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The ameliorative effects of ginger against heat-induced oxidative stress and mitochondrial dysfunction in *Caenorhabditis elegans*

Tingting Xu[a,b,c], Mingfang Tao[a,b,c], Rong Li[a,b,c], Shuxin Tang[a,b,c], Yuting Huang[a,b,c], Ting Wu[a,b,c], Senem Kamiloglu[d,e], Siyi Pan[a,b,c], Xiaoyun Xu[a,b,c,*]

[a] *Key Laboratory of Environment Correlative Dietology (Ministry of Education), Huazhong Agricultural University, Wuhan 430070, China*
[b] *Hubei Key Laboratory of Fruit & Vegetable Processing & Quality Control, Huazhong Agricultural University, Wuhan 430070, China*
[c] *College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China*
[d] *Department of Food Engineering, Faculty of Agriculture, Bursa Uludag University, Bursa 16059, Turkey*
[e] *Science and Technology Application and Research Center (BITUAM), Bursa Uludag University, Bursa 16059, Turkey*

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ABSTRACT

Ginger, rich in gingerols and shogaols, exhibits multiple biological properties. However, the mechanisms underlying its thermotolerance remain unclear. The study employed network pharmacology and experimental validation in Caenorhabditis elegans to investigate how gingerol-related compounds within ginger extract (GE) mitigated damage caused by heat stress (HS). A total of 18 types of gingerol analogues were identified in GE, among which 6-, 8-, and 10-gingerol, as well as 6-, 8-, and 10-shogaol were quantified. Collectively, these 6 compounds accounted for 54.4% of the total composition. Supplementation with 15 μg/mL GE significantly extended heat-stress lifespan by 20.30%, while the combination of the 6 major gingerols and shogaols at the same concentration prolonged lifespan by 18.93%. Additionally, pretreatment with GE and the combination alleviated HS-induced oxidative damage by eliminating reactive oxygen species (ROS) and upregulating antioxidant enzymes. Network pharmacology analysis suggested that the MAPK pathway may play a crucial role in thermotolerance. Experimental findings confirmed that ginger attenuated oxidative damage through the activation of SKN-1/Nrf2 and DAF-16/FOXO *via* the MAPK pathway. Moreover, GE stabilized mitochondrial membrane potential and restored ATP levels, thus preserving mitochondrial function during heat exposure. Further investigations using molecular docking and molecular dynamics simulations revealed that shogaols, with more stable binding affinities for Keap1 protein, exhibited more potent effects than gingerols in prolonging lifespan and reducing ROS levels under HS conditions. In short, gingerol analogues from ginger conferred thermal resistance to nematodes by mitigating oxidative damage and mitochondrial dysfunction.

1. Introduction

As global warming intensifies, ambient high temperatures become the most persistent threat, causing organisms suffering unprecedented thermal exposure. Heat stress (HS) primarily occurs when the thermoregulatory capacity of organisms is overwhelmed, resulting in various physiological abnormalities, such as cell and tissue damage and multi-organ dysfunction[1].

As a nonspecific stressor, heat stress has relatively complex potential pathogenic mechanisms[2]. Emerging evidence suggests that HSserves as an inducer of oxidative stress, as prolonged exposure in all organism sex acerbates the generation of intracellular reactive oxygen species (ROS)[3]. These highly reactive substances initiate free radical-mediated chain reactions, leading to phospholipid oxidation, protein degradation, and mitochondrial DNA fragmentation. Mitochondria, as the primary site of intracellular ROS production, are suspected to be the first organelles to be destroyedduring HS[4]. Dysfunctional mitochondria, in turn, aggravate ROS production

* Corresponding author at: College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China.
E-mail address: xuxiaoyun@mail.hzau.edu.cn (X.Y. Xu)
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and contribute to oxidative stress. Consequently, modulation of intracellular ROS levelsis critical forpreventing heat damage. More recently, functional antioxidant phytochemicals, such as various phenolic compounds, have been shown to ameliorate the deleterious effects of HS[5–6]. Therefore, nutritional intervention with phytochemicals may represent an effective strategy for alleviating the oxidative stress and mitochondria dysfunction induced by HS, thereby enhancing an organism's thermotolerance.

Ginger (*Zingiber officinale* Roscoe) is extensively utilized as a flavoring agent, food ingredient, and herbal remedy. Its usage spans a lengthy history as an effective and safe treatment for symptoms associated with asthma and hyperpyrexia[7]. Multiple studies have revealed that ginger exhibited significant antioxidant, anti-inflammatory, and regulatory effects on metabolic disorders, among other pharmacological actions[8-9]. The major bioactive constituents in ginger are collectively known as gingerols and their derivatives, such as shogaols and gingerdiols. It has been confirmed that gingerol analogues, as phenolic compounds, exert antioxidative bioactivities through both the direct scavenging of free radicals and the indirect activation of antioxidant response pathways[10]. Recently, these substances in ginger have been reported to enhance intestinal health by modulating mitochondrial function[11]. Moreover, ginger ethanol extract has been shown to mitigate severe heat-induced cell death in mouse fibroblast cells, but the underlying molecular mechanisms remain to be explored[12]. Our prior study investigating the anti-aging effects of ginger demonstrated that it could extend the HS lifespan of *Caenorhabditis elegans*[13]. Concurrently, previous researches on the anti-aging effects of 6-gingerol[14] and 6-shogaol[15] also indicated that these two compounds enhanced resistance to heat on lifespan in *C. elegans*. These studies support the hypothesis that gingerol analogues from ginger supplementation have potential benefits in mitigating heat damage. However, the mechanisms involved in thermotolerance mediated by ginger extract (GE) and its primary phenolic compounds have not yet been elucidated.

C. elegans has emerged as a prominent model organism for studying the molecular mechanisms underlying stress responses at both cellular and organismal levels due to its evolutionary conservation, the easy availability of mutant strains, and its sensitivity to minor temperature shifts[16]. Numerous conserved stress-responsive pathways in *C. elegans*, including the transforming growth factor-β pathway, the MAPK pathway, insulin/insulin-like growth factor signaling (IIS) pathway, and the mitophagy pathway, have been recognized across in various species[17]. Given the complexity of the mechanisms that respond to environmental stimuli, systematically exploring the underlying mechanisms of ginger in attenuating HS-induced damage was challenging. At present, network pharmacology has emerged as a comprehensive methodology for interpreting the complex mechanisms of pharmacodynamic components involved in disease prevention and treatment based on multiple targets and pathways[18]. There has been a notable increase in research focused on understanding the mechanisms of multi-target drugs, especially multi-component herbal medicines on complex diseases, through the application of network pharmacology[19-21]. Consequently, network pharmacology offers unique advantages in analyzing the potential pharmacological mechanisms of GE for preventing HS.

Therefore, this research investigated the mechanisms and targets of gingerol analogues from ginger in alleviating HS-induced damage, utilizing network pharmacology and experimental verification in *C. elegans*. This work may provide a theoretical basis for supporting ginger as a functional food with thermotolerance properties and health benefits.

2. Materials and methods

2.1 Chemicals and reagents

Dimethyl sulfoxide (DMSO), 5-fluoro-2-deoxyuridine (FUDR), and 2′,7′-dichloro-dihydrofluorescein diacetate (H_2DCF-DA) were purchased from Sigma Aldrich Chemical Co., (St. Louis, MO, USA). Standards (≥ 98%) of 6-, 8-, and 10-gingerol, 6-, 8-, and 10-shogaolwere products of YuanYe Bio-Technology Co., Ltd. (Shanghai, China). The glucose (Glu), bovine serum albumin (BSA), and Coomassie brilliant blue G250 were brought from YuanYe Bio-Technology Co., Ltd. Commercial kits for adenosine triphosphate (ATP) and mitochondrial membrane potential (MMP) assayswere obtained from Beyotime Biotechnology (Nanjing, China).

2.2 Extract preparation

Ginger, sourced from Jinan, was procured from a local market (Shandong, China). Fresh ginger was dried at 50 ℃ in a drying oven, then pulverized, and sifted through a 60-mesh sieve. The dried sample (300 g) was mixed with 50 mL of 80% ethanol and ultrasonicated at 30 ℃ for 1 h. After centrifugation at 8 000 r/min for 10 min at 4 ℃, the residue was extracted again. The supernatants were combined and concentrated using a rotary evaporator. For purification, the concentrate was loaded onto AB-8 microporous resin. Once the 100% water fraction was completely removed, the 80% ethanol fraction was collected, evaporated, and freeze-dried to obtain GE (3.26 g)[22].

2.3 Measurement of the content of total sugar and total protein in GE

To determine the total sugar content in GE, the phenol-sulfuric acid method was employed in accordance with the established protocol[23]. Briefly, 1 mL of GE (0.5 mg/mL) or standard solutions of Glu at concentrations of 0, 40, 80, 120, 160, and 200 μg/mL were mixed with 1 mL of 6% phenol and 5 mL of sulfuric acid. The mixture was then incubated for 20 min at 25 ℃, the absorbance was measured at 490 nm. The total sugar content was expressed as mg glucose equivalent/g GE.

The total protein content in GE was measured using the Bradford method[24]. In brief, 1 mL of GE (0.5 mg/mL) or standard solutions of BSA at concentrations of 0, 20, 40, 80, 120, 160, and 200 μg/mL were incubated with 5 mL of Coomassie brilliant blue. The absorbance was measured at 595 nm, and the result was presented as mg BSA equivalent/g GE.

2.4 Identification and quantification of gingerol analogues in GE

Gingerol-related compounds in GE were identified using a Waters UPLC-Q/TOF-MS/MS system (Waters Co., USA)[25] coupled with an

electrospray ionization source. Chromatographic separation was achieved using a Waters BEH-C_{18} column (2.1 mm × 100 mm, 1.7 μm) with a binary gradient elution composed of water containing 0.1% (*V/V*) formic acid (solvent A) and acetonitrile (solvent B). The gradient elution was performed as follows: 0–2 min, 5% B; 2–22 min, 5%–99% B; 22–25 min, 95%–5% B; and 25–30 min, 5% B. The injection volume was 1 μL, and the flow rate was set at 0.2 mL/min. The mass spectrometry operation parameters were as follows: ionization mode, positive-ion mode; scanning range of *m/z* 100–1 000; capillary voltage, 2.0 kV; source temperature, 120 ℃; desolvation temperature, 450 ℃; cone gas (nitrogen) flow, 50.0 L/Hr; low collision energy, 10 V; high collision energy, 30 V.

The quantification of 6 major compounds in GE was conducted by a Waters Acquity UPLC H-Class system (Waters Co., USA) equipped with a photodiode array detector. The sample of GE at a concentration of 5 mg/mL was injected into a Waters ACQUITY UPLC® HSS C_{18} column (2.1 mm × 100 mm, 1.8 μm) at a flow rate of 0.2 mL/min. The injection volume was 2 μL, and the detection wavelength was set to 280 nm. The mobile phase consisted of 0.1% (*V/V*) formic acid in water (A) and acetonitrile (B). The sample was eluted according to the following gradient program[13]: 0–8 min (20%–35% B); 8–15 min (35%–55% B); 15–18 min (55%–60% B); 18–26 min (60%–70% B); 26–28 min (70%–100% B), 28–30 min (100%–60% B), and 30–32 min (60%–20% B).

2.5 Worms strains and maintenance

Wild-type worms N2 (Bristol), mutant strains EU1, CF1038, AU3, VC8, KU4, KU25, CB1370, and transgenic strains CL2166, CF1553, CB7272, and *Escherichia coli* OP50 were provided by the *Caenorhabditis* Genetics Center (CGC, University of Minnesota, Minneapolis, MN, USA). Nematodes were incubated at 20 ℃ on nematode growth medium (NGM) plates with *E. coli* OP50, as described in the Worm Book.

2.6 Stress resistance assay

All age-synchronized strains grown to L4 larval stage. The worms were then incubated in S-completed medium with *E. coli* OP50 (1.2×10^9 bacteria per mL) and supplemented with different concentrations of GE (0, 3.75, 7.5, 15, 30, and 60 μg/mL). After 6 days of treatment with GE at 20 ℃, all *C. elegans* were exposed to 35 ℃, and the survival of the worms was recorded every 2 h until all worms died.

The motility of worms was measured by recording the number of head thrashes per 30 s after exposure to 35 ℃ for 3 h, and then they were returned to 20 ℃ for 3 h for recovery on day 5 of adulthood[26-28].

2.7 Measurement of endogenous ROS in C. elegans

After treatment with extracts for 6 days, the worms were exposed to 35 ℃ for 3 h, then returned to 20 ℃ for 3 h for recovery, washed with M9 buffer, and then incubated with a 50 μmol/L H_2DCF-DA probe in a black opaque 96-well plate (30 worms per well), subsequently. Fluorescence values were subsequently collected using a multimode microplate reader (Thermo Fisher, Waltham, MA, USA) at excitation/emission wavelengths of 485 nm and 535 nm every 15 min for 5 h at 25 ℃[29].

2.8 Network pharmacologyanalysis

2.8.1 Collation of ginger targets and the prediction of disease targets

Swiss Target Prediction (http://www.swisstargetprediction.ch/) was utilized to identify protein targets of gingerol-related compounds[30]. The targets associated with HS-induced oxidative stress were predicted by searching for the terms “heat shock” and “oxidative stress” on GeneCardsdatabase (https://www.genecards.org).

2.8.2 Analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment

KEGG pathway and GO enrichment analyses were conducted using all drug-disease intersection genes through the R software, utilizing the“clusterProfiler”, “org.Hs.eg.db”, “ggplot2”, and “BiocManager” packages.

2.8.3 Construction of a protein-protein interaction network

Drug-disease intersection targets were imported into the STRING database(https://string-db.org/) to construct the protein-protein interaction (PPI) network. Subsequently, the PPI network was then imported into Cytoscape 3.9.1 software for the evaluation of the core targets.

2.9 Quantification of ATP and measurement of MMP

On day 3, the heat-stressed nematodes were washed 3 times with M9 buffer solution and homogenized using a tissue grinder. Thereafter, the suspension was centrifuged at 12 000 r/min at 4 ℃ for 10 min. The supernatant was collected and then assayed for ATP content following the manufacturer’s instructions.

For MMP assay, pre-treatment worms were exposed to 35 ℃ for 3 h and 20 ℃ for 3 h, washed 3 times with M9 buffer, and then incubated with 2 μmol/L JC-1 dye for 1 h at 20 ℃. Subsequently, nematodes were anesthetized with 20 mmol/L sodium azide and photographed using a fluorescent microscope. The red fluorescence was captured at an excitation/emission of 585/590 nm, while the green fluorescence was observed at 514/529 nm.

2.10 Fluorescence quantification and visualization

Nematodes synchronized at the L1 stage were treated with or without GE at 20 ℃ for 3 days. After being exposed to acute HS for 3 h and then to 20 ℃ for 3 h, thenematodes were immobilized with sodium azide and photographed using an inverted fluorescent microscope (excitation at 470 nm and emission at 535 nm). The fluorescence intensities of SOD-3::GFP and GST-4::GFP were measured using ImageJ software. The quantification of CB7272 strain was consistent with that of the MMP assay.

2.11 Gene expression analysis by quantitative Real-Time PCR

Worms were exposed to 35 ℃ for 3 h and then returned to 20 ℃ for 3 h on day 3 of adulthood, as described in Section 2.9. Total RNA

was extracted using TRIzol reagent (Aidlab Biotechnologies Co., Beijing, China), synthesized into cDNA with a reverse transcription kit (Takara Biotechnology Co. Ltd., Dalian, China), and then analyzed by the Applied Biosystems™ QuantStudio™ 7 Flex PCR Detection System (Life Technologies, USA) with SYBR Green Mix (Takara). The expression levels of genes were analyzed using the comparative $2^{-\Delta\Delta CT}$ method, with β-actin as the normalizer. Primers used for RT-qPCR are listed in Table S1.

2.12 Molecular docking and molecular dynamics (MD) simulation

Molecular docking was performed using AutoDock 4.2.6 to predict the binding affinities of 6 gingerol-related compounds with the crystal structure of Keap1 (PDB ID: 4IQK) obtained from the RCSB Protein Data Bank. The 6 compounds, with the lowest energy, were docked into the grid box formed by the Keap1 protein. The docking results were visualized using PyMOL 2.5.

GROMACS software (version 2024.2) was utilized to perform MD simulations ofthe interaction between Keap1 and 6 compounds[31]. For this simulation, the protein topology file was generated using the CHARMM 36 force field, while the ligand topology file was created using CGenFF. The protein-ligand complex was situated within a dodecahedral water box (TIP3P), which contained approximately 7 868 water molecules to meet the minimum spatial constraints. Na^+ and Cl^- counterions were added to neutralize the solvation system. Prior to the simulation, the system underwent energy minimization using the steepest descent method for 50 000 steps, followed by NVT and NPT equilibration for 100 ps to stabilize the temperature at 300 K and the pressure at 1 bar. Subsequently, an MD simulation lasting 100 ns was conducted.

2.13 Statistical analysis

The graphical abstract was drawn using Figdraw. The survival curve for HS lifespan was plotted using Kaplan-Meier analysis with GraphPad Prism 8 software (San Diego, United States). The results were presented as mean ± SD (n=3), and statistical analysis was performed with IBM SPSS V. 22.0 software (SPSS Inc., Chicago, IL, USA). Differences between the control and treatment groups were analyzed by One-way ANOVA with Duncan's multiple range test or Student's t-test. In all comparisons, $P < 0.05$ was considered as statistically significant.

3 Results and discussion

3.1 Chemical composition of GE

Gingerol analogues, as distinctive non-volatile phenolic compounds in ginger, have been reported to possess multiple health-promoting functions with minimal side effects[32]. Due to the presence of the fundamental structure of 4-hydroxy-3-methoxyphenyl in gingerol compounds, characteristic fragment ions ($[C_8H_9O_2]^+$) were generated at m/z 137[33]. As shown in Table S2 and Fig. 1, 18 gingerol-related compounds were identified using UPLC-Q-TOF-MS/MS, of which 6 major compounds were quantified by UPLC. Consequently, 6-gingerol was the most abundant constituent in GE (305.27 ± 0.77 mg/g DW), followed by 10-gingerol, 8-gingerol, 8-shogaol, 10-shogaol, and 6-shogaol, which accounted for 30.52%, 11.90%, 4.85%, 2.50%, 2.34%, and 2.33% of the total composition, respectively. Consistent with previous research, 6-gingerol serves as a quality marker for ginger-related products, exhibiting a higher concentration than both 10-gingerol and 8-gingerol[34]. Among phenolic compounds, shogaols are formed during storage or thermal processing through the dehydration of the unstable β-hydroxyl ketone moiety in gingerols[35]. Hence, the amount of gingerols in fresh ginger is far higher than that of shogaols.

In GE, the content of total protein was (87.78 ± 5.48) mg BSA/g dry weight (DW) and the content of total sugar was (169.61 ± 6.05) mg Glu/g DW.

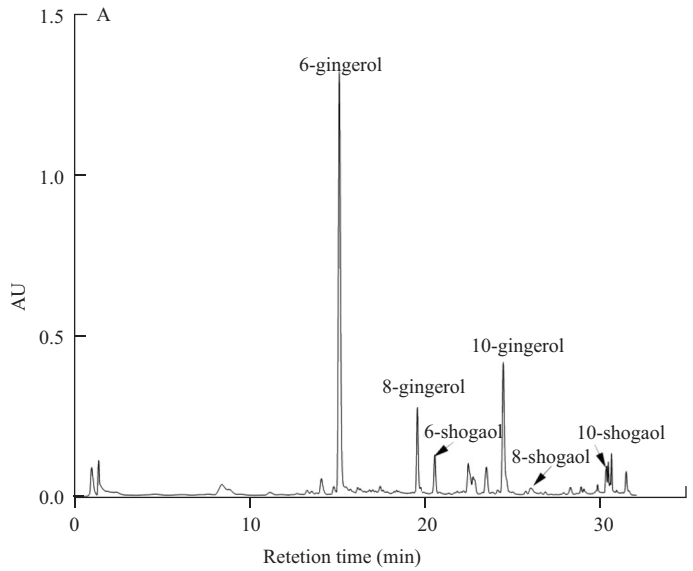

Fig. 1 The major components of GE. (A) UPLC elution profiles of gingerolanalogues in GE. The MS/MS information and chemical structure of (B) 6-gingerol, (C) 8-gingerol, (D) 10-gingerol, (E) 6-shogaol, (F) 8-shogaol, and (G) 10-shogaol.

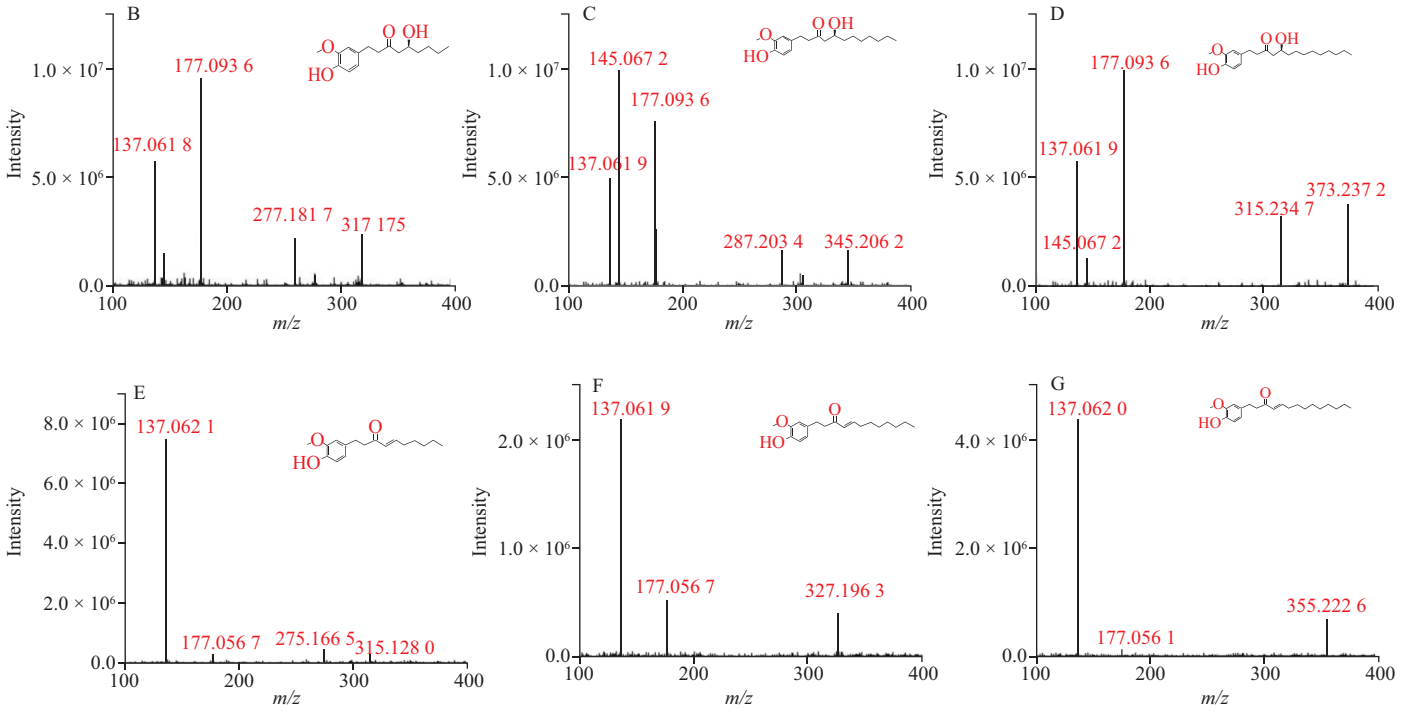

Fig. 1 (*Continued*)

3.2 GE extended lifespan and improved motion status under HS in C. elegans

Lifespan, as an intuitive and convenient indicator, is related to the ability of an organism to resist environmental stimuli in *C. elegans*. After incubating GE with increasing concentrations (3.75, 7.5, 15, 30, and 60 μg/mL) at 35 ℃, the mean lifespans were significantly increased ($P < 0.05$) by 6.41%, 17.24%, 20.30%, 12.10%, and 8.25% (Fig. 2A and Table S3). Notably, GE enhanced tolerance against HS in an inverted U-shaped dose-dependent manner. At a concentration of 15 μg/mL, GE maximally extended the lifespan of worms treated with HS. However, at concentrations of 30 and 60 μg/mL, GE exhibited inhibitory effects on thermal resistance compared to the 15 μg/mL concentration. Consistent with a previous study, no further increase in anti-nociceptive activity in mice was observed with the administration of a higher dose of GE[36]. The adverse effect of unabsorbed excess extract might be responsible for this observation[37]. In the study, the doses of 7.5, 15, and 30 μg/mL GE were considered as optimal concentrations for subsequent experiments.

To determine the effective constituents responsible for prolonging the lifespan of *C. elegans* under HS at a concentration of 15 μg/mL GE, the effect of a combination of 6 predominant ingredients was assessed: 15.55 μmol/L 6-gingerol, 2.25 μmol/L 8-gingerol, 1.26 μmol/L 6-shogaol, 5.09 μmol/L 10-gingerol, 1.23 μmol/L 8-shogaol, and 1.06 μmol/L 10-shogaol. As illustrated in Fig. 2B and Table S3, the mean lifespan of the combined group ((13.72 ± 0.68) h) showed an 18.93% increase ($P < 0.05$) compared to the control group ((11.54 ± 0.55) h). The combination group demonstrated similar efficiency to GE at 15 μg/mL ((13.96 ± 0.53) h), indicating that these 6 ingredients played a dominant role in mitigating the adverse effects of HS on *C. elegans*. It has been reported that both 6-gingerol and 6-shogaol, at a concentration of 25 μmol/L, extended the HS lifespan, which closely correlated with the content of the dominant bioactive components in 15 μg/mL of GE[14-15]. In contrast, polyphenols generally exhibit resistance to HS at a concentration of 100 μmol/L[38], indicating that gingerol-related compounds have a more pronounced anti-HS effect.

The degradation of coordinated movement is an obvious change during HS in *C. elegans*[39]. To clarify the effect of GE on motility in *C. elegans*, movement behavior was assessed after exposure to 35 ℃. The motility of *C. elegans* slowed down following 3 h of HS (Fig. 2C). Treatment with 7.5 and 15 μg/mL of GE notably ($P < 0.05$) minimized heat-induced damage in body bending, while no ameliorative effect was observed with 30 μg/mL of GE. Likewise, both 15 μg/mL of GE and the combination were superior to the lower and higher doses in enhancing mobility.

3.3 GE diminished ROS level and alleviated HS-induced oxidative stress in C. elegans

It is acknowledged that HS leads to mitochondrial dysfunction, triggering an excess of ROS, ultimately progressing into oxidative stress[40]. Hence, the ROS levels were measured in wild-type worms pre-incubated with GE under HS conditions. As expected, exposing *C. elegans* to HS significantly amplified the generation of intracellular ROS (Fig. 2D). However, the ROS levels with GE intervention were notably ($P < 0.05$) lower than those in the heat-stress group.

HS-induced oxidative damage is always accompanied by alterations in the antioxidant system, which involves in both non-enzymatic antioxidants (e.g., vitamin C and glutathione) and antioxidant enzymes (e.g., superoxide dismutase (SOD), catalase, and glutathione peroxidase). Normally, SOD plays a crucial role in decomposing superoxide anion radicals generated from the single-

electron reduction of oxygen in mitochondria, converting them into hydrogen peroxide (H_2O_2), which is then metabolized into H_2O by catalase and glutathione-peroxidase/glutathione[41]. The study utilized transgenic strains CF1553 (SOD-3::GFP, superoxide dismutase 3) and CL2166 (GST-4::GFP, glutathione transferase activity) to monitor the expression of SOD-3 and GST-4. After heat exposure, there was a decrease of 26.19% in SOD expression (Figs. 2E and F) and 5.36% in GST-4 expression (Figs. 2G and I) compared to the control. The alteration in antioxidant enzymes activities was found to be associated with the duration and magnitude of HS[42]. Under mild HS conditions, the activities of these enzymes increased sharply to protect cells from oxidative damage[43]. However, as HS intensifies, the generation of excessive H_2O_2 overwhelms antioxidant enzymes, disrupting the redox balance. Subsequently, the antioxidant enzyme activities were inhibited, which is consistent with our results[44]. Supplementation with 15 μg/mL of GE effectively ameliorated the adverse effect of HS on SOD-3 and GST-4 expression ($P < 0.05$), suggesting that ginger might strengthen the stress-response capabilities of *C. elegans*. It is noteworthy that the combination group did not restore the reduction in SOD-3 activity induced by HS. A previous study has preliminarily demonstrated that ginger polysaccharides could enhance the activities of antioxidant enzymes, including CAT and SOD[45], thereby attenuating oxidative stress. Perhaps that ginger polysaccharides in GE compensated for the SOD activity. Regarding the low-dose group, it is possible that an insufficient amount of active ingredients in GE resulted in a lower ROS removal rate[46].

Furthermore, the expression levels of several oxidative-stress marker genesin *C. elegans* (Fig. 2H), including *gcs-1* (involved in glutathione biosynthesis), *gst-4* (encoding glutathione transferase), *sod-1* (encoding SOD), *sod-2*, *sod-3*, *ctl-1* (encoding CAT), *ctl-2*, and *mtl-1* (metallothionein) were evaluated. Compared with the nonexposed control group, acute exposure to a high ambient temperature caused an obvious decrease ($P < 0.05$) in *sod-1*, *sod-3*, *ctl-1*, *ctl-2*, and *gst-4* mRNA expression, which were approximately 0.50-, 0.33-, 0.52-, 0.62-, and 0.41-fold, respectively. However, the expression of *mtl-1*, *gcs-1* and *sod-2* remained unchanged ($P > 0.05$). In contrast, the worms treated with 15 μg/mL GE and the combination compounds showed higher expressions of *sod-3* (1.90- and 1.62-fold)

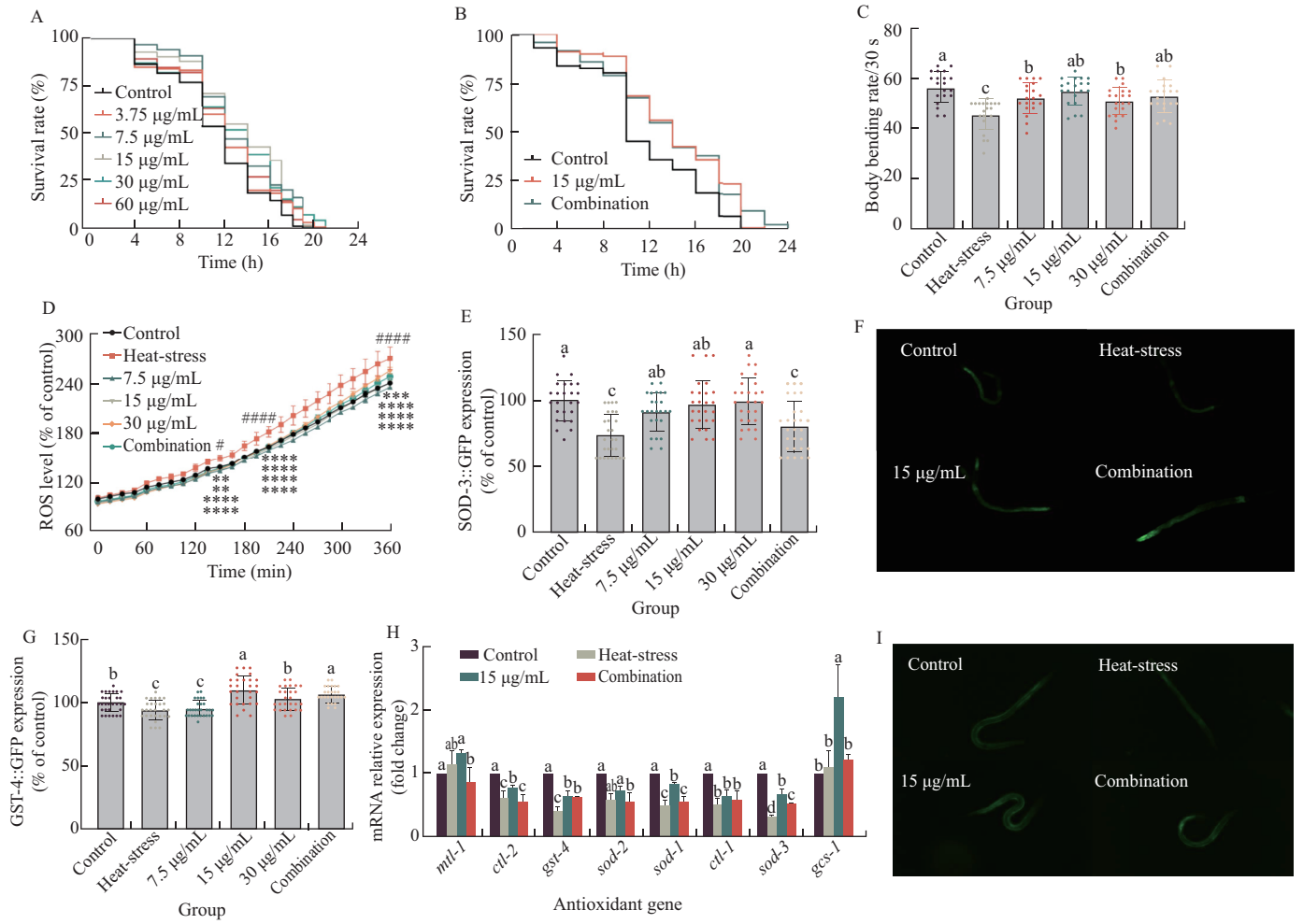

Fig. 2 GE improved the health status and alleviated oxidative damage under HS in wild-type worms. (A) HS lifespan curve of wild-type worms pretreated with GE (3.75, 7.5, 15, 30, and 60 μg/mL) or with 1‰ DMSO. (B) HS lifespan of *C. elegans* pretreated with 15 μg/mL GE, the combination of 6 major gingerols and shogaols at 15 μg/mL (the Combination), and 1‰ DMSO. The Control group in HS lifespan curves indicated that the worms were incubated 1‰ DMSO at 35 °C. (C) Body bending rates after HS for 3 h with GE pretreatment within 30 s. (D) ROS level after HS for 3 h with GE pretreatment or 1‰ DMSO in wild-type worms. $^{\#\#\#\#}P < 0.001$ represented the comparison between Control (at 20 °C) and Heat-stress group (at 35 °C for 3 h), while $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.001$ indicated the comparison between Heat-stress and GEs treatment groups. Fluorescence images and expressions of SOD-3 and GST-4 in (E, F) CF1553 and (G, I) CL2166 transgenic strains with GE pretreatment or without pretreatment under HS, respectively. (H) The mRNA expression of antioxidant genes of 15 μg/mL GE and the combination under HS in wild-type *C. elegans*. Different letters represent significant differences at $P < 0.05$.

and *gst-4* (1.60- and 1.55-fold) than heat-stress group. In additional, *sod-1* and *ctl-2* genes exhibited a significant upregulation in worms pretreated with 15 μg/mL GE, with increases of 1.26- and 1.69-fold, respectively, while no significant changes were observed between the combination group and the model group. Other components in GE, such as ginger polysaccharides, maylead to increase dsynergistic antioxidant activity. These results revealed that GE could remove HS-elicited ROS by enhancing the antioxidant enzyme system. Existing studies have reported that the phenolic group in gingerols and shogaols serves as an easily accessible source of "H" atoms[47], which can react with free radicals and exert antioxidant effects. Moreover, GE could mitigate oxidative damage by enhancing SOD and CAT activity and promoting GSH synthesis both *in vivo* and *in vitro*[11,48]. This suggests that phenolic compounds in ginger are natural antioxidants that can not only directly eliminate free radicals, but also indirectly activate antioxidant defense systems to maintain cellular homeostasis under HS.

3.4 Potential pathways and targets of GE against HS

Since it has been proven that phytochemicals from GE could attenuate HS-induced oxidative stress, network pharmacology was employed to identify the potential pathways and targets of GE in combating HS. The 382 targets of phenolic compounds were retrieved from the SwissTargetPrediction database with a criterion of probability ≥ 0.1. Meanwhile, 450 targets related to HS-induced oxidative stress were gathered from GeneCards, with relevance score ≥ 8. By constructing Venn diagrams using R software, 88 overlapping targets between ginger and HS were identified (Fig. 3A), indicating that GE mayinteract with these targets associated with HS.

KEGG and GO enrichment were carried out based on drug-disease intersection genes. Apart from the pathways linked to cancer, the analysis identified the MAPK pathway and the FOXO pathway with the highest gene counts (Fig. 3B), highlighting the

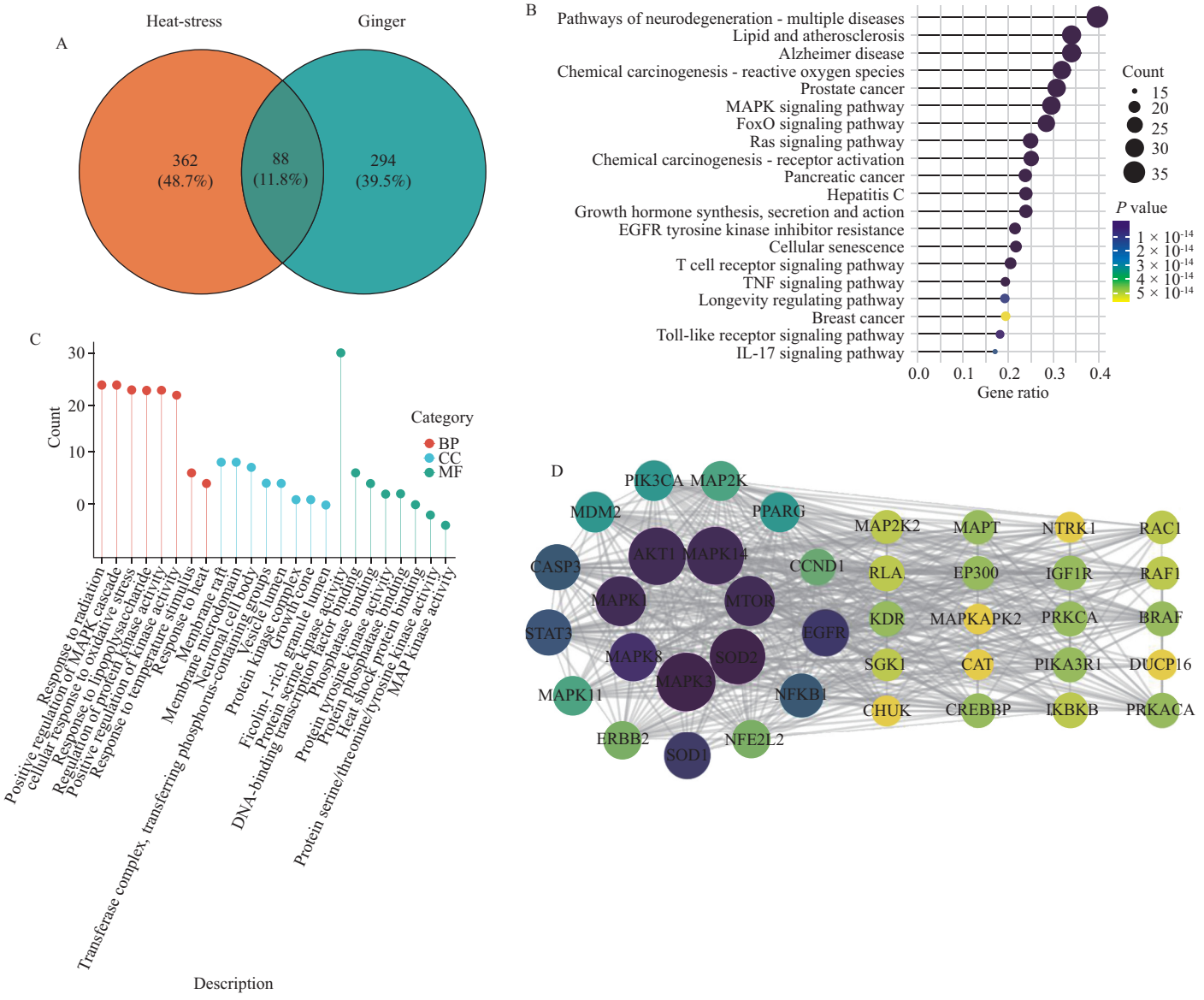

Fig. 3 Network pharmacology of GE in the prevention of HS. (A) The Venn diagram representingthe intersection between the genes associated with HS and the targets of GE. The lollipop charts for the (B) KEGG analysis and the (C) GO enrichment analysis. (D) The PPI network of the intersection targets between GE and HS-related pathways.

significant roles in ginger's modulation of HS[49-50]. In addition, the longevity regulation pathway is also relevant to HS, as resistance to environmental stressors is intricately associated with longevity[17].

The results from the GO enrichment analysis indicated that a total of 2 424 GO items that were statistically significant, comprising 2 122 in biological process (BP), 113 in cellular component (CC), and 189 in molecular function (MF). Subsequently, the 8 items from each categorythat exhibited the highest gene counts were selected for the construction of a visual lollipop plot by R software. As illustrated in Fig. 3C, the BP of the potential targets were primarily associated with the responses to environmental stressors, including radiation, oxidative stress, temperature stimuli, and heat, as well as the regulation of the MAPK cascade. Regarding the BP section, the potential targets were enriched in membrane raft, membrane microdomain, and neuronal cell body. In terms of the MF category, the result suggested that MAP kinase activitymay play a pivotal role in HS.

Following the collation of genes about HS related pathways, including the MAPK pathway, FOXO pathway, and Longevity regulation pathway, a total of 40 genes were uploaded to the STRING database. Subsequently, a PPI network was created in Cytoscape. As depicted in Fig. 3D, 7 core targets were identified *via* the Centiscape tool based on the criterion: degree > 28, betweenness > 33, and closeness > 0.015[51]. The identified targets included MAPK14, MAPK3, AKT1, MAPK1, SOD2, MTOR, and MAPK8, emphasizing the role of the MAPK pathway in the efficacy of GE against HS.

3.5 GE increased thermal resistance via MAPK pathway in C. elegans

Combined with the results from KEGG and GO enrichment analyses, this further supported that MAPK14/PMK-1, MAPK3/ERK1, MAPK1/ERK2, and MAPK8/JNK-1 may serve as core targets, and that the MAPK pathway may be the primary pathway through which GE exerted its effects against HS. Research has indicated that the ERK family of MAPKs is predominantly activated in response to cytokines and growth factors, while the p38 and JNK families of MAPKs primarily respond to environmental stimuli[49]. In *C. elegans*, PMK-1 and JNK-1 are orthologs of human MAPK14 and MAPK8, respectively. Thus, the roles of the p38 MAPK pathway and the JNK MAPK pathway in mediating the thermal resistance enhanced by GE were investigated. p38 MAPK signaling pathway is crucial for transducing extracellular stimuli and helping cells adapt to various environmental stressors. When *C. elegans* is subjected to oxidative stress, NSY-1/MAPKKK is initially activated, subsequently triggering the phosphorylation of SEK-1/MAPKK, PMK-1/MAPK14, and SKN-1/Nrf2. Upon phosphorylation at residues Ser70 and Ser340, the active SKN-1 translocates to the nucleus to transcribe the target genes, including those encoding phase II detoxification enzymes[52]. To elucidate whether the thermotolerance provided by GE is associated with MAPK signaling pathway, HS lifespan effect of GE was evaluated in mutants deficient in genes (*nsy-1*, *sek-1*, and *pmk-1*) involved in the p38 MAPK pathway. Our results showed that there was no significant difference ($P > 0.05$) in lifespan prolongation between control and GE pretreatment groups across the respective mutants at 35 ℃ (Figs. 4A-C and Table S4). These data indicated that GE enhanced thermal resistance in a manner dependent on p38 MAPK pathway.

SKN-1, the ortholog of mammalian Nrf2, has been identified as a key transcription factor that regulates the expression of anti-stress genes in nematodes exposed to HS in the p38 MAPK pathway[17]. Several phenolic compounds have been implicated in stress defense *via* SKN-1[53–54]. Furthermore, a prior study has demonstrated that GE prolonged lifespan *via skn-1* in *C. elegans*[13]. In this study, GE was found to upregulate antioxidant genes targeted by *skn-1* (*gst-4* and *gcs-1*) under HS. The involvement of *skn-1* in the GE-mediated extension of HS lifespan and the upregulation of stress-responsive genes was examined. The results showed that the lifespan of mutants lacking *skn-1* (Fig. 4D and Table S4) did not change significantly ($P > 0.05$), revealing that *skn-1* is crucial for GE-mediated lifespan extension in the context of HS. In addition, under HS, the expression of *skn-1* (0.59-fold, $P < 0.05$) was significantly inhibited compared to the normal conditions (Fig. 4E). Conversely, preincubating *C. elegans* with GE at 15 μg/mL and a combination of gingerols significantly increased *skn-1* (1.98- and 1.87-fold) mRNA levels. These results suggested that the changes in mRNA relative expressions of downstream genes in *C. elegans* were consistent with *skn-1*, indicating that GE enhances antioxidant capacity by modulating SKN-1.

Previous studies have demonstrated that the *jnk-1*/MAPK pathway acts in parallel with IIS pathway to respond to HS, with both pathways converging upon DAF-16/FOXO in *C. elegans*[46]. JNK-1 directly phosphorylates DAF-16, facilitating its translocation into the nucleus[55], and *daf-16* activity is modified by the upstream DAF-2/AGE-1 kinase cascade in the IIS pathway[56]. It was analyzed whether *daf-2* and *jnk-1* are involved in the thermotolerance induced by GE. As depict in Fig. 4G and Table S4, *daf-2* mutants exposed to 15 μg/mL GE and a combination of gingerols displayed 7.05% and 9.07% longer survival ($P < 0.05$) than the control group, respectively, while *jnk-1* and *daf-16* mutants did not show a lifespan prolongation ($P > 0.05$) under HS (Figs. 4F, H, and Table S4). What's more, it is obvious that HS inhibited the *daf-16* mRNA expression (Fig. 4I), whereas wild-type worms preincubated with GE (2.04-fold) and the combination group (2.73-fold) demonstrated an elevated the mRNA expression of *daf-16*. In contrast, there was no statistically significant difference in the mRNA expression of *daf-16* between the dose group and the model group in *jnk-1* mutants. However, *daf-2* mutants subjected to GE pretreatment displayed an increased mRNA expression of *daf-16*. These results suggested that *jnk-1*, but not *daf-2*, is essential for modulating the thermotolerance response of *daf-16* to GE supplementation. Generally, phytochemicals such as genistein and quercetin conferred resistance to environmental stress *via daf-2*, which serves as a key target for resistance responses[57-58]. A comparable case was observed where the heat resistance conferred by chlorogenic acid was independent of the IIS/DAF-2 pathway, animportant stress-responsive pathway in *C. elegans*[59]. What's more, sea red rice bran has been demonstrated to enhance health indicators through the *jnk-1* and *daf-2* pathways in *C. elegans*[37]. Furthermore, previous studies have confirmed that gingerols and shogaols elicited biological activities through the modulation of MAPKs, including ERK1/2, JNK1/2, and p38[36,60].

Overall, our results indicated that GE-mediated enhancement of oxidative activities is contingent upon the MAPK pathway.

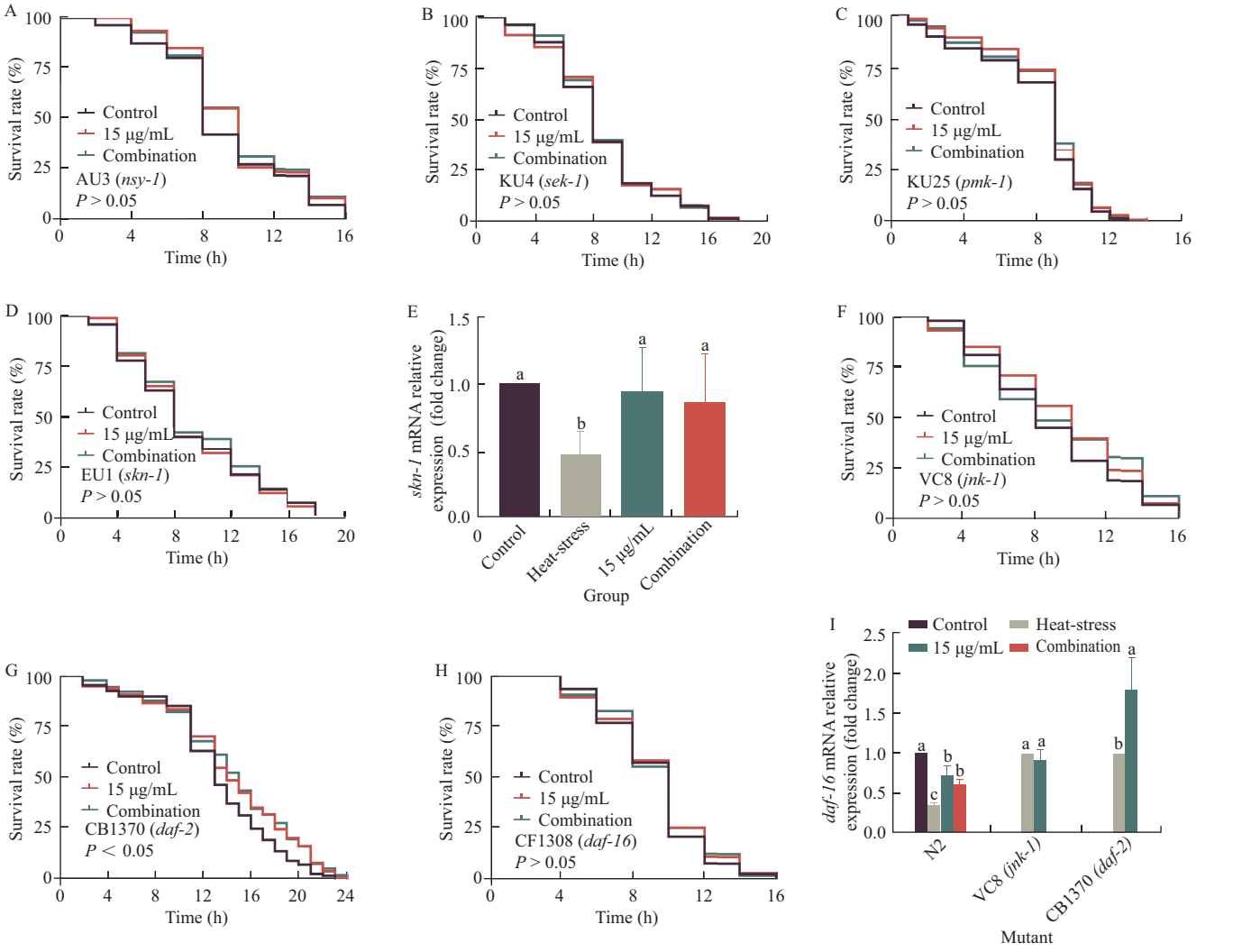

Fig. 4 GE improved thermal resistance *via* MAPK pathway in *C. elegans*. HS lifespan curves of the mutants (A) *nsy-1*, (B) *sek-1*, (C) *pmk-1*, (D) *skn-1*, (F) *jnk-1*, (G) *daf-2*, and (H) *daf-16* pretreated with 15 μg/mL GE, the combination of major gingerol-related compounds, and 1‰ DMSO. The Control group in HS lifespan curves indicated that the worms were incubated with 1‰ DMSO at 35 °C. (E) The mRNA expression of *skn-1* with treatment of 15 μg/mL GE, the combination and 1‰ DMSO under HS in wild-type worms. (I) The mRNA expression of *daf-16* with treatment of 15 μg/mL GE and 1‰ DMSO under HS in wild-type worms, as well as in *jnk-1* and *daf-2* mutants, respectively. $P > 0.05$ means no significance. Different letters represent significant differences at $P < 0.05$.

3.6 GE improved mitochondrial function under HS in C. elegans

When the severity or duration of HS surpasses mitochondrial homeostasis, it leads to a decline in mitochondrial membrane potential (MMP) and adenosine triphosphate (ATP) synthesis[61], along with the induction of mitochondrial permeability transition, resulting in increased ROS production. Thus, mitochondrial dysfunction underlies oxidative stress. Network pharmacology analysis has revealed that pathways associated with aging and responses to xenobiotic stressors may also have the potential to alleviate HS in relation to GE. Among these pathways, mitochondrial function is tightly linked to both aging and oxidative stress. What's more, emerging evidence shows that ginger attenuates mitochondrial energy metabolism disorder and respiratory dysfunction both *in vivo* and *in vitro*[11,62]. Thus, the involvement of ginger in the regulation of mitochondrial function under HS was investigated. MMP serves as a marker to assess mitochondrial function and oxidative stress levels. *C. elegans* JC-1 staining demonstrated a higher red to green fluorescence ratio in nematodes exposed to high temperature, indicating the formation of mitochondrial monomers and a reduction in MMP. Conversely, the ratio of red to green fluorescence level decreased ($P < 0.05$) in the GE treatment group (Fig. 5A), suggesting that GE could reverse MMP depolarizations and preserve mitochondrial function in *C. elegans*.

Mitochondrial damage is invariably accompanied by reduced ATP levels. As shown in Fig. 5B, the heat-stress group exhibited a notable 28.58% decrease ($P < 0.05$) in ATP levels compared to the control group. The demand for ATP increases under HS, while damaged mitochondria interfere with ATP synthesis, resulting in decreased ATP content[63]. However, GE intervention effectively restored ATP levels ($P < 0.05$). Notably, the low-dose group did not consistently display favorable effects on thermotolerance, but the combined group exhibited bioactivities comparable to the middle-dose group.

The respiratory chain complexes, located in the inner mitochondrial membrane, play a pivotal role in ATP generation, and maintaining mitochondrial function in cells. The mitochondrial electron transport chain combines electron transfer (from complex I to complex IV) and proton pumping, ultimately driving ATP

synthesis (complex V). A previous study has demonstrated that HS-induced ROS oxidizes complex I, II, IV, and V, causing a decrease in enzyme activities and ATP levels[42]. A recent investigation into whole-genome transcriptomics in humans with classic heat stroke found a downregulation of genes involved in mitochondrial electron transport chains and ATP synthase[64]. Comparable findings were noted when *C. elegans* was subjected to chronic HS (28 ℃ for 48 h)[65]. Therefore, to detect respiratory enzyme complex activities under HS with GE treatment, the transgenic strain CB7272 was employed. The strains featured GFP labeling for complexes I, II, and III, and RFP labeling for complexes IV and V. The fluorescence intensity represents the activity of complexes. Compared to the control group, the overall activities of complexes were downregulated ($P < 0.05$), indicating that HS has caused damage to the mitochondrial respiratory chain (Figs. 5C, D, F, and G). Nevertheless, the activities of the complexes increased moderately after pretreatment with GE ($P < 0.05$). Consistent with prior research, the mRNA expressions of *gas-1* (encoding complex I), *mev-1* (encoding complex II), *isp-1* (complex III), *clk-1* (encoding ubiquinone biosynthesis enzyme), *clk-2*, and *atp-2* (encoding ATP synthase) significantly decreased ($P < 0.05$) by 0.34-, 0.46-, 0.46-, 0.19-, 0.38-, and 0.13-fold under HS (Fig. 5E). However, treatment with 15 μg/mL of GE markedly ($P < 0.05$) up-regulated the mRNA expression of *gas-1* (1.70-fold), *mev-1* (1.53-fold), *isp-1* (1.61-fold), *clk-2* (1.69-fold), and *atp-2* (2.03-fold). The combination of gingerols also increased ($P < 0.05$) the mRNA expressions of *isp-1* (1.68-fold), *gas-1* (1.48-fold), and *atp-2* (1.54-fold). The results indicated that GE could maintain the integrity of the mitochondrial respiratory chain. More importantly, these observations suggested that the restoration of mitochondrial respiratory chain homeostasis by GE during HS was in line with the changes in MMP and ATP levels.

In brief, GE supplementation attenuated mitochondrial dysfunction by restoring the activities of respiratory enzyme complexes, MMP, and ATP.

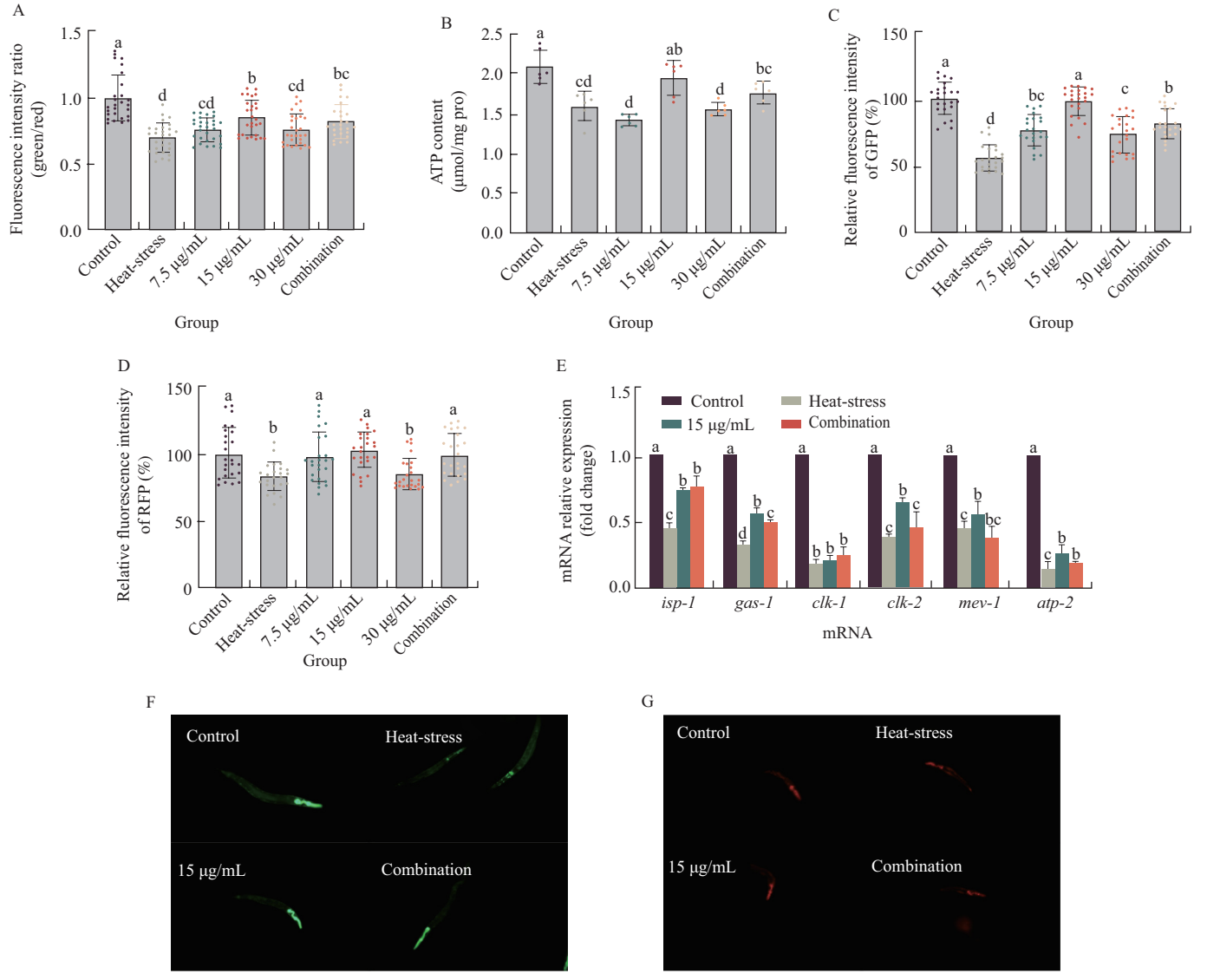

Fig. 5 GE improved mitochondrial function under HS in *C. elegans*. (A) MMP and (B) ATP levels with pretreatment (7.5, 15, and 30 μg/mL GE and the combination) or with 1‰ DMSO under HS in wild-type worms. Fluorescence images and expressions of respiratory enzyme (C, F) complex I, II, and III and (D, G) complex IV and V in transgenic strain CB7272 with GE or with 1‰ DMSO. (E) The mRNA expression of respiratory enzyme complexes of 15 μg/mL GE, the combination and 1‰ DMSO under HS in wild-type *C. elegans*. Different letters represent significant differences at $P < 0.05$.

3.7 Gingerols and shogaols enhanced HS lifespan and decreased ROS level in C. elegans

Considering that the combination group relieved thermal tolerance, we investigated whether 6 monomers could individually prolong HS lifespan and decrease ROS level. In the current study, the doses of 12.5, 25, and 50 μmol/L gingerols and shogaols were selected based on previous research that highlighting the anti-aging properties of 6-gingerol and 6-shogaol in *C. elegans*[14-15]. Under HS, all gingerol-related compounds exhibited a lifespan-promoting effect ($P < 0.05$) at 12.5 and 25 μmol/L compared to the control group (Figs. 6A, B and Table S5). However, the beneficial effect of these compounds at 50 μmol/L was not as significant as 25 μmol/L (Fig. 6C and Table S5). This was consistent with the above results that GE pretreatment with middle dose (15 μg/mL) had better bioactivities. Six components at 25 μmol/L, 6-, 8-, and 10-gingerol and 6-, 8-, and 10-shogaol, significantly extended the lifespan of worms ($P < 0.05$) by 10.10%, 13.84%, 20.94%, 11.21%, 18.11%, and 26.04%, respectively. The study found that 8-shogaol, 10-gingerol, and 10-shogaol had comparable life-extending properties, whereas 6-gingerol, 6-shogaol, and 8-gingerol showed minimal beneficial effects ($P > 0.05$). Notably, since 60% of the xenobiotics after GE metabolization by rats are derived from 6-shogaol and 6-gingerol[66], these 2 compounds are widely recognized as the most important active ingredients in ginger[67-68]. Only a limited number of studies suggested that 6-gingerol and 6-shogaol might not be as effective as other gingerols and shogaols[69]. Moreover, it appears that shogaols were more effective than gingerols in prolonging the HS lifespan of worms. Similar results were also observed at concentrations of 12.5 and 50.0 μmol/L.

Subsequently, the levels of ROS in worms preincubated with 25 μmol/L gingerols and shogaols were quantified. The results showed that the ROS level effectively decreased after treatment with gingerols and shogaols at 25 μmol/L under HS condition ($P < 0.05$), with shogaols demonstrating a moderately superior effect compared to gingerols (Fig. 6D). Furthermore, there was a relative improvement in the mRNA expression of antioxidant genes in wild-type worms pretreated with 25 μmol/L gingerols and shogaols under HS conditions, with an increase in the mRNA expression of upstream genes *skn-1* and *daf-16* (Fig. 6E). In general, shogaols exhibited a greater capacity to upregulate the mRNA expression of antioxidant genes in comparison with gingerols, especially for *skn-1* and its downstream gene *gst-4*. Notably, no significant increase was observed in the 6 monomer pretreatment groups compared to the control group in mutants lacking *skn-1* (Fig. 6F and Table S6), suggesting that all these gingerol analogues exhibited thermal tolerance that depended on *skn-1*.

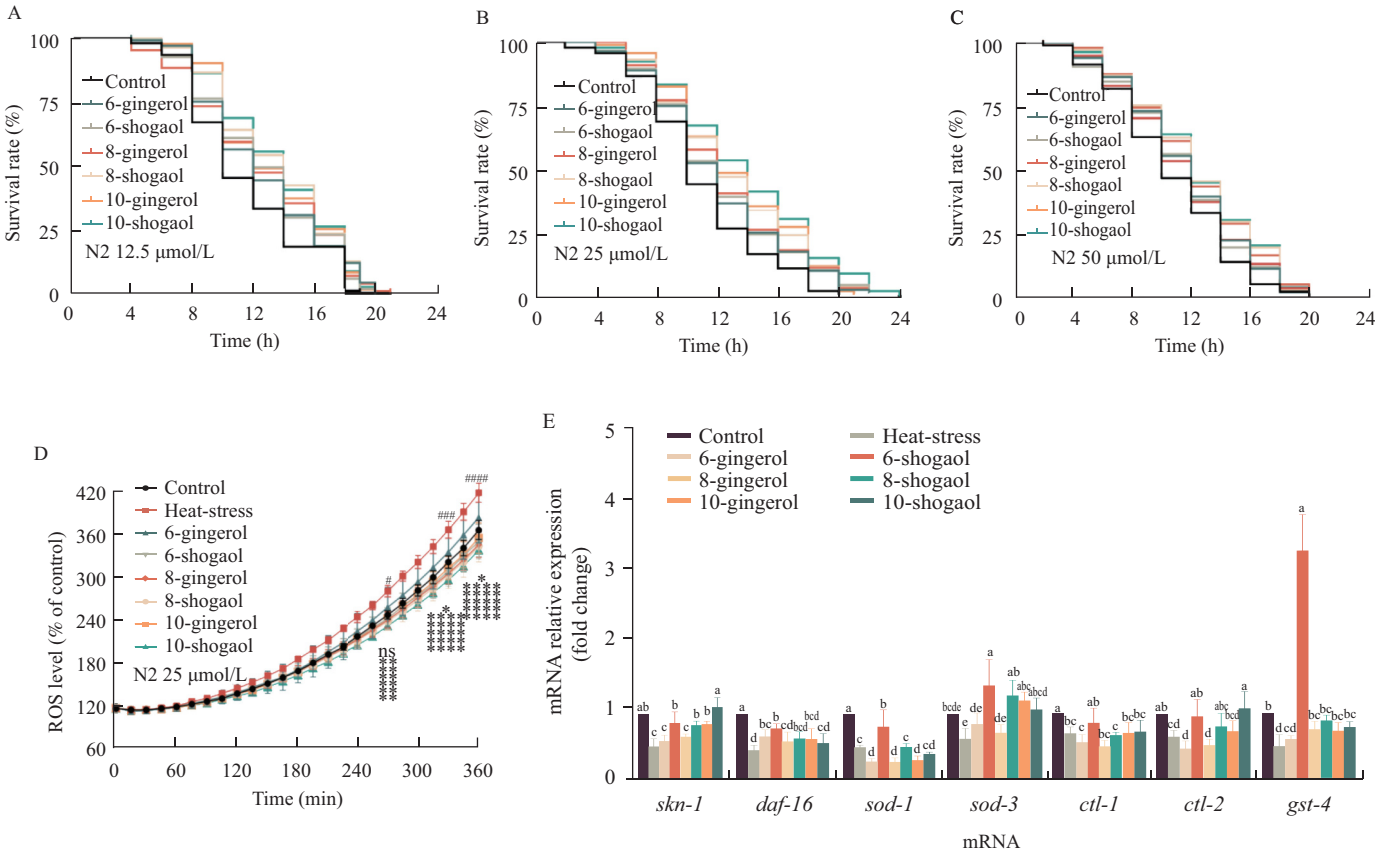

Fig. 6 Gingerols and shogaols enhanced HS lifespan and decreased ROS level in *C. elegans*. (A, B, C) HS lifespan curves treated with 6-, 8-, and 10-gingerol, and 6-, 8-, and 10-shogaol at 12.5, 25, and 50 μmol/L or with 1‰ DMSO in wild-type worms. The Control group in HS lifespan curves indicated that the worms were incubated with 1‰ DMSO at 35 °C. (D) ROS levels treated with 3 gingerols and shogaols at 25 μmol/L or with 1‰ DMSO under HS. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.01$, and $^{\#\#\#\#}P < 0.001$ represented the comparison between Control (at 20 °C) and Heat-stress group (HS for 3 h), while $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ indicated the comparison between Heat-stress and GEs treatment groups. (E) The mRNA expression of antioxidant genes of the 25 μmol/L gingerols and shogaols under HS in wild-type *C. elegans*. Different letters represent significant differences at $P < 0.05$. (F) HS lifespan curves treated with 6-, 8-, and 10-gingerol, and 6-, 8-, and 10-shogaol at 25 μmol/L or with 1‰ DMSO in *skn-1* mutants. The Control group in HS lifespan curve indicated that the worms were incubated with 1‰ DMSO at 35 °C. (G) Correlation analysis of 6 constituents and thermal tolerance in ginger.

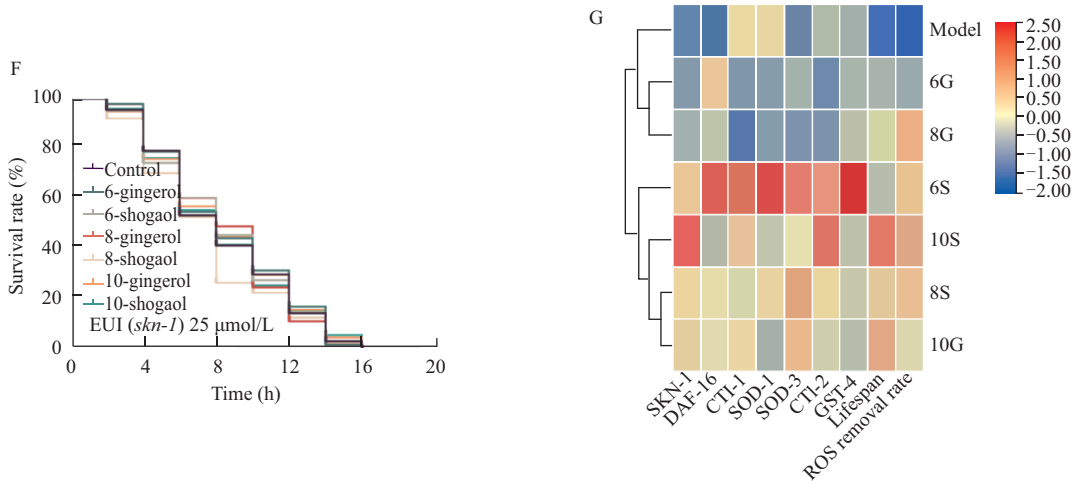

Fig. 6 (*Continued*)

To further determine the difference in thermotolerance effects between gingerols and shogaols, a cluster analysis was conducted using the data on efficacy and mRNA expression of each group. As shown in Fig. 6G, the model group and the dose pretreatment groups were divided into 2 categories. Specifically, the model group, the 6-gingerol group, and 8-gingerol group were classified into one category, while the 6-shogaol, 8-shogaol, 10-shogaol, and 10-gingerol groups were categorized differently. Taken together, these results indicated that GE enhanced protection against HS through the reduction of oxidative damage, which was attributed to gingerols and shogaols, particularly shogaols.

3.8 Molecular docking and MD simulation verification of 6 compounds with Keap1

Based on the results above, molecular docking and MD simulations were conducted to validate the affinity between Keap1 (a known Nrf2 inhibitor) and the 6 predominant constituents, with the aim of determining variations in antioxidant activities under HS. Previous reports have indicated that phytochemicals such as pterostilbene and lycopene, can bind competitively to Keap1, thereby facilitating the activation of Nrf2[70-71]. In the present study, our findings demonstrated that both gingerols and shogaols interact spontaneously with the active site of the Keap1 protein, which is comprised of specific residues (Arg415, Phe478, Arg483, Phe577, Tyr572, Tyr334, Tyr525)[72]. Among them, Asn414, Ser602, and Arg415 were identified as the key amino acid residues interacting with Keap1 and the 6 ligands (Figs. 7A–F). Moreover, shogaols also exerted a significantly greater effect on binding energy compared to gingerols, with 6-gingerol (–5.01 kcal/mol), 6-shogaol (–5.81 kcal/mol), 8-gingerol (–4.58 kcal/mol), 8-shogaol (–6.07 kcal/mol), 10-gingerol (–5.23 kcal/mol), and 10-shogaol (–6.01 kcal/mol).

MD simulations were conducted on Keap1 and 6 phenolic compounds for 100 ns to further analysis the binding stability. Root mean square deviation (RMSD) serves as an indicator of the complex's mobility. The RMSD values ranged from 1.5 Å to 2 Å (< 3 Å) following the binding of phenolic compounds (Fig. 7G), indicating that these ligands formed stable complexes with Keap1 *via* non-covalent interactions[73]. The 6-shogaol, 8-shogaol, and 10-shogaol exhibited consistent RMSD patterns after 75 ns. In contrast, the Keap1-6-gingerol and Keap1-8-gingerol complexes displayed fluctuations between 80 and 100 ns, indicating that these ligands induced local conformational changes in Keap1. These observations were aligned with the lifespan results.

The root mean square fluctuation (RMSF) reflects the local variations of amino acid residues. The RMSF values for the Keap1-ligand complexes were slightly higher than those of the initial protein structure (Fig. 7H). Moreover, the RMSF values for the protein-ligand complexes were lower in proximity to the protein domain structure (Asn415, Val463, Ser555), suggesting a stable interaction between the ligands and these amino acid residues[74]. Overall, the changes in specific residues of Keap1 and the 6 compounds do not show significant differences in binding stability.

The radius of gyration (Rg) indicates the compactness of protein structure. As shown in Fig. 7I, the Rg of the 6 complexes showed slight fluctuations, which implies stable protein folding. The Rg values for the complexes formed by Keap1 with 6-, 8-, and 10-gingerol, as well as 6-, 8-, and 10-shogaol were measured at 1.787 23, 1.794 28, 1.787 65, 1.796 67, 1.786 66, and 1.788 39 nm, respectively, suggesting no significant differences among them. The average number of hydrogen bonds for the complexes formed by Keap1 with 6-, 8-, and 10-gingerol, and 6-, 8-, and 10-shogaol were recorded at 0.74, 0.94, 0.91, 0.79, 0.94, and 1.61, respectively (Fig. 7J). Furthermore, the total interaction energy, encompassing both short-range Coulombic energy (Fig. 7L) and short-range Lennard-Jones energy (Fig. 7K) for the complexes formed by Keap1 with 6-, 8-, and 10-gingerol, and 6-, 8-, and 10-shogaol, was measured at (–115.33 ± 7.33), (–106.67 ± 17.53), (–148.78 ± 6.30), (–132.34 ± 4.16), (–148.43 ± 8.83), and (–189.02 ± 5.60) kJ/mol, respectively. The results showed that the affinities of Keap1 for shogaols were more stable than those for gingerols, thereby conferring more potent antioxidant effects to shogaols, which supported our experimental findings. It is well known that shogaols are crucial bioactive phenolic compounds in dried ginger, whereas gingerols are the primary constituents of fresh ginger. Consequently, the findings offered new insights into the precise use of ginger.

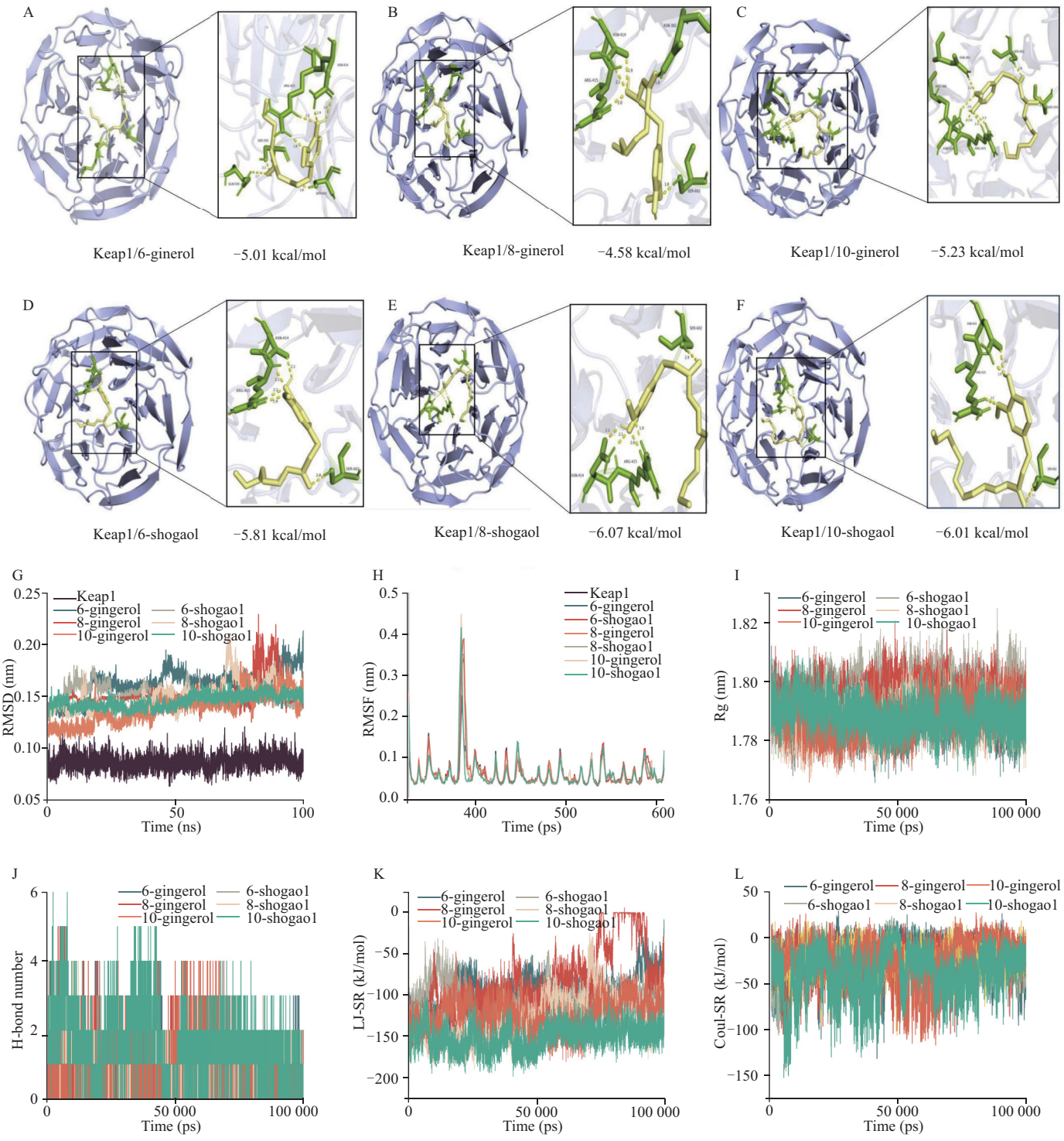

Fig. 7 Molecular docking and MD simulation of Keap1 and 6 compounds. Visualization of molecular docking of (A) 6-gingerol- Keap1, (B) 8-gingerol- Keap1, (C) 10-gingerol- Keap1, (D) 6-shogaol- Keap1, (E) 8-shogaol- Keap1, (F) 10-shogaol- Keap1. MD simulation (100 ns) results of Keap1-ligands complexes: (G) RMSD, (H) RMSF, (I) Rg, (J) H-bond number, (K) LJ-SR energy, (L) Coul-SR energy.

4. Conclusion

In brief, this study preliminarily explored the underlying mechanisms of GE in alleviating oxidative damage and mitochondrial dysfunction induced by HS. The ameliorative effect of GE was attributed to 6 major gingerols and shogaol, especially shogaols. In future studies, more attention should be devoted to elucidating the potential mechanisms of thermal tolerance of shogaols in mammals. Additionally, our work supports the contention that phytochemicals with antioxidant properties provide a potential alternative for the mitigation of heat damage.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/F SHW.2024.9250448.

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