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Dehydrocostus lactone ameliorates lipid accumulation, insulin resistance, and endoplasmic reticulum stress in palmitate-treated hepatocytes

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Abstract

Fatty liver disease is caused by lipid accumulation in the liver, insulin resistance (IR), reactive oxygen species (ROS), and endoplasmic reticulum (ER) stress. Dehydrocostus lactone (DHE) has anticancer, anti-inflammatory, and anti-ulcer effects. However, its effects on hepatic steatosis and IR remain unclear. In this study, we investigated whether DHE has antisteatotic effect on fatty liver in vitro. Hepatocytes HepG2 and SNU-449 cells were exposed to 0.25 mM palmitate (PA), and then antisteatotic effect was evaluated by treatment with 10 μ M DHE. DHE treatment reduced lipid accumulation and lipogenesis factor protein levels, compared with PA-treated hepatocytes. DHE treatment also decreased gluconeogenesis marker expression and recovered IR in PA-treated hepatocytes, and promoted glucose uptake in PA-treated HepG2 cells. Additionally, the levels of ROS and ER stress factors in PA-treated HepG2 cells were reduced by DHE treatment, compared with PA-treated HepG2 cells. Overall, DHE decreased lipid accumulation and lipogenesis factors as well as recovered IR, gluconeogenesis, and glucose uptake by reducing ER stress and ROS levels in PA-treated hepatocytes. Thus, DHE is a potential antisteatotic agent.

Keywords: Dehydrocostus lactone, Hepatocytes, Lipid accumulation, Insulin resistance, Endoplasmic reticulum stress, Reactive oxidative species

Introduction

The incidence of non-alcoholic fatty liver disease (NAFLD), a chronic liver disease, is increasing worldwide, and NAFLD occurs in 45–75% of patients with type 2 diabetes (T2D) (Masarone et al. 2014; Lonardo 2015). NAFLD can present as simple steatosis to the more aggressive and serious non-alcoholic steatohepatitis. Excess lipid accumulation in the liver, which is strongly related to hepatic insulin resistance (IR), is essential in the pathogenesis of NAFLD (Marusic et al. 2021). Hepatic

lipid accumulation occurs because of the absorption and synthesis of excess free fatty acids (FFAs), which exceeds the oxidation of FFAs. This abnormal FFA metabolism is caused by excess dietary FFAs as well as synthesis of adipocytes (Taylor et al. 2019). Excessive fat accumulation in the liver weakens insulin responsiveness, resulting in IR and increased glucose production (Qiao et al. 2022). In addition, increased ectopic lipid accumulation in the liver leads to hepatic endoplasmic reticulum (ER) and oxidative stress. With the activation of the unfolded protein response by free fatty acids, hepatic ER stress triggers IR and apoptosis (Kallen et al. 2009).

Saussurea lappa is known to have anticancer, anti-inflammatory, anti-ulcer, and anti-obesity effects, and is used as an herbal medicine in Asian countries including Korea, India, and China (Wang et al. 2020; Yu et al. 2007).

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Dehydrocostus lactone (DHE) is a single compound isolated from *S. lappa* and is known to have anti-inflammatory, anti-ulcer, and antiviral effects (Kim and Choi 2019; Yuan et al. 2022; Zheng et al. 2016). A recent study has reported that DHE attenuates osteoclastogenesis via the downregulation of the I κ B kinase (IKK)/c-Jun N-terminal kinase (JNK) signaling pathways (Lee et al. 2020). Moreover, the extract of *S. lappa*, which includes DHE, decreases adipogenesis and lipid accumulation in 3T3-L1 cells, resulting in a therapeutic effect against obesity (Karamfilova et al. 2022; Yoon et al. 2010). Although DHE has been shown to have various therapeutic effects, its anti-steatotic effects are not known. In this study, we aimed to investigate whether DHE has an anti-steatotic effect and recover IR in palmitate (PA)-treated hepatocytes.

Methods

Cell culture and treatment

Hepatocytes, HepG2 and SNU-449 cells, were purchased from the Korean Cell Line Bank (KOREA) and grown in high-glucose Dulbecco's modified Eagle medium (Hyclone, San Angelo, TX, USA) with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, MA, USA) and 1% penicillin/streptomycin (Hyclone) at 37 °C in a humidified atmosphere of 5% CO₂. The cells were transferred to the other cell culture dish every 3 days and maintained.

Cell viability

For the cell viability assay, HepG2 cells were seeded at 4×10^3 cells in a 96-well plate and incubated at 37 °C in CO₂ for 24 h. The cells were treated with 0.25 mM PA as well as with different concentrations of DHE (0, 1, 5, 10, 15, 20, and 40 μ M) and incubated at 37 °C with CO₂. After 24 h, Cell Counting Kit-8 (CCK-8) (Dojindo Molecular Technologies, Inc., Rockville, MD, USA) was added to the cells in a 96-well plate and incubated for 1 h. The treated cells were evaluated for cell viability using SpectroMax M4 (Molecular Devices, San Jose, USA) at an optical density of 450 nm.

Nile red staining

HepG2 cells were seeded at 1×10^4 cells in a 96-well plate and incubated at 37 °C with CO₂ for 24 h. To measure the effective concentration of DHE, HepG2 cells were treated with 0.25 mM PA or PA and various concentrations of DHE (0, 1, 5, and 10 μ M), and then incubated at 37 °C with CO₂ for a further 24 h. In addition, to confirm the treatment time of DHE, HepG2 cells were treated with 0.25 mM PA or PA and 10 μ M DHE, and then incubated at 37 °C with CO₂ for 0, 6, 12, 24, and 48 h.

For the assay of lipid accumulation in PA-treated HepG2 and SNU-449 cells, the cells were seeded at

1×10^4 cells in a 96-well plate and incubated at 37 °C with CO₂ for 24 h. The seeded cells were treated with 0.25 mM PA or 0.25 mM PA and 10 μ M DHE and incubated at 37 °C with CO₂ for 24 h.

The treated hepatocytes were fixed with 4% paraformaldehyde fixation solution (Thermo Fisher Scientific) for 15 min at 25 °C. The fixed cells were washed twice with phosphate-buffered saline (PBS) and incubated with Nile Red dye (1 μ g/mL) (Invitrogen™, MA, USA) at 37 °C for 10 min in the dark. The cells were visualized using the Opera Quadruple Enhanced High Sensitivity (QEHS) microscope (PerkinElmer, MA, USA) with water immersion objective lens at 20 \times , and the relative intensity was quantified using Columbus software (PerkinElmer).

Glucose uptake

The Glucose Uptake Cell-Based Assay Kit (Cayman, Ann Arbor, MI, USA) was used to evaluate glucose uptake, following the manufacturer's protocol. The treated HepG2 cells were incubated with 100 μ g/mL 2-NBDG (a fluorescent glucose analog) mixed with glucose-free buffer at 37 °C for 30 min in the dark. The cells were washed once with the cell-based assay buffer, and the final cell-based assay buffer was added to each well. The intensity of glucose uptake was measured using SpectroMax M4.

ROS production

For the analysis of ROS production, the treated HepG2 cells were washed twice with PBS and replaced with PBS containing the cell-permeant 2,7-dichlorodihydrofluorescein diacetate (Thermo Fisher Scientific). The cells were visualized using the Opera QEHS microscope with water immersion objective lens at 20 \times , and the relative intensity was quantified using Columbus software.

Western blotting

Proteins were isolated from the PA-treated hepatocytes (HepG2 and SNU-449) with/without DHE in RIPA lysis and extraction buffer (ThermoFisher Scientific) containing a proteinase inhibitor cocktail (Thermo Fisher Scientific) and phosphatase inhibitor cocktail (GenDEPOT, TX, USA). The protein concentration was measured using a BCA protein assay kit (ThermoFisher Scientific). The proteins were loaded in SDS-PAGE gels and a western blot analysis was performed as described previously (Baldwin 1996). The following antibodies were used: anti-actin (sc7210, Santa Cruz Biotechnology, CA, USA); lipogenesis factors: anti-fatty acid synthase (FAS) (sc20140, Santa Cruz Biotechnology), anti-stearoyl-CoA desaturase-1 (SCD1) (sc14719, Santa Cruz Biotechnology), anti-lipin1 (sc98450, Santa Cruz Biotechnology), and anti-diacylglycerol O-Acyltransferase 2 (DGAT2) (ab102831, Abcam, MA, USA); IR factors: anti-p-insulin

receptor substrate 1 (p-IRS1) (sc33956 (S307), Santa Cruz Biotechnology), anti-IRS1 (sc559, Santa Cruz Biotechnology), anti-p-protein kinase B (p-AKT) (4060 (S473), Cell Signaling Technology), and anti-AKT (9272, Cell Signaling Technology); gluconeogenesis markers: anti-glucose-6-phosphatase (G6Pase) (sc25840, Santa Cruz Biotechnology), anti-phosphoenolpyruvate carboxykinase (PEPCK) (sc32879, Santa Cruz Biotechnology), and anti-peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC1 α) (2178 s, Cell Signaling Technology, MA, USA); ER stress markers: anti-binding immunoglobulin protein, (Bip) (3177, Cell Signaling Technology), anti-activating transcription factor 6 (ATF6) (65880, Cell Signaling Technology), anti-inositol-requiring transmembrane kinase/endoribonuclease 1 α (IRE1 α) (3294, Cell Signaling Technology), anti-protein kinase R-like endoplasmic reticulum kinase (PERK) (3191 s, Cell signaling), anti-p-eukaryotic initiation factor 2 α (p-eIF2 α) (3597 (S51), Cell Signaling Technology), anti-eIF2 α (Cell Signaling Technology), anti-p-c-Jun N-terminal kinases (p-JNK) (9251 s (T1183/Y185) Cell Signaling Technology), and anti-JNK (9252, Cell Signaling Technology). The band intensity was evaluated using ImageJ software (Schneider et al. 2012).

Statistical analysis

All data are presented as mean \pm standard deviation (SD). Statistical analysis was performed using a one-way ANOVA with the GraphPad Prism 5 analysis package (GraphPad, CA, USA). Statistical significance was set at $p < 0.05$. All data were obtained from at least three independent experiments.

Results

DHE decreases lipid accumulation and lipogenesis factors in PA-treated HepG2 cells

We treated HepG2 cells with PA, one of the FFAs, to generate an in vitro model with steatosis features (Meng et al. 2017; Xiao et al. 2019). First, to evaluate cytotoxicity caused by DHE, the PA-treated HepG2 cells were treated with DHE at different doses, and then cell viability was measured. DHE exerted cytotoxicity of DHE at concentrations in the range of 15–40 μ M (Fig. 1a). We measured lipid accumulation in PA-treated HepG2 cells after DHE treatment at each concentration and time. Lipid accumulation significantly reduced when PA-treated HepG2 cells were treated with 10 μ M DHE for 24 h (Fig. 1b, c). Based on these results, 10 μ M DHE, which did not exert cytotoxicity and reduce lipid accumulation, was used in the subsequent experiments to study the therapeutic efficacy of DHE in fatty liver disease.

We investigated whether DHE affects lipid accumulation. Lipid accumulation increased in PA-treated HepG2

cells, but was significantly decreased by DHE treatment (Fig. 1d, e). To examine the lipogenesis factors related to lipid accumulation, these factors were evaluated in PA-treated HepG2 cells with/without DHE. Lipogenesis factors including FAS, SCD1, Lipin1 and DGAT2 were downregulated in PA-treated HepG2 cells with DHE compared with PA-treated HepG2 cells without DHE (Fig. 1f, g). These results indicate that the low concentration of DHE had no cytotoxicity, and that DHE's effect on PA-induced lipid accumulation was accompanied with the reduction in lipogenesis factors in PA-treated HepG2 cells.

DHE recovers IR and glucose metabolism in PA-treated HepG2 cells

Insulin promotes glucose uptake from the blood to the liver through the insulin signaling pathway. However, insulin does not function properly owing to IR, resulting in hyperglycemia due to the impaired suppression of glucose production and the reduction in hepatic glucose uptake (Jiang et al. 2020). We examined whether DHE regulates IR and glucose metabolism in PA-treated HepG2 cells. DHE treatment increased the phosphorylated AKT (Ser473) protein level in PA-treated HepG2 cells, whereas it decreased the phosphorylated IRS1 (Ser307) level compared with those in just PA-treated HepG2 cells, resulting in improved IR (Fig. 2a, b). Gluconeogenesis markers such as G6Pase, PEPCK, and PGC1 α , were downregulated by DHE treatment in PA-treated HepG2 cells compared with those in PA-treated HepG2 cells without DHE (Fig. 2c, d). Moreover, glucose uptake was promoted by DHE treatment compared with that in PA-treated HepG2 cells without DHE (Fig. 2e). These findings show that DHE recovers the protein levels of IR factors and gluconeogenesis markers as well as glucose uptake in PA-treated HepG2 cells.

DHE suppresses ROS levels and ER stress in PA-treated HepG2 cells

Studies have reported that PA increases ER stress and ROS levels in HepG2 cells (Gao et al. 2010; Tang et al. 2015). We examined whether DHE regulates ROS levels in PA-treated HepG2 cells with or without DHE using an ROS assay kit. DHE treatment significantly suppressed ROS levels in PA-treated HepG2 cells compared with those in PA-treated HepG2 cells without DHE treatment (Fig. 3a, b). Moreover, we evaluated ER stress markers such as PERK, IRE1 α , ATF6, Bip, phosphorylated eIF2 α (Ser51), and phosphorylated JNK (Tyr1183/Y185) in PA-treated HepG2 hepatocytes with/without DHE. DHE treatment suppressed ER stress marker levels in PA-treated HepG2 cells compared with that in PA-treated HepG2 cells without DHE (Fig. 3c, d). These results

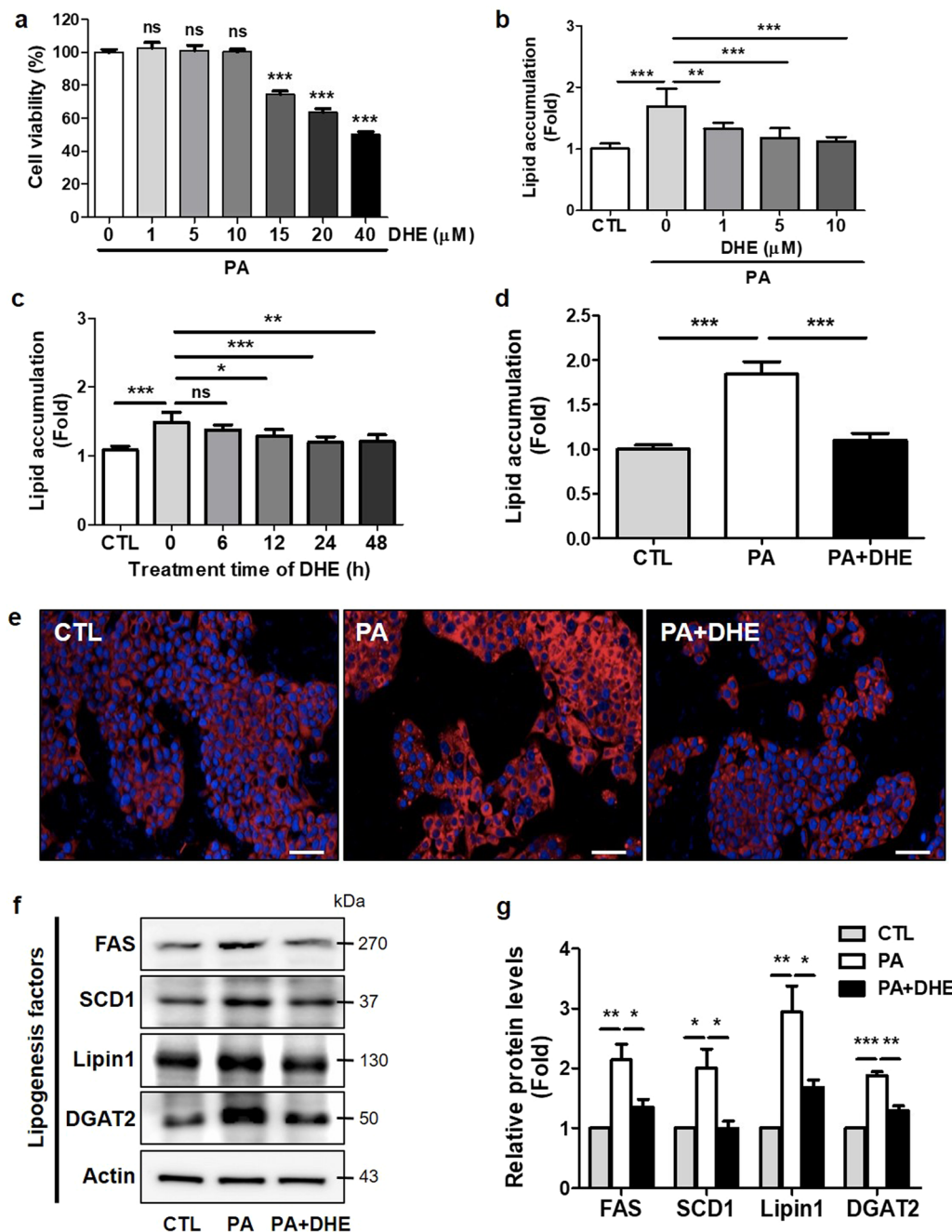


Fig. 1 DHE is not cytotoxic and decreases lipid accumulation and lipogenesis factors in PA-treated HepG2 cells. **a** HepG2 cells were treated with 0.25 mM PA and different concentrations of DHE (0, 1, 5, 10, 15, 20, and 40 μM) for 24 h, and then cell viability was analyzed using Cell Counting Kit-8. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ compared with 0 μM DHE. $n = 9$. **b** HepG2 cells were treated with 0.25 mM PA and 0, 1, 5, 10 μM DHE for 24 h, and then lipid accumulation was evaluated using Nile Red staining. **c** HepG2 cells were treated with 0.25 mM PA and 10 μM DHE for 0, 1, 6, 12, 24, and 48 h, and lipid accumulation was examined using Nile Red staining. **d** HepG2 cells were treated with 0.25 mM PA or with 0.25 mM PA and 10 μM DHE for 24 h, and were stained using Nile Red. The stained cells were analyzed using Columbus software. **e** Lipid accumulation was detected using Nile Red staining (Scalebar = 50 μm). **f** The protein expression of lipogenesis factors, including FAS, SCD1, Lipin1, and DGAT2, was confirmed in treated HepG2 cells using western blotting. **g** Relative protein levels of lipogenesis factors were assessed using the Image J program. All data are indicated as mean \pm SD of at least three independent experiments. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. $n = 3-4$. CTL; control

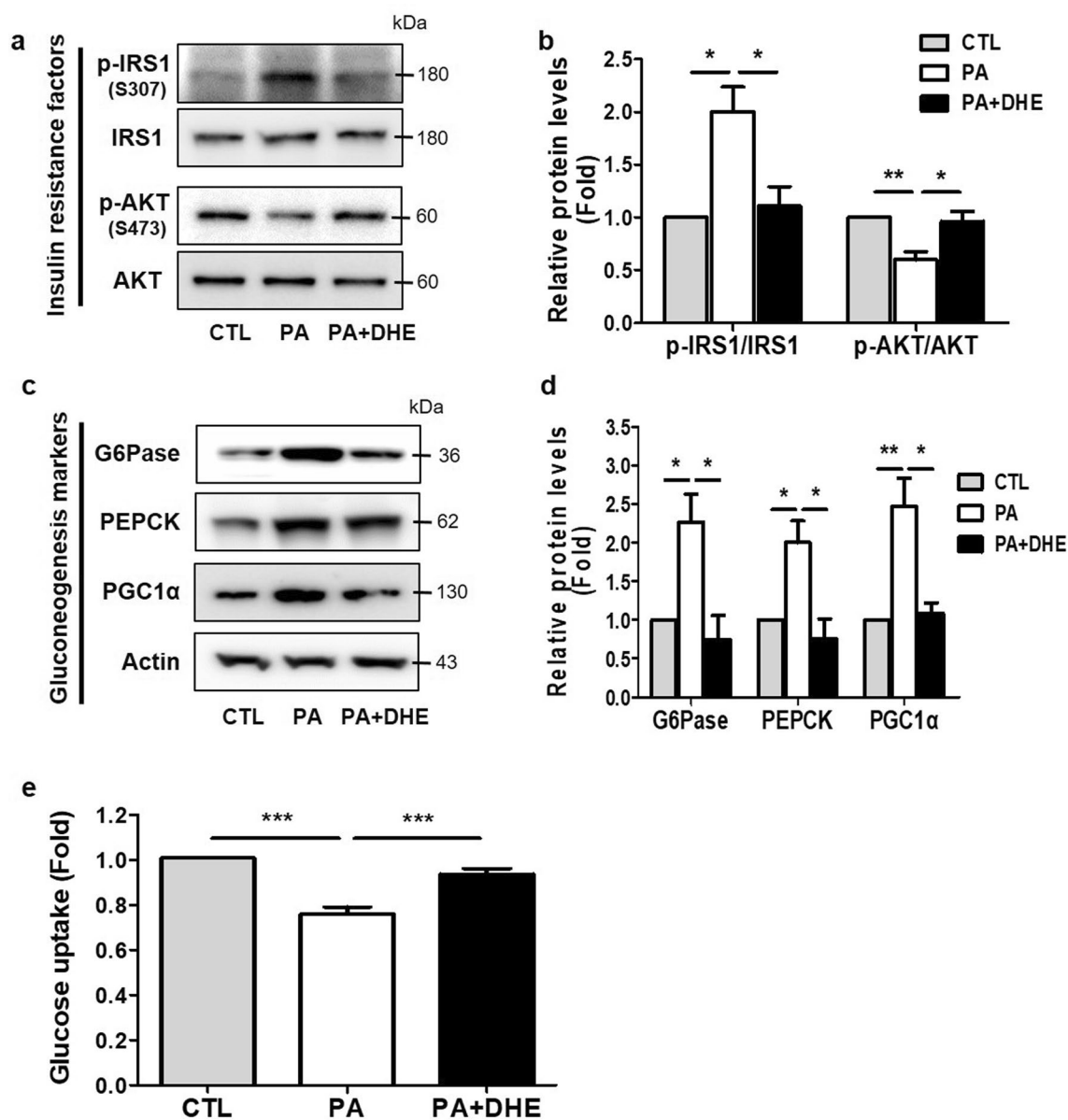
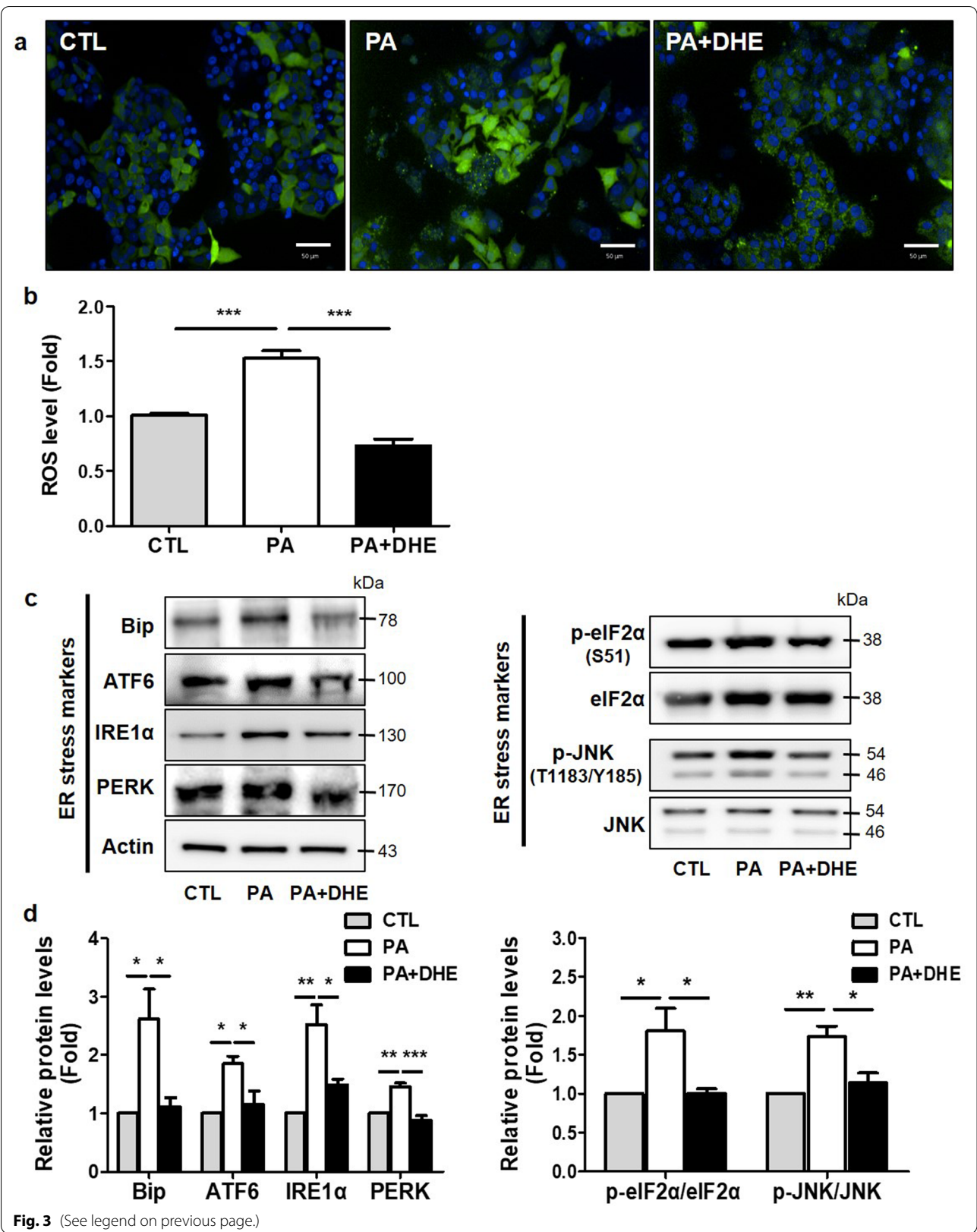


Fig. 2 DHE influences insulin resistance and glucose metabolism in PA-treated HepG2 cells. HepG2 cells were treated with 0.25 mM PA or with 0.25 mM PA and 10 μ M DHE for 24 h, and then the cells were harvested and protein was isolated. **a** The insulin resistance factors, including p-AKT (S473) and p-IRS1 (S307), were evaluated using western blotting. **b** Relative protein levels of insulin resistance factors were assessed using the Image J program. Each value was normalized to AKT or IRS1. **c** The gluconeogenesis markers, such as G6Pase, PEPCK, and PGC1 α , were examined using western blotting. **d** Relative protein levels of gluconeogenesis markers were assessed using the Image J program. Values were normalized to actin. **e** Glucose uptake in the treated cells was assessed using a glucose uptake cell-based assay kit. All data were indicated as mean \pm SD of at least three independent experiments. *** p < 0.001, ** p < 0.01, * p < 0.05. n = 3–4. CTL; control

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Fig. 3 DHE decreases ROS levels and protein levels of ER stress markers in PA-treated HepG2 cells. **a** HepG2 cells were treated with 0.25 mM PA or with 0.25 mM PA and 10 μ M DHE for 24 h, and ROS levels were assessed (Scale bar = 50 μ m). **b** ROS levels were evaluated in the treated cells using SpectroMax M4. **c** The treated HepG2 cells were assessed for the protein expression of ER stress markers (PERK, IRE1 α , ATF6, Bip, p-JNK(T1183/Y185), and p-eIF2 α (S51)) using western blotting. **d** Relative protein expression levels of ER stress markers were assessed using the Image J program. Values were normalized to β -actin, JNK, or eIF2 α . All data are indicated as mean \pm SD of at least three independent experiments. *** p < 0.001, ** p < 0.01, * p < 0.05. n = 3–5. CTL; control



indicate that DHE decreased ER stress and ROS levels in PA-treated HepG2 cells.

DHE decreases lipid accumulation, and recuperates IR and glucose metabolism in PA-treated SNU-449 cells

We investigated whether DHE also has antisteatotic effects on SNU-449 cells, one of human hepatocyte. Previous studies have reported that PA induces steatosis in SNU-449 cells (Sullivan et al. 2022). We measured lipid accumulation in PA- or PA and DHE-treated SNU-449 cells using Nile Red staining. DHE treatment significantly decreased lipid accumulation in PA-treated SNU-449 cells (Fig. 4a, b). The increased lipogenesis factors in PA-treated SNU-449 cells were decreased by DHE treatment, compared with those in PA-treated SNU-449 cells (Fig. 4c, d). Besides, DHE treatment restored IR factors (Fig. 4e, f), and consequently decreased gluconeogenesis markers in PA-treated SNU-449 cells (Fig. 4g, h). These findings indicate that DHE decreases lipid accumulation and protein levels of lipogenesis factors as well as recovers IR and glucose metabolism in PA-treated SNU-449 cells.

Discussion

Natural products, as well as their structural analogs and natural product-based drugs, have historically made a great contribution to the treatment of various diseases (Atanasov et al. 2021). DHE is a natural product obtained from the roots of *S. lappa*, that has therapeutic effects against inflammatory and cancer diseases (Zhou et al. 2020; Sheng et al. 2018). High concentrations of DHE, such as 15 and 30 μ M, are toxic to HepG2 cells owing to the inhibition of cell proliferation and promotion of apoptosis (Jeong et al. 2007). Consistent with these findings, our results also showed cytotoxicity of DHE at over 15 μ M. Furthermore, our results indicated that 10 μ M DHE was not cytotoxic but had an antisteatotic effect. Another study reported that a low concentration of DHE upregulated the viability of oxidative stress-induced HepG2 cells and increased cellular resistance as an inducer of cytoprotective HO-1 expression (Yadollah et al. 2017). Therefore, we suggest that a high concentration of DHE is cytotoxic owing to its anticancer effect on HepG2 cells, but that treatment

with a low concentration of DHE is not toxic to PA-treated HepG2 cells and has antisteatotic effects.

NAFLD is currently one of the most common liver diseases, affecting up to 25% of the adult population (Tanase et al. 2020). Hepatic steatosis refers to a form in which more than 5% of the liver's is accounted by lipids (Nassir et al. 2015), and it has been reported that DHE inhibits lipogenesis in adipocytes (Yoon et al. 2010). We expected that DHE would affect lipid accumulation and lipogenesis in hepatic steatosis. To induce fatty liver conditions, we treated HepG2 and SNU-449 cells with PA, a free fatty acid. PA-treated hepatocytes have been used in previous studies as a fatty liver model to simulate lipid accumulation and IR (Xiao et al. 2019; Gao et al. 2010; Liu et al. 2019; Zhang et al. 2019). As shown in Figs. 1d–g and 4a–d, DHE treatment decreased lipid accumulation and protein levels of lipogenesis factors in PA-induced steatotic hepatocytes.

Insulin is an important factor in regulating blood glucose and mediates hepatic glucose metabolism by suppressing the production of hepatic glucose and stimulating glucose uptake (Hatting et al. 2018). Insulin binds to the insulin receptor on the cell surface activates IRS1, intracellularly, which in turn activates PI3K and AKT to increase insulin sensitivity (Dong et al. 2006). In addition, the IRS1-AKT pathway is a major signaling pathway mediating the effects of insulin on anabolic metabolism and insulin-stimulated glucose uptake (Huang et al. 2018). These signaling pathways regulate gluconeogenesis genes such as *G6Pase* and *PEPCK* (Huang et al. 2018; Guo et al. 2009). However, under IR condition, insulin action becomes dysfunctional, gluconeogenesis increases, and glucose uptake decreases due to the weakening of insulin signaling, including IRS1 and AKT (Sangwung et al. 2020). Furthermore, FFAs inhibit insulin-stimulated glucose uptake (Boden et al. 1994; Homko et al. 2003). Our observations indicate that DHE recovered IR by activating IRS1/AKT signaling in PA-treated HepG2 and SNU-449 cells (Figs. 2a, b and 4e, f). Moreover, our results indicate that DHE downregulated gluconeogenesis markers in PA-treated HepG2 and SNU-449 cells (Figs. 2c, d and 4g, h) and promoted glucose uptake in PA-treated HepG2 cells (Fig. 2e). The effect of DHE on

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Fig. 4 DHE decreases lipid accumulation and restores IR and glucose metabolism in PA-treated SNU-449 cells. SNU-449 cells were treated with 0.25 mM PA or 0.25 mM PA and 10 μ M DHE for 24 h. **a** Lipid accumulation was confirmed in the treated SNU-449 using Nile Red staining (Scale bar = 50 μ m). **b** The stained cells were analyzed using Columbus software. **c** The treated cells were harvested and protein was isolated, and then the lipogenesis factors, including FAS, SCD1, Lipin1, and DGAT2, were measured using western blotting. **d** Relative protein levels of lipogenesis factors were assessed using the Image J program. Values were normalized to actin. **e** The insulin resistance factors, including p-AKT (S473) and p-IRS1 (S307), were evaluated using western blotting. **f** Relative protein levels of insulin resistance factors were assessed using the Image J program. Each value was normalized to AKT or IRS1. **g** The gluconeogenesis markers, such as *G6Pase*, *PEPCK*, and *PGC1 α* , were examined using western blotting. **h** Relative protein levels of gluconeogenesis markers were assessed using the Image J program. Values were normalized to actin. All data are indicated as mean \pm SD of at least three independent experiments. *** p < 0.001, ** p < 0.01, * p < 0.05. n = 3–5. CTL; control

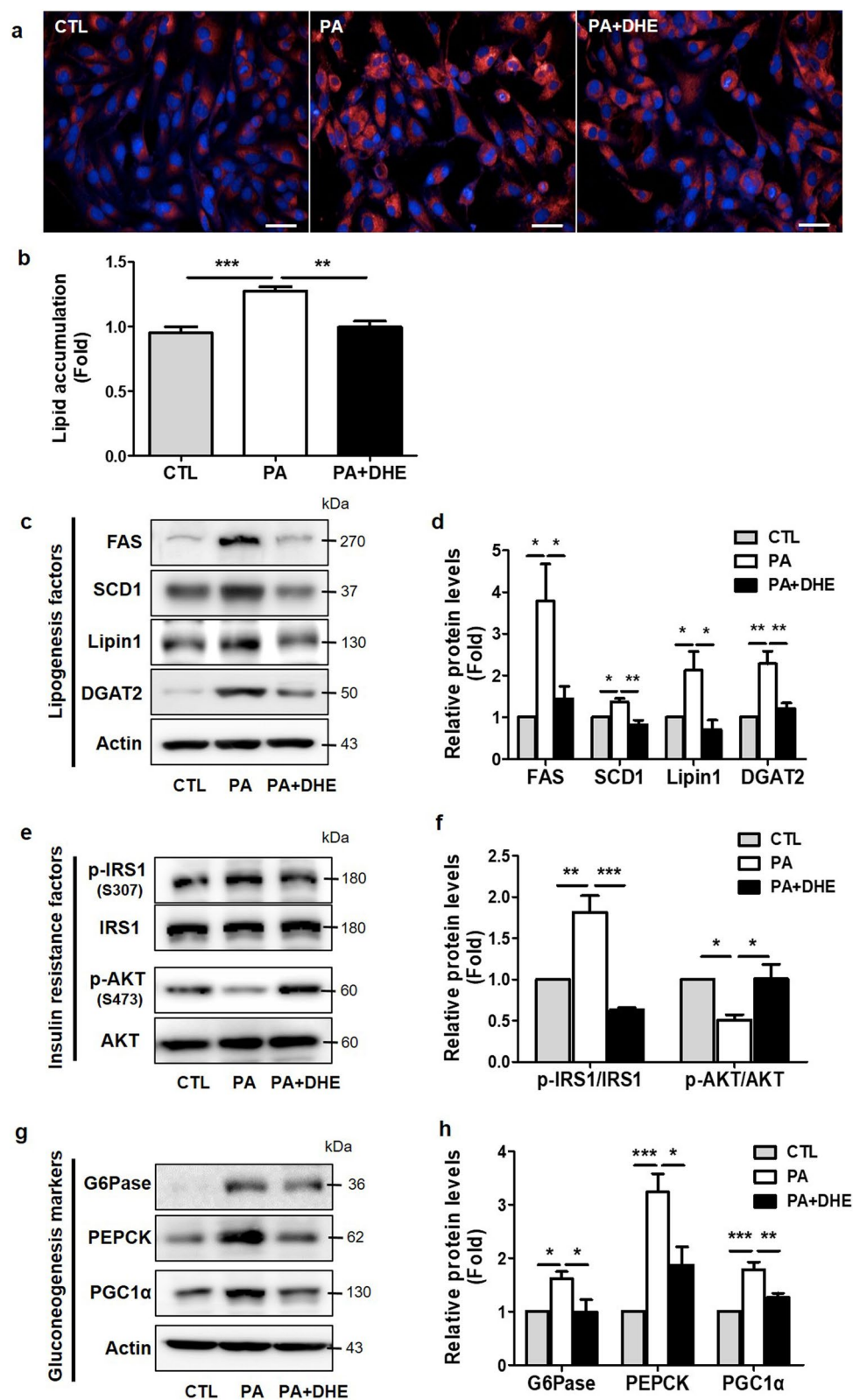


Fig. 4 (See legend on previous page.)

glucose uptake might result from decreased FA uptake and utilization.

ER stress has been reported to play an important role in both pathogenesis of steatosis and progression to NAFLD (Zhang et al. 2014). The various stress pathways by which ER stress activates IR and inflammation, or pathologically induces physiological mechanisms include fat accumulation, enhanced ROS production, inflammation, and apoptosis that play an important role in NAFLD (Sreejayan et al. 2008; Rahman et al. 2007). ROS production has been linked to ER stress and unfolded protein reaction, and redox homeostasis induces ER stress and can induce ROS production in the ER and mitochondria (Sangwung et al. 2020; Bhattarai et al. 2021). Moreover, the increased FFAs induce protein unfolding, and increased levels of unfolded proteins bind to PERK, IRE1 α , and ATF6 in the ER lumen, consequently inducing ER stress and activating the JNK pathway (Kaneto et al. 2010). Therefore, we hypothesized that DHE affects the ROS and ER stress signaling pathways. Consistent with this hypothesis, our results indicated that DHE inhibited ROS levels and ER stress, including the JNK pathway (Fig. 3). We propose that DHE reduces lipid accumulation with a decrease in ER stress and ROS level, and influences the regulation of IR and glucose metabolism. Future studies are needed to determine whether DHE has the same therapeutic effect in animal models with fatty liver.

Conclusions

In conclusion, we used an in vitro model of fatty hepatocytes by treating two types of hepatocytes, HepG2 and SNU-449, with PA to measure the antisteatotic efficacy of DHE. Our results showed that DHE decreases lipid accumulation and recovers IR and glucose metabolism by reducing ER stress and ROS levels in PA-induced fatty hepatocytes. Furthermore, treatment with a low concentration of DHE had no cytotoxicity and exerted antisteatotic effects on PA-induced fatty hepatocytes. Therefore, we propose that DHE could be used as a treatment for hepatic steatosis.

Abbreviations

DHE: Dehydrocostus lactone; ER: Endoplasmic reticulum; FFA: Free fatty acid; IR: Insulin resistance; NAFLD: Non-alcoholic fatty liver disease; PA: Palmitate; ROS: Reactive oxygen species; T2D: Type 2 diabetes; QEHS: Quadruple enhanced high sensitivity.

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Author contributions

YH, ML, and GK conceptualized and designed the study. YH, ML, and CK carried out the experiments. YH and ML drafted the manuscript. GK guided the research and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials are available on request.

Declarations

Competing interests

The authors declare that they have no conflict of interest.

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