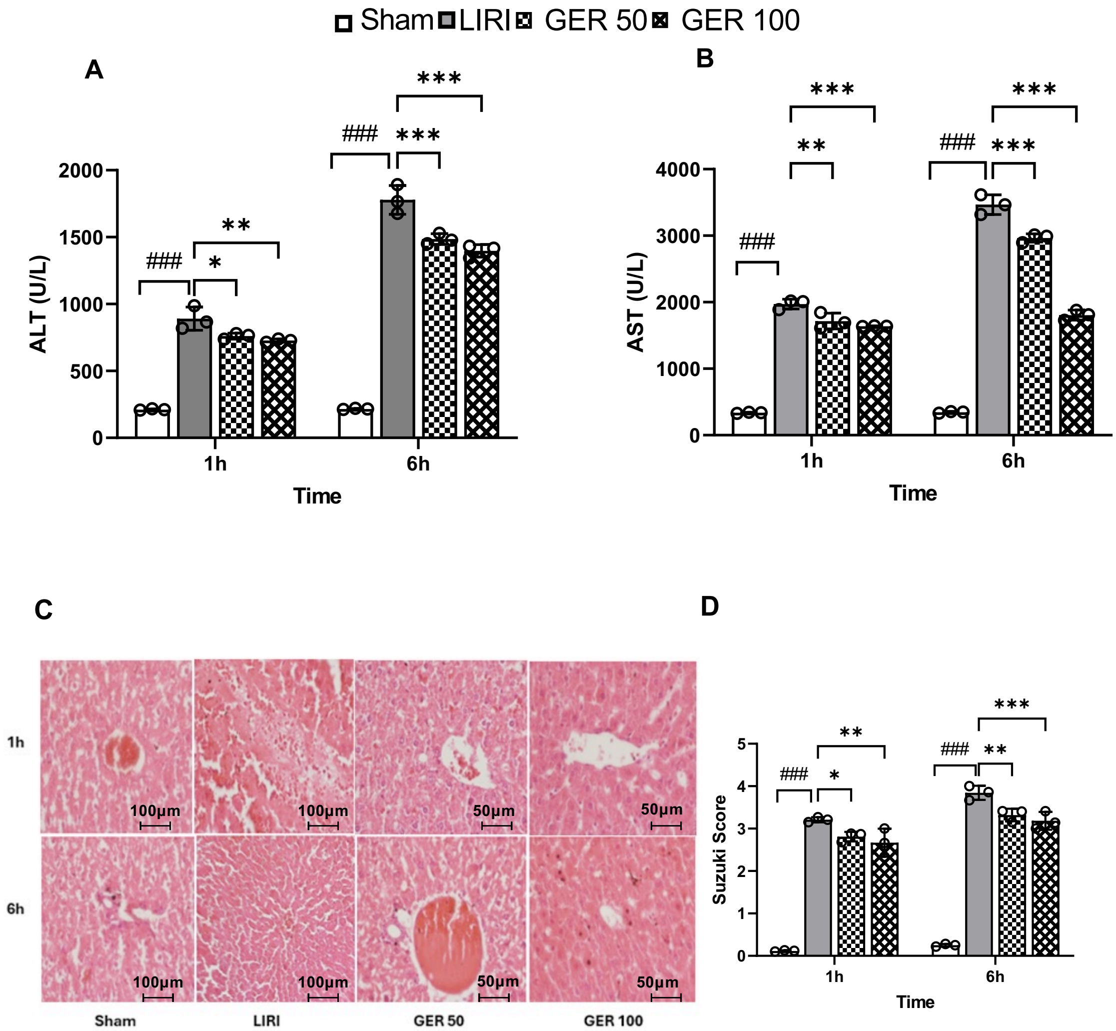


Figure 2. Effects of geraniol (GER) on ischemia-reperfusion-induced liver injury. A) Serum Alanine aminotransferase (ALT) levels, B) Aspartate aminotransferase (AST) levels, C) histopathological changes with hematoxylin, and eosin (H&E) staining (magnification ×200; bar = 100 µm, magnification ×400; bar = 50 µm), in ischemia-reperfusion-induced liver injury. Rats received injections of GER at a 50 and 100 mg/kg dose, 1 and 6 hrs following reperfusion. Rats were then euthanized after 6 hrs. D) Suzuki scores are presented in the sham group, Liver ischemia–reperfusion injury (LIRI) control group LIRI +GER (50 and 100 mg/kg) groups at 1h and 6 hrs after reperfusion. Data were presented as mean ± Standard error of the mean (SEM), n = 7/group. # $p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs sham group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs LIRI group.

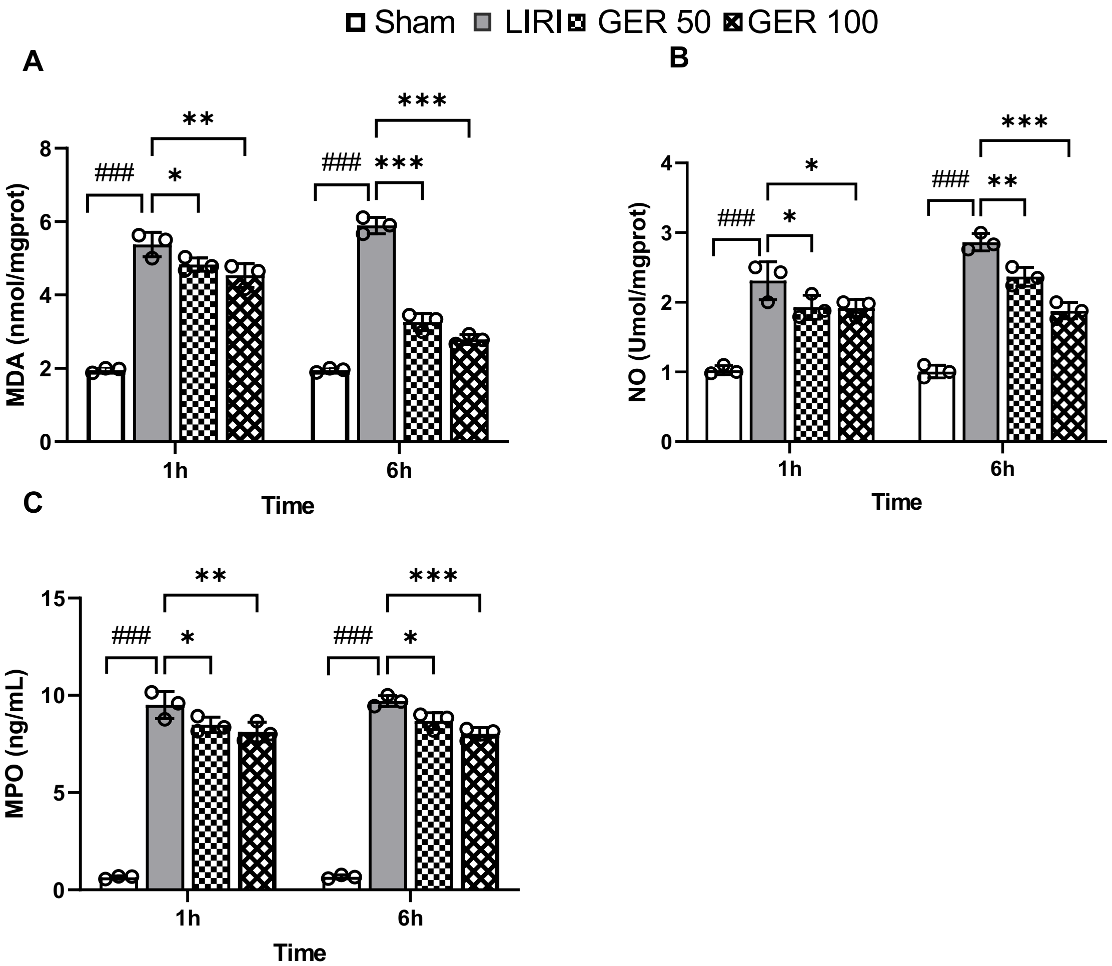
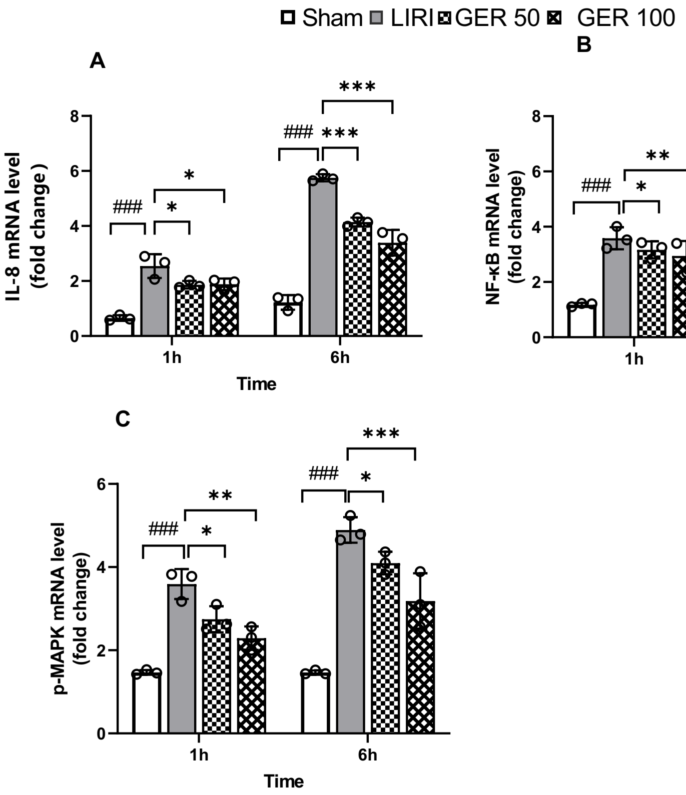
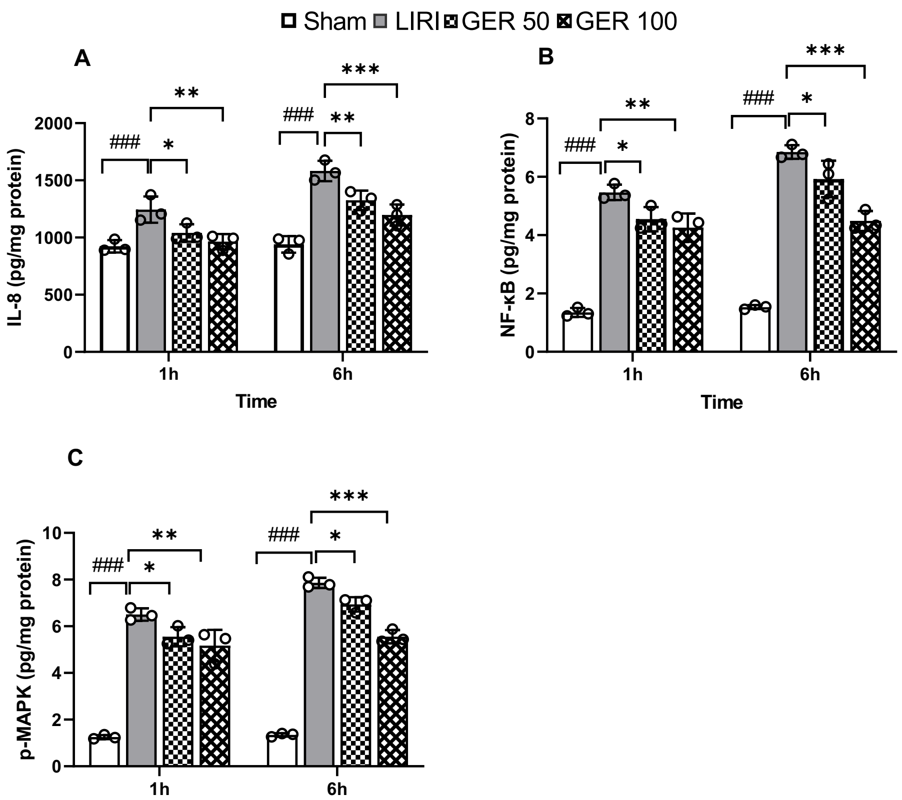


Figure 3. Effect of GER treatment on oxidative stress and neutrophil infiltration induced by ischemia-reperfusion. The tissue levels of Malondialdehyde (MDA), Nitric oxide (NO), and Myeloperoxidase (MPO) were analyzed. Data are presented as mean ± Standard error of the mean (SEM), n = 7/group. # $p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs sham group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs Liver ischemia–reperfusion injury (LIRI) group.


Sham LIRI GER 50 GER 100
A
IL-8 mRNA level (fold change)
Time
B
NF-κB mRNA level (fold change)
Time
C
p-MAPK mRNA level (fold change)
Time

a


Sham LIRI GER 50 GER 100
A
IL-8 (pg/mg protein)
Time
B
NF-κB (pg/mg protein)
Time
C
p-MAPK (pg/mg protein)
Time

b

Figure 4. Effects of GER on the pro-inflammatory mediators. Panel a: aA) The Interleukin-8 (IL-8) mRNA expression, aB) Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and aC) Phosphorylated mitogen-activated protein kinase (p-MAPK) in rat liver were determined by Reverse transcription polymerase chain reaction (RT-PCR). Panel b: bA) The protein expression of Interleukin-8 (IL-8), bB) Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and bC) Phosphorylated mitogen-activated protein kinase (p-MAPK) in rat liver was determined by Enzyme-linked immunosorbent assay (ELISA). Data were presented as mean ± Standard error of the mean (SEM), $n = 7$/group. # $p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs sham group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs Liver ischemia–reperfusion injury (LIRI) group.

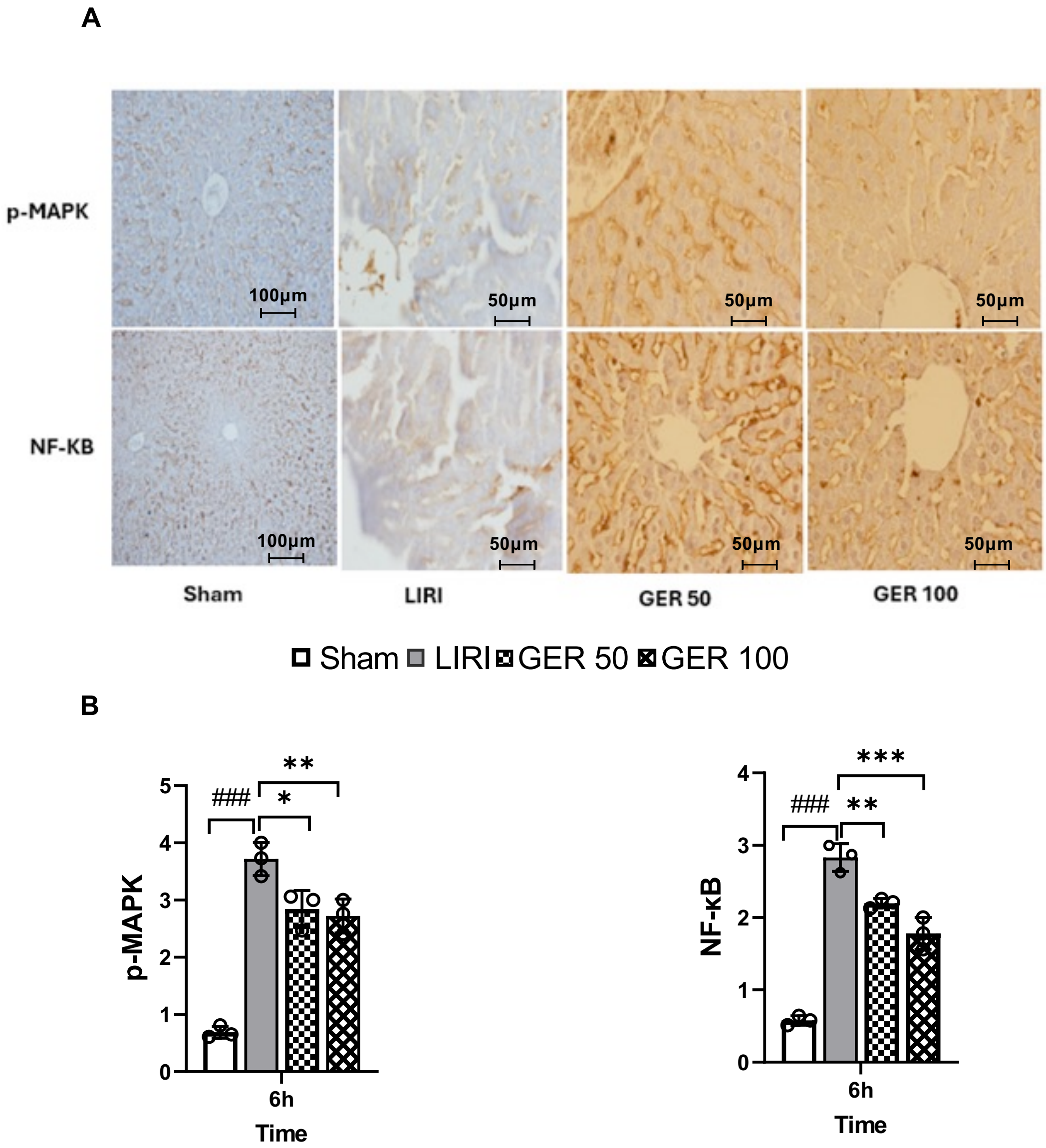


Figure 5. Immunohistochemistry was used to detect the expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Phosphorylated mitogen-activated protein kinase (p-MAPK) in liver tissue after 6h reperfusion. A) Immunohistochemical staining of NF-κB and p-MAPK (magnification: ×200, scale bar =100 µm; ×400, scale bar =50 µm). B) Relative positive expression analysis of p-MAPK and NF-κB. Stained cells were scored as semiquantitative: no staining (score: 0), mild staining (score: 1), moderate staining (score: 2), and severe staining (score: 3). Data were presented as mean ± Standard error of the mean (SEM), n = 7/group. # $p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs sham group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs Liver ischemia–reperfusion injury (LIRI) group.

Contributions Seyedeh Mahdieh Khoshnazar, conceptualization, methodology, software, formal analysis, investigation, writing-original draft preparation, writing-review and editing, project administration; Saeedeh Khajoei Nejad, software, formal analysis; Shahriyar Dabiri, methodology; Alireza Amirbeigi, revising, writing-review and editing; Nader Shahrokhi, investigation, editing; Mohammad Khaksari Hadad, investigation, editing; Sara Shafieipour, conceptualization, methodology, writing-review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

Competing interests: the authors declare no competing interests.

Ethics approval: the ethics committee for animal care at Kerman University of Medical Sciences, under the identification number IR KMU.AH.REC.2023.129, approval date: 22 February 2024, approved the animal protocols. The study is reported in accordance with Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines (https://arriveguidelines.org).

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

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