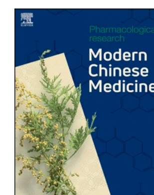
Pharmacological Research - Modern Chinese Medicine 12 (2024) 100470

Contents lists available at ScienceDirect

Pharmacological Research - Modern Chinese Medicine

journal homepage: www.elsevier.com/locate/prmcm



6-Gingerol anti-inflammatory and antioxidant properties protect against heart and liver dysfunction in rats with sepsis

Helia Keivanpour [a,b,1], Reihaneh Zamzam [a,b,1], Mojtaba Mojtahedzadeh [c], Mohammad-Reza Delnavazi [b], Amin Sharifan [d,*], Omid Sabzevari [a,e,*]

[a] Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[c] Department of Clinical Pharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[d] Department of Pharmaceutical Care, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran
[e] Toxicology and Poisoning Research Centre, Tehran University of Medical Sciences, Tehran, Iran

A R T I C L E I N F O

Keywords:
Sepsis
Gingerol
Anti-inflammatory agents
Antioxidants
Liver
Heart

A B S T R A C T

Objective: To investigate the effect of 6-gingerol, active component of *Zingiber officinale (Shēngjiāng),* on sepsis-induced inflammation, oxidative injury, and organ damage in vivo.
Methods: 6-gingerol was extracted from ginger, and its identity was confirmed with ^{1}H NMR, ^{13}C NMR, and TLC. Sepsis was induced via cecal ligation and puncture (CLP) in all groups except for sham. Thirty male Wistar rats were randomly divided into sham, CLP alone, 6-gingerol, ginger extract, hydrocortisone, and solvent. Treatments were administered intraperitoneally with a dose of 25 mg/kg. Biomarkers for inflammation, oxidative injury, and organ damage were assessed. Furthermore, heart and liver organs were stained with hematoxylin-eosin to determine the extent of sepsis-induced organ damage.
Results: 6-gingerol with 98.9 % purity was extracted. Compared with CLP-alone, biomarker analyses indicated that 6-gingerol significantly attenuated sepsis-induced inflammation by decreasing the levels of tumor necrosis factor-α, interleukin-6, and nuclear factor-κB. Furthermore, this bioactive constituent markedly reduced oxidative stress during sepsis by replenishing the levels of glutathione, increasing catalase, and decreasing malondialdehyde and superoxide. Moreover, organ damage assays showed that 6-gingerol substantially reduced the levels of cardiac troponin I, alanine aminotransaminase, aspartate aminotransferase, and lactate dehydrogenase, showing protective effects against cardiac and hepatic dysfunction. Similarly, rats treated with 6-gingerol had normal hepatic lobules, fewer Kupffer cells in minor regions of the liver, and lower necrosis and apoptosis in the myocardium.
Discussion: 6-gingerol protects both heart and liver organs through its anti-inflammatory and antioxidant effects in septic rats.

Introduction

Sepsis (classified with code 1G40 in WHO International Classification of Diseases version 11) is a life-threatening medical condition due to the dysregulated inflammatory response to an infection with some degree of organ dysfunction, which contributes to 48.9 million incident cases and 11 million deaths worldwide in 2017 [1,2]. Sepsis is associated with inflammatory and oxidative stress, and these pathological pathways may lead to various degrees of organ dysfunction. After immune system stimulation in sepsis, early cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)−1β initiate a cytokine cascade, which results in the production of IL-6, that in turn has a modulatory effect on TNF-α and IL-1β. Nuclear transcriptional factor (NF)-κB plays a critical role in activating and regulating this cascade. NF-κB activates in response to elevation of TNF-α and IL-1β, which induces the transcription of several cytokines, namely TNF-α, IL-1β, IL-6, and IL-8, leading to excessive inflammatory response [3]. In addition, sepsis may induce oxidative stress. In the process of lipid peroxidation,

* Corresponding authors.
E-mail addresses: aminsharifan@gmail.com (A. Sharifan), omid@tums.ac.ir (O. Sabzevari).
[1] Helia Keivanpour and Reihaneh Zamzam contributed equally to this study and are shared first authors.

https://doi.org/10.1016/j.prmcm.2024.100470
Received 27 November 2023; Received in revised form 15 June 2024; Accepted 18 June 2024
Available online 19 June 2024
2667-1425/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

reactive oxygen metabolites are generated, which may lead to sepsis-induced tissue damage and multiple organ dysfunction. Malondialdehyde (MDA), a major end product of lipid peroxidation, is an indicator of oxidative stress, and studies suggest that MDA is significantly elevated in hepatic tissue in response to sepsis, as well as in the heart. [4, 5]. On the contrary, glutathione (GSH), the main endogenous thiol antioxidant and an anti-inflammatory agent, has a protecting role against oxidative damage caused by sepsis, and evidence show that this condition also decreases GSH concentration in the heart [6,7]. Superoxide dismutase (SOD) is an antioxidant enzyme that catalyzes the dismutation of the oxygen radical species to hydrogen peroxide and oxygen, and catalase (CAT) rapidly cleaves hydrogen peroxide to water and oxygen, which plays a critical role in protecting cells against oxidative damage. SOD/CAT ratio is increased in lethal sepsis, which in turn results in the accumulation of hydrogen peroxide in cells and oxidative damage [8]. Sepsis can induce myocardial dysfunction and changes in circulating volume and vessel tone. The serum level of cardiac troponin I (cTnI) has the highest specificity and sensitivity to diagnose myocardial injury, and the elevation of this biomarker in septic patients is correlated with higher rates of mortality [9,10]. Furthermore, hepatic dysfunction may be another manifestation of sepsis. This organ failure may be manifested as elevation of certain liver enzymes, namely alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [11]. Moreover, lactate dehydrogenase (LDH) serum levels are elevated in response to sepsis, making it a helpful biomarker in assessing the degree of tissue damage and cell injury [12].

Zingiber officinale Roscoe, a member of the Zingiberaceae family, is a well-known plant commonly referred to as ginger. It has been suggested to possess a range of psychophysiological and clinical properties. The use of ginger as a medicinal herb and spice dates back to prehistoric times, and by the second century, it was traded from China to Rome via the Silk Road. The consumption of ginger is believed to confer protection against chronic diseases in adults, and its popularity is expected to rise in the future [13]. Evidence suggest that ginger may reduce nausea, diarrhea, subjective experience of pain, and, when added to non-steroidal anti-inflammatory medicines, it may help with migraine headaches [14–16]. Ginger, showcasing its anti-inflammatory and antioxidant properties that position ginger as a valuable component in addressing a spectrum of health issues such as nausea, arthritis, and menstrual discomfort. In addition, ginger has shown antioxidant and anti-inflammatory effects, which are attributed to its active phenolic compounds, including gingerol, paradol, and shogaol. 6-Gingerol is a major constituent of ginger, and it encompasses the capacity to inhibit the production of inflammatory cytokines, as well as reduce the burden of oxidative damage [17]. Additionally, 6-gingerol has shown to improve the quality of life and effectively preventing chemotherapy-induced nausea and vomiting in cancer patients who received adjuvant chemotherapy [18].

Considering the strong historic evidence behind the medical use of ginger and the association of organ dysfunction induced by sepsis with inflammation and oxidative stress, we aimed to investigate the 6-gingerol effects on sepsis-induced cardiac and hepatic injury in a male rat model.

Material and methods

All experiments in this study were performed in line with the guidelines outlined in the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals [19]. In addition, this study is reported in accordance with the Animal Research: Reporting In Vivo Experiments guidelines (ARRIVE) 2.0 [20], and its experimental procedures were approved by the Animal Ethics Committee of Tehran University of Medical Sciences (Codes: IR.TUMS.MEDICINE.REC.1400.038 and IR.TUMS.MEDICINE.REC.1400.039).

Animals

Thirty 8-week-old healthy adult male albino Wistar rats (*Rattus norvegicus*) weighing 250 to 300 g that did not go under any previous procedures were received from the animal house of the Faculty of Pharmacy, Tehran University of Medical Sciences. The animals were housed in a temperature-controlled environment (25 ± 3 °C) on a 12 h light-12 h dark cycle and were provided with a standard diet and had free access to water and food. The acclimatization period for the subjects was 7 days before the initiation of the procedure. In addition, rats were kept in the same environmental condition until the end of the experiment.

In vivo sepsis model

Sepsis was induced in rats through cecal ligation and puncture (CLP) following the protocol introduced by Wichterman et al. [21] with minor modifications. The animals were anesthetized using intraperitoneal administration of ketamine 10 % (80 mg/kg, Alfasan, Germany) and xylazine 2 % (10 mg/kg, Alfasan, Germany), followed by shaving and disinfecting the abdomen of the rats. Cecal exposure was performed by a 2-cm midline laparotomy on the anterior abdomen. The cecum was tightly ligated below the ileocecal valve with a 3.0 silk suture (Supasil, Iran) and perforated twice with a 20-gauge needle (Shenzhou, China). Then cecum was gently squeezed to extrude feces from the perforation sites and then was returned to the abdominal cavity. The peritoneum and skin were sutured, respectively. All groups received 1 mL of injectable saline solution (0.9% W/V) subcutaneously into the neck to ensure adequate fluid therapy after surgery. The sham group was subjected to surgery without cecal ligation and puncture procedures. All animals were sacrificed 48 h after induction of CLP, and whole blood was collected by cardiac puncture. Heart and liver tissues were excised and washed with cold phosphate-buffered saline (PBS, 0.1 M) to remove blood residues. A part of each tissue was fixed in formalin buffer 10 % for histopathological examination.

Study design

Rats were randomly divided into six groups (5 rats per group) using a random number generator, four of which received medications. The treated groups comprised CLP + 6-gingerol, CLP + ginger extract, CLP + hydrocortisone, and CLP + solvent, which received 6-gingerol, hydroalcoholic ginger extract, hydrocortisone (Afa Chemi Company, Iran), and solvent (2% V/V Tween 80, Merck; 20% V/V ethanol 96 %, Merck; and 78% V/V distilled water for injection) by intraperitoneal injection, respectively. The solvent was used to dissolve 6-gingerol and ginger extract. All agents of interest were administered at a dose of 25 mg/kg, 2 h prior to surgery, and then again at 12 h and 24 h after surgery [22]. This approach allowed the agents to act both as a preventive and a curative treatment. The sham and CLP alone groups received no treatment and solvent, and while the sham group went under surgery, sepsis was not induced. Rats with spontaneous vestibular syndrome were excluded from the study. Age, gender, weight range, and environmental conditions were the same across all treatment groups. Those responsible for surgical procedures, administrating the treatments, extracting serum and tissue samples, and analyzing histopathology slides were blinded during the experiment.

Extraction and isolation of 6-gingerol

6-Gingerol was extracted from the rhizomes of *Zingiber officinale* Rosc. that were purchased from a local market in Tehran, Iran. Fresh rhizomes (10 kg) were grated and macerated in methanol (3 × 10 L) at room temperature. The obtained extract was concentrated and fractionated successively with *n*-hexane and chloroform. The chloroform fraction (2.1 g) was moved on a silica gel (mesh 230–400) column and

eluted using a gradient mixture of *n*-hexane: ethyl acetate (9:1 to 7:3) to get 6-gingerol. The identity of 6-gingerol was confirmed by proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectral analysis (500 MHz, Varian Inova, Darmstadt, Germany), as well as thin-layer chromatography (TLC) on silica gel 60 F254 size 20 × 20 cm (Merck, Darmstadt, Germany), and the results were compared with data published in the literature [23,24]. The purified 6-gingerol was stored at −20 °C until analysis.

Measurement of serum TNF-α level

The TNF-α levels were measured via an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Quantikine, Minneapolis, MN, USA) as per the manufacturer's instructions. The kit's microplate contained monoclonal antibodies against TNF-α pre-coated in each well. Firstly, 100 μL of serum sample was added to the wells, and the microplate was allowed to incubate for 2 h at room temperature. After that, the wells were emptied, and each well was washed four times with 400 μL of wash buffer. Then, 100 μL of antibody conjugated with streptavidin-HRP B was added to the wells, and the microplate was incubated for an additional 2 h at room temperature. The wash procedure was then repeated four times. Afterward, 100 μL of substrate solution was added to each well, and the microplate was incubated for 30 min at room temperature, while being protected from the light. Finally, an ELISA plate reader (BioTek, Winooski, VT, USA) was used to measure the optical density of the wells at 450 nm. The serum level of TNF-α was expressed as pg/mL.

Serum IL-6 assay

The concentration of IL-6 in rat serum was determined through the use of IL-6 ELISA kits (R&D Systems Quantikine, Minneapolis, MN, USA) in accordance with the manufacturer's instructions. In brief, 50 μL of diluting solution was added to a 96-well microplate followed by the addition of 50 μL of serum sample to each well. The plate was then incubated for 2 h at room temperature. The wells were emptied and washed four times with 400 μL of a wash buffer. After that, 100 μL of enzyme-linked polyclonal antibody specific for rat's IL-6 was added to the wells, and the plate was incubated for 2 h at room temperature. The washing phase was then repeated. Following this, 100 μL of substrate solution was added to the wells, and the microplate was incubated for 30 min at room temperature. Finally, the optical density was measured at 450 nm using the ELISA plate reader. The serum level of IL-6 was expressed as pg/mL.

NF-κB concentrations assessment

The levels of NF-κB in the liver were determined using NF-κB ELISA kits (Cusabio Technology LLC in Houston, TX, USA) as per the manufacturer's instructions. The procedure involved adding 100 μL of liver sample and standard to the wells of a microplate, followed by incubation at 37 °C for 2 h. Next, the wells were emptied, a biotin-conjugated antibody specific for NF-κB was added to each well, and the microplate was incubated at 37 °C for 1 h. After emptying and washing them with 200 μL of wash buffer three times, the wells were treated with a biotinylated monoclonal antibody specific for NF-κB and avidin-HRP, and the microplate was incubated at 37 °C for 1 h. The wells were washed again, and then 90 μL of 3,3′,5,5′ tetramethylbenzidine (TMB)-substrate was added, followed by incubation at 37 °C for 30 min. Finally, 50 μL of stop solution was added to the microplate, and the optical density was measured at 450 nm using the ELISA plate reader. The tissue NF-κB was expressed as pg/mL.

Determination of GSH content

Cardiac GSH level was determined using Ellman's method [25] with minor modifications. Briefly, 4 mg DTNB [5,5′-dithiobis-(2-nitrobenzoic acid), Sigma-Aldrich] was dissolved in 10 mL sodium citrate 10% V/V. After centrifugation, 100 μL of the supernatant, i.e., the heart's homogenized tissue, was transferred to a 96-well microplate. Next, 200 μL of DTNB reagent was added to the wells. After that, the absorbance of each well was measured at 412 nm using the ELISA plate reader. The tissue GSH was expressed as nmol/mg.

Lipid peroxidation assay

The levels of MDA, one of the most valuable markers of oxidative stress, in liver tissue were measured using a commercial assay kit (Navand Salamat Inc., Urmia, West Azerbaijan, Iran) according to the method by Buege and Aust [26] with minor modifications. Briefly, 200 μL of the sample, i.e., supernatant of the homogenized liver tissue, was mixed with 800 μL of TBA-HCl-TCA reagent (0.375% W/V thiobarbituric acid, 0.25 N hydrochloric acid, and 15% W/V trichloroacetic acid). The mixture was then put into the water bath (95 °C) for 45 min. After that, the mixture was cooled down for 10 min in water and ice. Next, the mixture was centrifuged at 3000 rpm for 15 min. Subsequently, the supernatant was transferred to a dark 96-well microplate, and the optical density was measured at 550 nm with the ELISA reader. The tissue MDA was expressed as nmol/mg/mL.

SOD activity assessment

This experiment was done using a commercial assay kit (Navand Salamat Inc., Urmia, West Azerbaijan, Iran) according to the pyrogallol autoxidation method described by Marklund SL [27]. Briefly, 50 μL of the sample, i.e., supernatant of the homogenized liver tissue after being centrifuged at 12000 rpm for 5 min, was mixed with 200 μL of Tris–HCl buffer 50 mM, pH 8.2, in a 96-well microplate. Then, 50 μL of pyrogallol was added to this mixture. 50 μL deionized water with the same amount of buffer and pyrogallol was used as a control. The mixtures were then incubated for 5 min at room temperature. The optical density was then measured at 405 nm using the ELISA plate reader. The tissue SOD was expressed as U/mg/mL with the following formula:

$$Activity\ (U/mg\ protein) = \left(\frac{OD\ test}{OD\ control}\right) * 200$$

CAT activity assessment

This procedure was conducted using a commercial assay kit (Navand Salamat Inc., Urmia, West Azerbaijan, Iran) according to the methods previously described [28,29] with some modifications. Firstly, 100 μL of assay buffer (100 mM potassium phosphate, pH 7.0) with 30 μL of methanol and 20 μL of the sample (homogenized liver) were added to a 96-well microplate. Following this, 20 μL of diluted hydrogen peroxide 0.882 M was added to the wells to initiate the reaction. The plate was then incubated at room temperature for 20 min on a shaker. After incubation, 30 μL of potassium hydroxide 10 M was added to the wells to terminate the reaction. Subsequently, 30 μL of purpald (4-amino-3--hydrazino-5-mercapto-1,2,4-triazole), a chromogen, was added to each well, and the microplate was incubated for 10 min at 18 °C. Finally, 10 μL of potassium periodate was added to the wells, and the optical density was measured at 550 nm with the ELISA plate reader. The tissue MDA was expressed as nmol/mg/min.

Serum cTnI concentration assessment

Serum cTnI levels were measured by an assay kit (Cusabio Technology LLC, Houston, USA) in accordance with the manufacturer's instructions. To perform the assay, 100 μL of the samples were added to the wells of a 96-well microplate and incubated for 2 h at 37 °C. Following this, 100 μL of biotinylated monoclonal antibodies specific to

cTnI were added to the wells. The microplate was then incubated for 1 h at 37 °C. Afterward, the wells were washed three times with 200 μL of a wash buffer, which was included in the kit. Then, 100 μL of avidin conjugated Horseradish Peroxidase (HRP) was added to the sample wells and the plate was again incubated for 1 h at 37 °C. Wash buffer was applied three times to the wells before the plate was incubated for 30 min at 37 °C with 90 μL of substrate solution provided by the kit. Finally, 50 μL of the stop solution was added to the wells, and the optical density of the wells was measured at 450 nm with the ELISA plate reader. The concentration of CTnI in serum samples was expressed in pg/mL.

LDH activity quantification

LDH assays were performed by a commercial ELISA kit (Pars Azmoon, Karaj, Alborz, Iran) in accordance with the method proposed by the German Society for Clinical Chemistry and Laboratory Medicine [30]. To perform the LDH assay, 240 μL of reagent 1 consisting of phosphate buffer and pyruvate were added to the wells along with 3 μL of serum sample. After 25 s, 60 μL of reagent 2 which contained Good's buffer and nicotinamide adenine dinucleotide hydrogen (NADH) were added to the wells. The plate was then incubated for another 25 s, following which the difference between absorption during 100 s was measured at 340 nm using the ELISA reader. Finally, the level of LDH in the serum was expressed as U/L using the given formula:

$$Activity\ (U/L) = (\Delta A/\min) * 11496$$

Serum AST and ALT assessment

Serum levels of ALT and AST, biomarkers of liver function, to assess sepsis-induced liver damage, were measured using a commercial assay kit (Pars Azmoon Inc., Karaj, Iran) in accordance with the IFCC methods [31,32] without pyridoxal-5-phosphate and some minor modifications. To determine the ALT levels, 200 μL reagent 1 (tris[hydroxymethyl] aminomethane, l-alanine, and LDH) and 50 μL reagent 2 (2-oxoglutarate and NADH) were added to the wells with 25 μL liver sample, 25 s apart. After incubating for 50 s, the change in absorbance was measured in 50 s at 340 nm using the ELISA plate reader. The same procedure was taken to measure the AST levels except that reagent 1 (tris(hydroxymethyl) aminomethane, l-aspartate, LDH, and malate dehydrogenase) was slightly different from the preceding method. The activity of ALT and AST was determined using the following formula:

$$Activity\ (U/L) = (\Delta A/\min) * 1746$$

Histopathological examination

Samples were taken from the hearts and livers of rats and preserved with 10% V/V buffered formaldehyde. Afterward, the samples were embedded in paraffin wax and sectioned at 5 μM thickness on slides. These slides were then stained with hematoxylin-eosin and examined using a light microscope. The procedure includes deparaffinization and rehydration of slides to distilled water. Then Staining slides in Mayers Hematoxylin and washing until blue stops coming off slides. Decolorization with acid alcohol and washig again. Counterstain in Alcoholic-Eosin. Dehydrate through 3 changes of 95 % EtOH and 2 changes of 100 % EtOH.

Statistical analysis

Once the data was normalized, a one-way analysis of variance (ANOVA) was conducted to compare the groups, followed by Tukey's post hoc test. The statistical significance level was set at p-values below 0.05. The statistical analysis was performed using GraphPad Prism 9 for Windows (GraphPad Software, Inc., San Diego, CA, USA). All data from randomized rats were included in the analysis. The data is presented in the form of mean ± standard error of the mean (SEM) and comparisons are made between different groups, with a focus on treatment groups and the positive control values (i.e., CLP).

Results

Ginger methanol extract chemical composition

The identity of 6-gingerol was confirmed with ^{1}H NMR, ^{13}C NMR, and TLC (Fig. 1). The following were obtained after nuclear magnetic resonance spectroscopy: ^{1}H NMR (500 MHz, CDCl3): δ_H 6.82 (1H, d, $J =$ 8.0 Hz, H-5′), 6.68 (1H, d, $J = 1.6$ Hz, H-2′), 6.53 (1H, dd, $J = 8.0$, 1.6 Hz, H-6′), 5.58 (OH-5), 4.02 (1H, m, H-5), 3.86 (3H, s, OCH3), 2.84 (2H, t, J $= 7.5$, H-1), 2.73 (2H, t, $J = 7.5$, H-2), 2.57 (2H, dd, $J = 17.5$, 2.1 Hz, H-4a), 2.49 (2H, dd, $J = 17.5$, 9.0 Hz, H-4b), 1.2–1.5 (8H, overlapped signals, H-6 to H-9), 0.88 (3H, t, $J = 6.86$, H-10); ^{13}C NMR (125 MHz, CDCl3): δ_C 14.01 (C-10), 22.57 (C-9), 25.11 (C-8), 29.27 (C-7), 31.71 (C-6), 36.42 (C-1), 45.42 (C-2), 49.34 (C-4), 55.90 (OCH3), 67.70 (C-5), 111.01 (C-2′), 114.42 (C-5′), 120.71 (C-6′), 132.62 (C-1′), 143.96 (C-4′), 146.44 (C-3′), 212.43 (C-3). The purity of 6-gingerol extracted from ginger rhizomes was determined to be 98.9 %.

6-Gingerol shows anti-inflammatory effects by reducing TNF-α, IL-6, and Nf-κb

Compared with CLP alone, rats in the 6-gingerol group had significantly lower levels of TNF-α (29.44 ± 0.79 vs 10.71 ± 1.01, $p < 0.0001$, respectively). Similarly, treatment with hydrocortisone and ginger extract resulted in a statistically significant reduction in TNF-α concentration in comparison with the CLP group (23.48 ± 1.56, $p < 0.05$; and 16.06 ± 1.10, $p < 0.0001$, respectively). Furthermore, 6-gingerol was more effective in reducing TNF-α levels than hydrocortisone ($p <$ 0.0001), but there was no statistically significant difference between this group and ginger extract (Fig. 2A). Moreover, serum levels of IL-6 were significantly reduced in the 6-gingerol-treated group compared with the CLP group (35.27 ± 2.65 vs 61.72 ± 3.07, $p < 0.0001$, respectively). In addition, hydrocortisone and ginger extract significantly reduced IL-6 compared with the CLP group (72.27 ± 1.98, $p < 0.01$ and 95.05 ± 3.06, $p < 0.0001$, respectively). 6-Gingerol could further reduce the level of IL-6 compared with hydrocortisone and ginger extract ($p <$ 0.0001 and $p < 0.01$, respectively) (Fig. 2B). Furthermore, the 6-gingerol group had significantly lower NF-κB values compared with the CLP group (2.19 ± 0.21 vs 6.34 ± 0.61, $p < 0.0001$, respectively). Hydrocortisone and ginger extract also caused a significant reduction in NF-κB levels compared with the CLP group (4.41 ± 0.57, $p < 0.05$ and 2.80 ± 0.12, $p < 0.0001$, respectively). A statistically significant difference was observed between 6-gingerol and hydrocortisone ($p < 0.01$); however, no difference was seen between 6-gingerol and the ginger extract treatment groups (Fig. 2C). Additionally, there were no significant differences between the 6-gingerol group and the sham group regarding the levels of anti-inflammatory markers mentioned above; however, all other intervention groups exhibited statistically significant differences compared with the sham groups (7.79 ± 0.69, 32.27 ± 2.92, and 1.79 ± 0.16 for TNF-α, IL-6, and NF-κB, respectively).

6-Gingerol exhibits antioxidant potency via elevating GSH and CAT, and reducing MDA, and SOD levels

Both 6-Gingerol and the ginger extract markedly ($p < 0.0001$) increased GSH content (85.30 ± 3.57 and 66.93 ± 3.79, respectively) compared with the CLP group (22.15 ± 2.14); however, ginger extract was not as effective as 6-gingerol. On the other hand, hydrocortisone did not alter GSH depletion caused by sepsis (21.25 ± 1.93) (Fig. 3A). Additionally, the lipid peroxidation assay showed that MDA content in rats that received 6-gingerol was similar to the sham group (4.08 ± 0.25 vs 1.20 ± 0.19, respectively) and was significantly ($p < 0.0001$) reduced

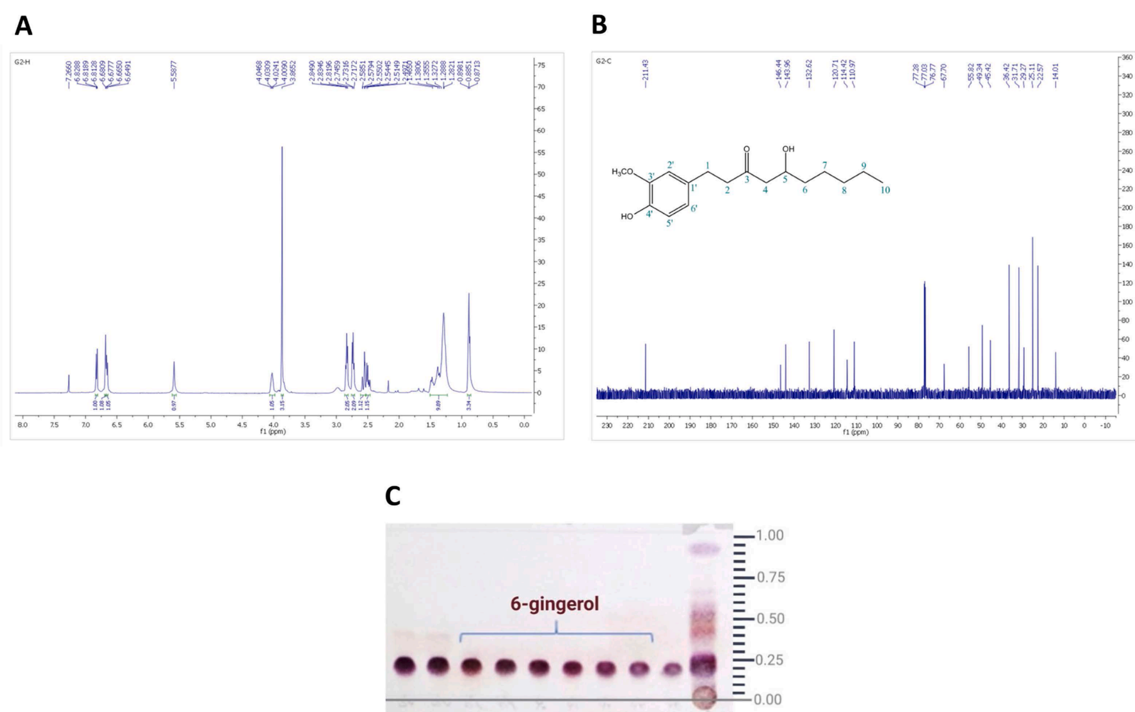


Fig. 1. The chemical identity of 6-gingerol, extracted from *Zingiber officinale* Roscoe (Zingiberaceae), was determined using a combination of analytical techniques. Proton nuclear magnetic resonance spectral analysis (A) and carbon-13 nuclear magnetic resonance (B) for 6-gingerol, the structure of which was determined afterward. Additionally, thin-layer chromatography revealed that 6-gingerol has an Rf value of approximately 0.24 (C).

compared with the CLP group (18.91 ± 1.01). Ginger extract was less efficient in decreasing MDA levels compared with 6-gingerol (7.38 ± 0.24 vs 4.08 ± 0.25, respectively) and hydrocortisone groups (7.38 ± 0.24 vs 3.58 ± 0.31, respectively) (Fig. 3B). Furthermore, rats that received 6-gingerol had the most significantly reduced levels of SOD among other intervention groups compared with the CLP group (43.88 ± 4.35 vs 102.8 ± 5.49, $p < 0.0001$, respectively). Additionally, hydrocortisone and ginger extract resulted in a significant ($p < 0.001$) reduction of SOD activity (52.60 ± 1.98, $p < 0.001$; 61.00 ± 3.74, respectively) in comparison with the CLP group (Fig. 3C). Although there was no significant difference between the sham and CLP groups in the levels of CAT (16.02 ± 0.94 vs 18.35 ± 1.36, respectively), the intervention groups, including 6-gingerol, ginger extract, and hydrocortisone, had significantly increased levels of CAT compared with the CLP group (58.85 ± 0.83, $p < 0.0001$; 34.88 ± 0.85, $p < 0.01$; and 34.41 ± 1.45, $p < 0.01$, respectively). In addition, the 6-gingerol group had markedly increased levels of CAT compared with ginger extract and hydrocortisone (both $p < 0.01$) (Fig. 3D).

6-Gingerol diminishes sepsis-associated tissue damage by reducing the levels of cTnI, LDH, AST, and ALT

Compared with the CLP group, 6-gingerol treatment significantly prevented increased levels of cTnI as a result of sepsis (592.6 ± 22.72; 300.8 ± 16.76, $p < 0.05$, respectively), and no statistically significant differences were seen between the sham, 6-gingerol-treated, and ginger extract-treated groups (269.0 ± 9.79; 300.8 ± 16.76; 384.4 ± 16.34, respectively). Nevertheless, hydrocortisone administration resulted in a non-significant reduction in cTnI levels compared with the CLP group (481.5 ± 9.63; 592.6 ± 22.72, respectively) (Fig. 4A). Furthermore, lipid peroxidation assays indicated that 6-gingerol significantly reduced LDH serum levels compared with the CLP group (1553 ± 18.48 vs 5218 ± 240.8, $p < 0.0001$, respectively), and ginger extract and hydrocortisone also significantly decreased LDH levels (1575 ± 67.6; 1770 ± 54.4, both $p < 0.0001$, respectively) compared with the CLP group, and there was no statistically significant difference between the treatment groups and the sham group (1255 ± 51.96) (Fig. 4B). Moreover, 6-gingerol, ginger extract, and hydrocortisone significantly reduced the levels of ALT and AST in comparison with the CLP group (12.50 ± 1.06; 18.15 ± 0.60; 7.30 ± 0.45; 29.25 ± 1.06, all $p < 0.0001$, respectively for ALT; and 10.50 ± 1.63; 18.70 ± 0.54; 4.57 ± 0.63; 33.30 ± 0.99, all $p < 0.0001$, respectively for AST). Nevertheless, 6-gingerol was superior to ginger extract in decreasing ALT and AST levels (both $p < 0.05$), but the hydrocortisone-treated group had the most reduction compared with other interventions (Fig. 4C and 4D).

Histopathology

Heart

The histological analysis of the myocardial tissue revealed that the sham group had a normal arrangement of myofibrils (Fig. 5A). In contrast, the CLP and solvent groups exhibited necrotic areas, poor morphology, and a high number of inflammatory and apoptotic cells, which were abnormally aligned (Fig. 5B and 5C, respectively). However, samples treated with 6-gingerol showed a significantly lower severity of necrosis in the myocardium than the CLP group (Fig. 5D). Although the heart of the extract group had apoptotic and necrotic cells, it was less abundant compared to the CLP group. Additionally, the hearts of rats treated with 6-gingerol had a more regular arrangement compared to the extract group (Fig. 5E). On the other hand, the hydrocortisone group exhibited no significant difference and was similar to the CLP group (Fig. 5F).

Liver

The liver parenchyma of the sham group showed normal sinusoids and hepatocytes, as observed in Fig. 6A. However, both the CLP and the solvent groups exhibited sinusoidal dilation, Kupffer cell aggregation, and deformation in hepatocytes' morphology, including ballooning

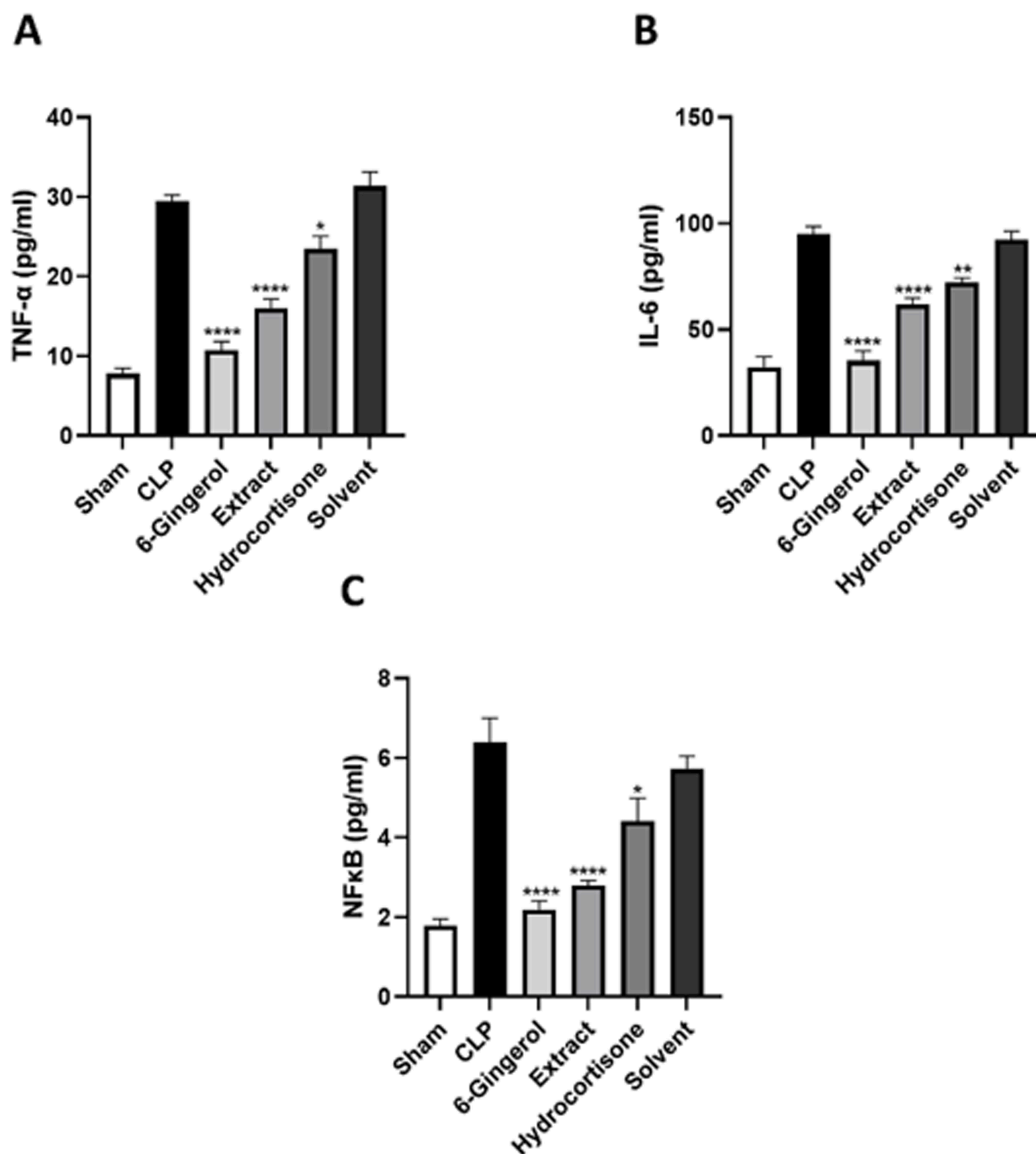


Fig. 2. The levels of inflammatory markers tumor necrosis factor (TNF)-α (A), interleukin (IL)−6 (B), and transcriptional factor (NF)-κB (C) in septic Wistar rats are displayed. Sepsis was induced by cecal ligation and puncture (CLP), and subjects were randomly assigned ($N = 5$ per group) to receive treatment. From left to right, the bars represent results for sham (negative control), CLP alone, CLP + 6-gingerol (CLP rats treated with 6-gingerol), CLP + ginger extract (CLP rats treated with ginger extract), CLP + hydrocortisone (CLP rats treated with hydrocortisone), CLP + solvent (CLP rats treated with solvent) in 48 h after sepsis induction by CLP. The data are presented as mean ± SEM. The statistically significant difference from the CLP group is reported as *, **, and **** indicating $p < 0.05$, $p < 0.01$, and $p < 0.0001$, respectively.

(Fig. 6B and 6C, respectively). On the other hand, the hepatic lobules of the 6-gingerol group appeared normal, with few Kupffer cells observed in minor regions of the liver (Fig. 6D). Furthermore, sinusoids were dilated in a few hepatic lobules, and several Kupffer cells were observed in the ginger extract-treated group. In addition, a number of hepatocytes exhibited abnormal morphology (Fig. 6E). Moreover, the hydrocortisone group showed a similar histopathology exhibition, except that liver damage was more pronounced (Fig. 6F).

Discussion

It has been reported that ginger extract may have a median lethal dose of 2000 mg/kg, with a suggested no observed adverse effect level of 1000 mg/kg/day in rats [33]. While the toxicity level of 6-gingerol has not been studied in rats, an investigation has shown that 50 μM and 100 μM doses of this bioactive compound can have toxic effects on sea urchin (*Paracentrotus lividus*) [34]. However, a clinical trial found that a daily dose of 2 g of ginger extract, which contains a C_{max} of 0.85 μg/mL for 6-gingerol, was well-tolerated by participants [35]. It is worth noting that no mortalities were observed in rats that received 6-gingerol.

During the course of this study, rats were treated with intraperitoneal injections. Since patients with sepsis often face dysphagia either during their hospitalization or after hospital discharge [36,37], oral administration of pharmacologically active agents may be challenging.

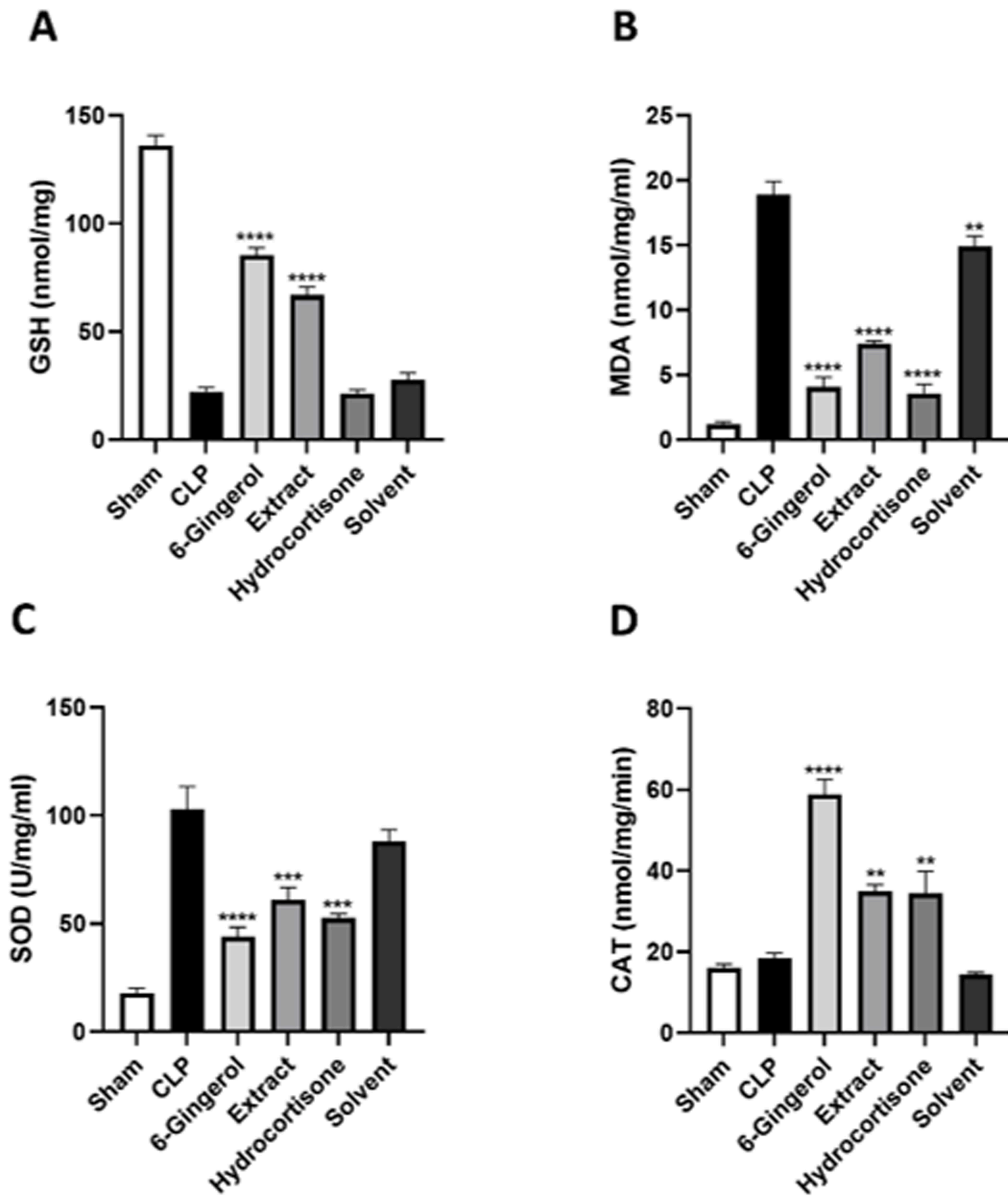


Fig. 3. The levels of oxidative stress markers glutathione (GSH) (A), malondialdehyde (MDA) (B), superoxide dismutase (SOD) (C), and catalase (CAT) (D) in Wistar rats are shown. Sepsis was induced by cecal ligation and puncture (CLP), and subjects were randomly assigned ($N = 5$ per group) to receive treatment. From left to right, the bars represent results for sham (negative control), CLP alone, CLP + 6-gingerol (CLP rats treated with 6-gingerol), CLP + ginger extract (CLP rats treated with ginger extract), CLP + hydrocortisone (CLP rats treated with hydrocortisone), CLP + solvent (CLP rats treated with solvent) in 48 h after sepsis induction by CLP. The data are presented as mean ± SEM. The statistically significant difference from the CLP group is reported as *, **, and **** indicating $p < 0.05$, $p < 0.01$, and $p < 0.0001$, respectively, compared with the CLP group.

Moreover, hydrocortisone is typically administered intravenously to septic patients due to the unpredictable absorption of steroids [38]. To maintain consistency between the groups, we chose the intraperitoneal route of administration for hydrocortisone, as well as other agents, resulting in a stronger resemblance to the clinical setting. Overall, this methodology allowed for a more robust study with greater clinical relevance.

Our results showcase that 6-gingerol, with a dose of 25 mg/kg, has the capacity to reduce inflammation, decrease oxidative stress injury, attenuate organ damage, and act as a cardioprotective and hepatoprotective bioactive compound in a rat model of sepsis.

Several inflammatory biomarkers have established roles in the pathogenesis of sepsis [3]. TNF-α is the prime mediator of the inflammatory response seen in sepsis and septic shock, and its elevation correlates with mortality in septic patients. IL-6, a cytokine with a wide range of biological activities, helps to control the induction of acute-phase response and is a sensitive indicator of inflammation. NF-κB plays a central role in regulating the transcription of several genes,

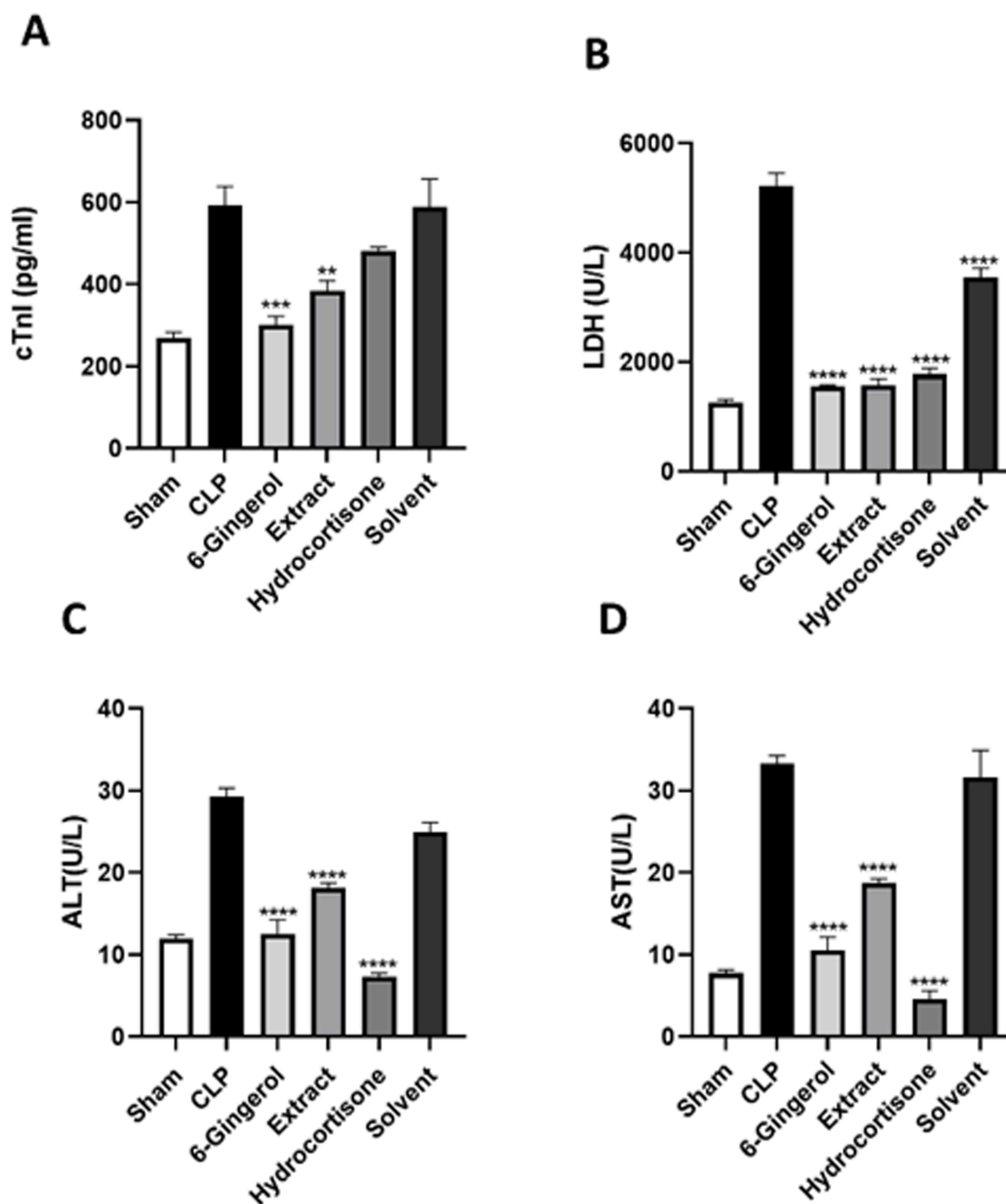


Fig. 4. The levels of organ damage markers cardiac troponin I (cTnI) (A), lactate dehydrogenase (LDH) (B), alanine aminotransferase (ALT) (C), and aspartate aminotransferase (AST) (D) in Wistar rats are demonstrated. Sepsis was induced by cecal ligation and puncture (CLP), and subjects were randomly assigned ($N = 5$ per group) to receive treatment. From left to right, the bars represent results for sham (negative control), CLP alone, CLP + 6-gingerol (CLP rats treated with 6-gingerol), CLP + ginger extract (CLP rats treated with ginger extract), CLP + hydrocortisone (CLP rats treated with hydrocortisone), CLP + solvent (CLP rats treated with solvent) in 48 h after sepsis induction by CLP. The data are presented as mean ± SEM. The statistically significant difference from the CLP group is reported as *, **, and **** indicating $p < 0.05$, $p < 0.01$, and $p < 0.0001$, respectively, compared with the CLP group.

including the encoding of pro-inflammatory cytokines involved with severe sepsis and septic shock. It has been suggested that 6-gingerol, as well as ginger extract, inhibit the production of pro-inflammatory cytokines mainly produced by macrophages through inhibition of the NF-κB pathway [39]. Our findings indicate that sepsis-induced inflammation is ameliorated by 6-gingerol, which is in line with other investigations [22,40,41]. Furthermore, our data indicate that 6-gingerol is superior to hydrocortisone and ginger extract in reducing TNF-α, IL-6, and NF-κB levels.

Oxidative stress is another mechanism that is presumed to be responsible for organ dysfunction in sepsis. As one of the major first-line antioxidants, GSH levels in the host with sepsis are depleted, and consequently, the synthesis rate of this antioxidant molecule is increased in the heart, liver, and other organs [42,43]. Moreover, serum levels of MDA, a marker for lipid peroxidation, are suggested to be elevated in patients with sepsis, making it an important biomarker in oxidative stress and sepsis pathophysiology [44]. Furthermore, the imbalance of SOD and CAT in sepsis has been attributed to oxidative stress [45]. In

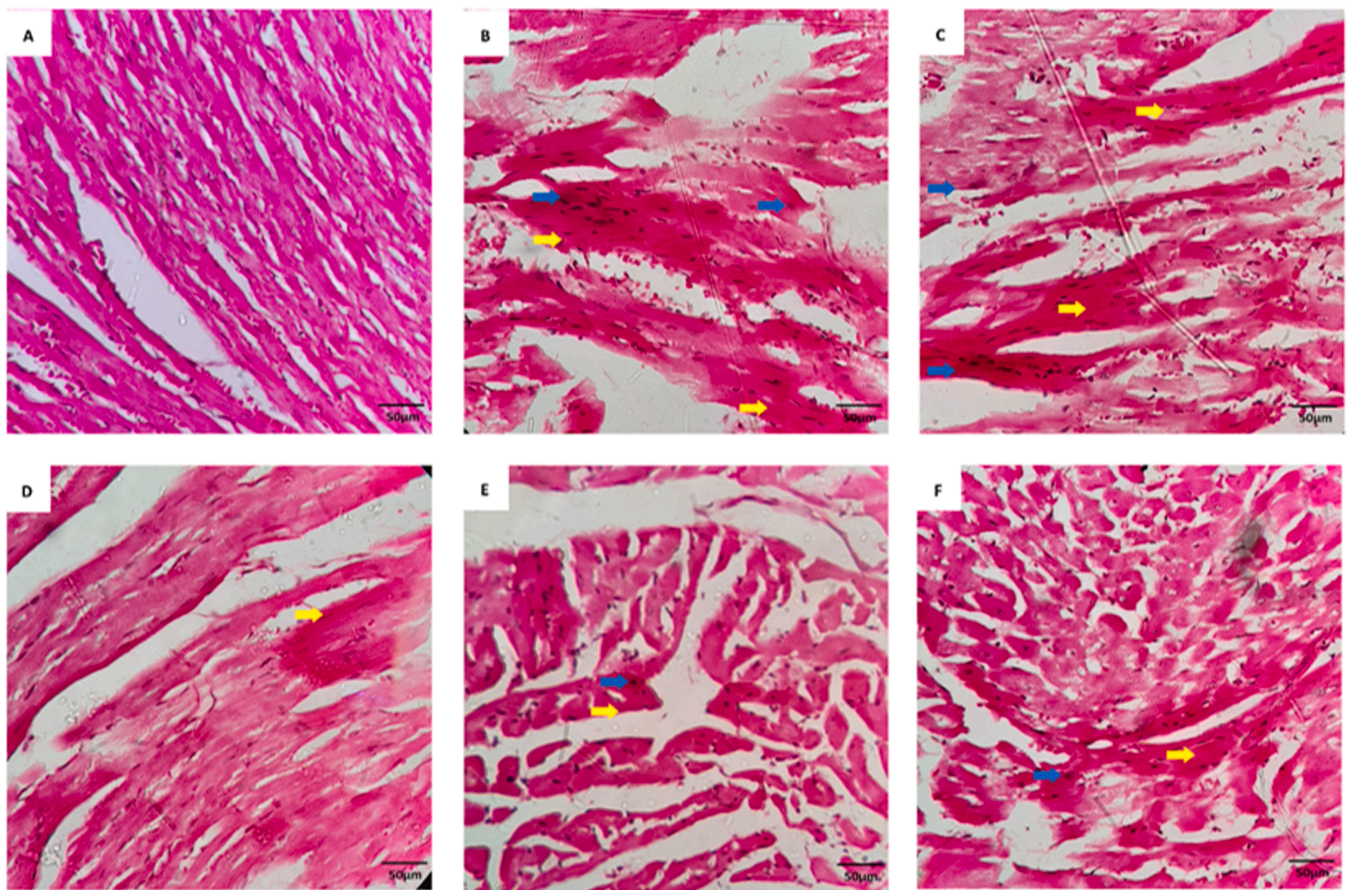


Fig. 5. The images presented here showcase the histopathology of the heart organ in five groups of Wistar rats with cecal ligation and puncture (CLP)-induced sepsis, and the sham group. In the sham group, the myocardial cells and myofibrils arrangement appear normal (A). However, in the CLP group, necrotic areas (indicated by yellow arrows) and apoptotic cells (indicated by blue arrows) are observed (B). The solvent group also shows necrosis and apoptosis, which is not significantly different from the CLP group (C). In the group treated with 6-gingerol, myocardial cells exhibit minor necrosis (D). The group treated with ginger extract shows the presence of necrotic and apoptotic cells, but not as much as the CLP group (E). Finally, in the group treated with hydrocortisone, myocardial cells exhibit necrosis and apoptosis similar to the CLP group (F). The myocardial tissue slides were stained with hematoxylin-eosin, and pictures were taken with x40 magnification. The bar size was indicated under each picture for reference.

this study, rats were sacrificed during the acute phase of sepsis, and those that were treated with 6-gingerol had increased levels of GSH compared with other groups, implying that this agent has promising antioxidant capacity *in vivo*. Of note, 6-gingerol performed better than ginger extract in this experiment. Furthermore, our findings show that 6-gingerol can prevent lipid peroxidation by decreasing MDA levels in the liver of rats with sepsis, and it performed better than ginger extract in this assay. Additionally, our results suggest that elevated levels of SOD, which may be markedly increased in severe sepsis [46], can be attenuated by the administration of 6-gingerol, and, on the other hand, this biochemical constitute has the capacity to increase CAT levels. These results are in accordance with other studies that showed ginger can attenuate oxidative stress in the lung and kidney tissues of rat models of sepsis [22,47]. Although SOD acts as the first line of defense against oxidative stress, the higher levels of this enzyme are associated with increased mortality in sepsis, which questions the role of SOD in this condition [48–51]. In our study, rats that had undergone CLP and did not receive any interventions had the highest levels of SOD compared with those that received treatments. Furthermore, an interesting finding of our study was that the sham group, rats that did not go under CLP, and the CLP group had similar levels of CAT. This may be due to the fact that the active site of CAT may be oxidized and inactivated by either excessive amounts of superoxide caused by sepsis or increased levels of hydrogen peroxide in response to SOD activity. Following that, oxidative stress as a result of SOD/CAT imbalance occurs [45]. Our results show that the administration of 6-gingerol effectively corrected this imbalance, further decreasing the oxidative stress burden on rats with sepsis. Overall, 6-gingerol displayed promising antioxidant efficacy in various assays conducted in this study, and it performed better than ginger extract.

The release of inflammatory markers and oxidants is responsible for organ dysfunction in sepsis, and cTnI, ALT, AST, and LDH are biomarkers that are elevated in this medical condition, each of which represents tissue damage [11,52–54]. Our findings show that 6-gingerol treatment can alleviate myocardial injury and reduce the levels of cTnI in vivo, which may be related to the anti-inflammatory and antioxidant activity of this bioactive compound [55]. Additionally, the results of this study demonstrate that ALT and AST levels were significantly reduced in rats treated with 6-gingerol, indicating the hepatoprotective effect of this pharmacologically active component of ginger against sepsis. This outcome may be related to the Nrf2 pathway, which also has a major regulatory role in sepsis-induced inflammation and oxidative stress [40] (reviwer2.response4). Furthermore, rats that received 6-gingerol had significantly lower levels of LDH, indicating less damage to and destruction of cells. This was also seen in other studies that had investigated the role of gingerol and its analog in bacterial infection and sepsis [22,56]. On the other hand, according to our histopathological analyses, 6-gingerol treatment demonstrated a significant protective effect against sepsis-induced heart damage. In addition, it is noteworthy that 6-gingerol not only prevented hepatotoxicity caused by the solvent

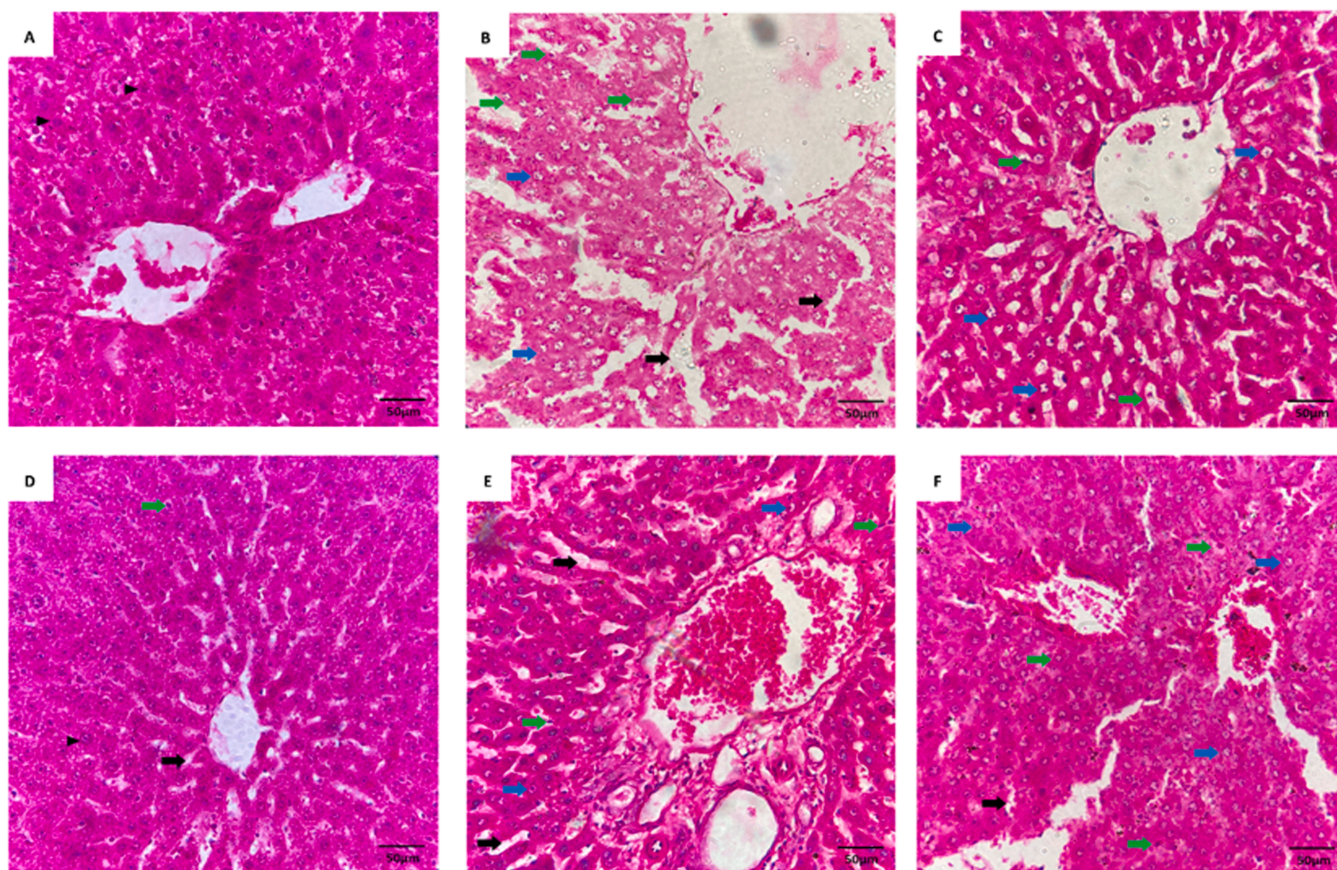


Fig. 6. These images depict the histopathology of the liver organ from five groups of Wistar rats with sepsis induced through cecal ligation and puncture (CLP), and a sham group. The sham group appears to have normal sinusoids and hepatocytes with regular morphology (A). On the other hand, the sepsis (CLP) group shows signs of sinusoidal duct dilation (black arrow), hepatocyte ballooning (blue arrow), and stimulated Kupffer cells (green arrow) (B). The sepsis group that received solvent exhibits hepatocytes and Kupffer cells similar to the CLP group (C). Meanwhile, the sepsis group that was treated with 6-gingerol shows normal hepatic lobules and minor Kupffer cells (D). The sepsis group treated with ginger extract displays dilated sinusoids, a few abnormal hepatocytes, and several Kupffer cells (E). Finally, in the sepsis group treated with hydrocortisone, hepatic tissue damage is not significantly different from the sepsis group (F). The hepatic parenchyma slides were stained with hematoxylin-eosin, and pictures were taken with x40 magnification, and the bar size was indicated under the pictures.

(ethanol), but it also protected the liver against damage caused by CLP-induced sepsis. In short, due to its anti-inflammatory and antioxidant effect, 6-gingerol successfully prevented cardiac and hepatic dysfunction, and this bioactive constituent performed better than ginger extract in these assays.

Our study yields highly promising results, clearly indicating that the isolated fraction of ginger, 6-gingerol, offers significant and robust cardioprotective and hepatoprotective effects against CLP-induced sepsis in rats. This bioactive compound works by effectively compensating the damage induced by inflammation and oxidative stress, thereby providing a strong foundation for further research and exploration. These findings raise genuine hopes for 6-gingerol as a potential clinical therapeutic intervention for patients with sepsis in clinical trial settings.

Limitations

This study has some limitations. Dose-response relationship and toxicity study is important and valuable, but we had to prioritize our objectives and make sure compromises in order to successfully complete the project. Therefore, we used the 6-gingerol dose from an investigation that employed the same route of administration. Limitations in the available budget prevented us from fully exploring the specific molecular pathways and mechanisms; additional research with greater financial support would be valuable. However, there are investigations that have conducted for these purposes which guide us to achieve meaningful result.

CRediT authorship contribution statement

Helia Keivanpour: Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Reihaneh Zamzam:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Mojtaba Mojtahedzadeh:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Mohammad-Reza Delnavazi:** Supervision, Software, Resources, Methodology. **Amin Sharifan:** Writing – review & editing, Writing – original draft, Validation, Software. **Omid Sabzevari:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

Funding

This study was supported by two grants (project nos. 51344 and 51345) from Vice Chancellor for research, Tehran University of Medical Sciences, Tehran, Iran.

References

[1] M. Singer, C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, et al., The third international consensus definitions for sepsis and septic shock (sepsis-3), JAMA 315 (2016) 801–810, https://doi.org/10.1001/jama.2016.0287.

[2] K.E. Rudd, S.C. Johnson, K.M. Agesa, K.A. Shackelford, D. Tsoi, D.R. Kievlan, et al., Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study, Lancet 395 (2020) 200–211, https://doi.org/10.1016/S0140-6736(19)32989-7.

[3] R.S. Hotchkiss, L.L. Moldawer, S.M. Opal, K. Reinhart, I.R. Turnbull, J.L. Vincent, Sepsis and septic shock, Nat. Rev. Dis. Prim. 2 (2016) 16045, https://doi.org/10.1038/nrdp.2016.45.

[4] G.M. Koksal, C. Sayilgan, S. Aydin, H. Oz, H. Uzun, Correlation of plasma and tissue oxidative stresses in intra-abdominal sepsis, J. Surg. Res. 122 (2004) 180–183, https://doi.org/10.1016/j.jss.2004.07.246.

[5] L. Lorente, M.M. Martín, P. Abreu-González, A. Domínguez-Rodríguez, L. Labarta, C. Díaz, et al., Prognostic value of malondialdehyde serum levels in severe sepsis: a multicenter study, PLoS One 8 (2013) e53741, https://doi.org/10.1371/journal.pone.0053741.

[6] P. Villa, A. Saccani, A. Sica, P. Ghezzi, Glutathione protects mice from lethal sepsis by limiting inflammation and potentiating host defense, J. Infect. Dis. 185 (2002) 1115–1120, https://doi.org/10.1086/340042.

[7] M. Tan, Y. Yin, X. Ma, J. Zhang, W. Pan, M. Tan, et al., Glutathione system enhancement for cardiac protection: pharmacological options against oxidative stress and ferroptosis, Cell Death. Dis. 14 (2023) 131, https://doi.org/10.1038/s41419-023-05645-y.

[8] M. Andrades, C. Ritter, J.C.F. Moreira, F. Dal-Pizzol, Oxidative parameters differences during non-lethal and lethal sepsis development, J. Surg. Res. 125 (2005) 68–72, https://doi.org/10.1016/j.jss.2004.11.008.

[9] J.F. Frencken, D.W. Donker, C. Spitoni, M.E. Koster-Brouwer, I.W. Soliman, D.S. Y. Ong, et al., Myocardial injury in patients with sepsis and its association with long-term outcome, Circ. Cardiovasc. Qual. Outcomes 11 (2018) e004040, https://doi.org/10.1161/CIRCOUTCOMES.117.004040.

[10] M.A. Garcia, J.M. Rucci, K.K. Thai, Y. Lu, P. Kipnis, A.S. Go, et al., Association between troponin i levels during sepsis and postsepsis cardiovascular complications, Am. J. Respir. Crit. Care Med. 204 (2021) 557–565, https://doi.org/10.1164/rccm.202103-0613OC.

[11] P. Strnad, F. Tacke, A. Koch, C. Trautwein, Liver — Guardian, modifier and target of sepsis, Nat. Rev. Gastroenterol. Hepatol. 14 (2017) 55–66, https://doi.org/10.1038/nrgastro.2016.168.

[12] J.G. Zein, G.L. Lee, M. Tawk, M. Dabaja, G.T. Kinasewitz, Prognostic significance of elevated serum lactate dehydrogenase (LDH) in patients with severe sepsis, Chest 126 (2004) 873S, https://doi.org/10.1378/chest.126.4_MeetingAbstracts.873S.

[13] C Spence, Ginger: the pungent spice, Int. J. Gastron Food Sci. 33 (2023) 100793, https://doi.org/10.1016/j.ijgfs.2023.100793.

[14] E. Viljoen, J. Visser, N. Koen, A. Musekiwa, A systematic review and meta-analysis of the effect and safety of ginger in the treatment of pregnancy-associated nausea and vomiting, Nutr. J. 13 (2014) 20, https://doi.org/10.1186/1475-2891-13-20.

[15] R. Terry, P. Posadzki, L.K. Watson, E. Ernst, The use of ginger (zingiber officinale) for the treatment of pain: a systematic review of clinical trials, Pain Med. 12 (2011) 1808–1818, https://doi.org/10.1111/j.1526-4637.2011.01261.x.

[16] L.B. Martins, S. Rodrigues AM dos, D.F. Rodrigues, L.C. dos Santos, A.L. Teixeira, A. V.M. Ferreira, Double-blind placebo-controlled randomized clinical trial of ginger (Zingiber officinale Rosc.) addition in migraine acute treatment, Cephalalgia 39 (2018) 68–76, https://doi.org/10.1177/0333102418776016.

[17] M. Zhang, R. Zhao, D. Wang, L. Wang, Q. Zhang, S. Wei, et al., Ginger (Zingiber officinale Rosc.) and its bioactive components are potential resources for health beneficial agents, Phyther Res. 35 (2021) 711–742, https://doi.org/10.1002/ptr.6858.

[18] J. Konmun, K. Danwilai, N. Ngamphaiboon, B. Sripanidkulchai, A. Sookprasert, S. Subongkot, A phase II randomized double-blind placebo-controlled study of 6-gingerol as an anti-emetic in solid tumor patients receiving moderately to highly emetogenic chemotherapy, Med. Oncol. 34 (2017) 69, https://doi.org/10.1007/s12032-017-0931-4.

[19] National Research Council, Guide For the Care and Use of Laboratory Animals, National Academies Press, Washington, D.C., 2011, https://doi.org/10.17226/12910.

[20] N. Percie du Sert, A. Ahluwalia, S. Alam, M.T. Avey, M. Baker, W.J. Browne, et al., Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0, PLoS Biol. 18 (2020) e3000411, doi:10.1371/journal.pbio.3000411.

[21] K.A. Wichterman, A.E. Baue, I.H. Chaudry, Sepsis and septic shock–a review of laboratory models and a proposal, J. Surg. Res. 29 (1980) 189–201, https://doi.org/10.1016/0022-4804(80)90037-2.

[22] P. Rodrigues FA de, C. Santos AD da, P.H.Q.S. de Medeiros, M.G. Prata M de, S. Santos TC de, J.A. da Silva, et al., Gingerol suppresses sepsis-induced acute kidney injury by modulating methylsulfonylmethane and dimethylamine production, Sci. Rep. 8 (2018) 12154, https://doi.org/10.1038/s41598-018-30522-6.

[23] Z. Gan, Z. Liang, X. Chen, X. Wen, Y. Wang, M. Li, et al., Separation and preparation of 6-gingerol from molecular distillation residue of Yunnan ginger rhizomes by high-speed counter-current chromatography and the antioxidant activity of ginger oils in vitro, J. Chromatogr. B 1011 (2016) 99–107, https://doi.org/10.1016/j.jchromb.2015.12.051.

[24] C.N.A. Salmon, Y.A. Bailey-Shaw, S. Hibbert, C. Green, A.M. Smith, L.A. D. Williams, Characterisation of cultivars of Jamaican ginger (Zingiber officinale Roscoe) by HPTLC and HPLC, Food Chem. 131 (2012) 1517–1522, https://doi.org/10.1016/j.foodchem.2011.09.115.

[25] G.L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys. 82 (1959) 70–77.

[26] J.A. Buege, S.D. Aust, [30]Microsomal lipid peroxidation, in: S Fleischer, LBT-M in E Packer (Eds.), Biomembr. - Part C Biol. Oxidations, vol. 52, Academic Press, 1978, pp. 302–310, https://doi.org/10.1016/S0076-6879(78)52032-6.

[27] S.L. Marklund, Pyrogallol autoxidation. Handb. Methods Oxyg. Radic. Res., CRC press, 2018, pp. 243–248.

[28] L.H. Johansson, L.A Håkan Borg, A spectrophotometric method for determination of catalase activity in small tissue samples, Anal. Biochem. 174 (1988) 331–336, https://doi.org/10.1016/0003-2697(88)90554-4.

[29] C.R. Wheeler, J.A. Salzman, N.M. Elsayed, S.T. Omaye, D.W. Korte, Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity, Anal. Biochem. 184 (1990) 193–199, https://doi.org/10.1016/0003-2697(90)90668-Y.

[30] G.S. Chemistry, for C. Recommendations of the German Society for Clinical Chemistry: standardisation of methods for the estimation of enzyme activities in biological fluids—Experimental basis for the optimized standard conditions, Z. Klin. Chem. Klin. Biochem. 10 (1972) 281–291.

[31] Bergmeyer H.U., Hørder M., Rej R. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (L-. J Clin Chem Clin Biochem Zeitschrift Fur Klin Chemie Und Klin Biochem 1986;24:481–95.

[32] Bergmeyer H.U., Hørder M., Rej R. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (. J Clin Chem Clin Biochem Zeitschrift Fur Klin Chemie Und Klin Biochem 1986;24:497–510.

[33] N. Promdam, P. Panichayupakaranant, [6]-Gingerol: a narrative review of its beneficial effect on human health, Food Chem Adv. 1 (2022) 100043, https://doi.org/10.1016/j.focha.2022.100043.

[34] M. Chiaramonte, R. Bonaventura, C. Costa, F. Zito, R. Russo, [6]-Gingerol dose-dependent toxicity, its role against lipopolysaccharide insult in sea urchin (Paracentrotus lividus Lamarck), and antimicrobial activity, Food Biosci. 39 (2021) 100833, https://doi.org/10.1016/j.fbio.2020.100833.

[35] S.M. Zick, Z. Djuric, M.T. Ruffin, A.J. Litzinger, D.P. Normolle, S. Alrawi, et al., Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects, Cancer Epidemiol. Biomarkers Prev. 17 (2008) 1930–1936, https://doi.org/10.1158/1055-9965.EPI-07-2934.

[36] J. Zielske, S. Bohne, F.M. Brunkhorst, H. Axer, O. Guntinas-Lichius, Acute and long-term dysphagia in critically ill patients with severe sepsis: results of a prospective controlled observational study, Eur. Arch Oto-Rhino-Laryngol. 271 (2014) 3085–3093, https://doi.org/10.1007/s00405-014-3148-6.

[37] T. Hongo, T. Yumoto, H. Naito, T. Fujiwara, J. Kondo, S. Nozaki, et al., Frequency, associated factors, and associated outcomes of dysphagia following sepsis, Aust. Crit. Care. 36 (2023) 521–527, https://doi.org/10.1016/j.aucc.2022.06.003.

[38] F. Lamontagne, B. Rochwerg, L. Lytvyn, G.H. Guyatt, M.H. Møller, D. Annane, et al., Corticosteroid therapy for sepsis: a clinical practice guideline, BMJ 362 (2018) k3284, https://doi.org/10.1136/bmj.k3284.

[39] S. Tripathi, K. Maier, D. Bruch, D. Kittur, Ginger and its active ingredient 6-gingerol down regulate pro-inflammatory cytokine release by macrophages, J. Surg. Res. 130 (2006) 318, https://doi.org/10.1016/j.jss.2005.11.456.

[40] M.K. Hong, L-L Hu, Y.X. Zhang, Y.L. Xu, X.Y. Liu, P.K. He, et al., 6-Gingerol ameliorates sepsis-induced liver injury through the Nrf2 pathway, Int. Immunopharmacol. 80 (2020) 106196, https://doi.org/10.1016/j.intimp.2020.106196.

[41] W. Hong, F.X. Zhi, T.H. Kun, F.J. Hua, L. Huan Ling, F. Fang, et al., 6-Gingerol attenuates ventilator-induced lung injury via anti-inflammation and antioxidative stress by modulating the PPARγ/NF-κBsignalling pathway in rats, Int. Immunopharmacol. 92 (2021) 107367, https://doi.org/10.1016/j.intimp.2021.107367.

[42] G. Biolo, R. Antonione, M. De Cicco, Glutathione metabolism in sepsis, Crit. Care Med. 35 (2007) S591–S595, https://doi.org/10.1097/01.CCM.0000278913.19123.13.

[43] O. Huet, C. Cherreau, C. Nicco, L. Dupic, M. Conti, D. Borderie, et al., Pivotal role of glutathione depletion in plasma-induced endothelial oxidative stress during sepsis, Crit. Care Med. 36 (2008) 2328–2334, https://doi.org/10.1097/CCM.0b013e3181800387.

[44] L. Lorente, M.M. Martín, P. Abreu-González, A. Domínguez-Rodriguez, L. Labarta, C. Díaz, et al., Sustained high serum malondialdehyde levels are associated with severity and mortality in septic patients, Crit. Care 17 (2013) R290, https://doi.org/10.1186/cc13155.

[45] C. Ritter, M. Andrades, M.L.C. Frota, F. Bonatto, R.A. Pinho, M. Polydoro, et al., Oxidative parameters and mortality in sepsis induced by cecal ligation and perforation, Intensive Care Med. 29 (2003) 1782–1789, https://doi.org/10.1007/s00134-003-1789-9.

[46] A. Warner, A. Bencosme, D. Healy, C. Verme, Prognostic role of antioxidant enzymes in sepsis: preliminary assessment, Clin. Chem. 41 (1995) 867–871.

[47] Q. Pan, P. Liu, M. Wan, 6-Gingerol attenuates sepsis-induced acute lung injury by suppressing NLRP3 inflammasome through Nrf2 activation, Folia. Histochem. Cytobiol. 61 (2023) 68–80, https://doi.org/10.5603/FHC.a2023.0002.

[48] B.W. Warner, P.O. Hasselgren, J.H. James, H. Bialkowska, D.F. Rigel, C. Ogle, et al., Superoxide dismutase in rats with sepsis: effect on survival rate and amino acid transport, Arch. Surg. 122 (1987) 1142–1146, https://doi.org/10.1001/archsurg.1987.01400220052010.

[49] M.O. Guerreiro, F. Petronilho, M. Andrades, L. Constantino, F.G. Mina, J.C. F. Moreira, et al., Plasma superoxide dismutase activity and mortality in patients with septic, J. Trauma Acute Care Surg. 69 (2010) E102–E106, https://doi.org/10.1097/TA.0b013e3181dbb289.

[50] J.A. Leff, C.E. Day, J.M. McCord, J.E. Repine, P.E. Parsons, F.A. Moore, et al., Serum antioxidants as predictors of adult respiratory distress syndrome in patients with sepsis, Lancet 341 (1993) 777–780, https://doi.org/10.1016/0140-6736(93)90558-X.

[51] M.É. Andrades, A. Morina, S. Spasić, I. Spasojević, Bench-to-bedside review: sepsis - from the redox point of view, Crit. Care 15 (2011) 230, https://doi.org/10.1186/cc10334.

[52] H.J. Forman, H. Zhang, Targeting oxidative stress in disease: promise and limitations of antioxidant therapy, Nat. Rev. Drug Discov. 20 (2021) 689–709, https://doi.org/10.1038/s41573-021-00233-1.

[53] A. Duman, A. Akoz, M. Kapci, M. Ture, S. Orun, K. Karaman, et al., Prognostic value of neglected biomarker in sepsis patients with the old and new criteria: predictive role of lactate dehydrogenase, Am. J. Emerg. Med. 34 (2016) 2167–2171, https://doi.org/10.1016/j.ajem.2016.06.012.

[54] K. Thygesen, J.S. Alpert, A.S. Jaffe, B.R. Chaitman, J.J. Bax, D.A. Morrow, et al., Fourth universal definition of myocardial infarction (2018), Circulation 138 (2018) e618–e651, https://doi.org/10.1161/CIR.0000000000000617.

[55] X. Han, P. Liu, M. Liu, Z. Wei, S. Fan, X. Wang, et al., [6]-Gingerol ameliorates ISO-induced myocardial fibrosis by reducing oxidative stress, inflammation, and apoptosis through inhibition of TLR4/MAPKs/NF-κB pathway, Mol. Nutr. Food Res. 64 (2020) 2000003, https://doi.org/10.1002/mnfr.202000003.

[56] H. So-Young, K. Han-Shin, J.M. Jee, L. Jeong-Hoon, B. Youngjoo, K. Gang-Jee, et al., Combined treatment of 6-gingerol analog and tobramycin for inhibiting pseudomonas aeruginosa, Infections. Microbiol Spectr. 9 (2021) e00192–e00221, https://doi.org/10.1128/Spectrum.00192-21.