Dansameum regulates hepatic lipogenesis and inflammation in vitro and in vivo

Sang Hyun Ahn¹ · Kang Pa Lee² · Kibong Kim³ · Jun-Yong Choi⁴ · Sun-Young Park⁵ · Jin Hong Cheon^{3,6,7}

Received: 12 June 2018 / Revised: 28 December 2018 / Accepted: 5 February 2019 / Published online: 14 February 2019
© The Korean Society of Food Science and Technology 2019

Abstract Although the clinical guidelines for nonalcoholic fatty liver disease (NAFLD) therapy recommended hepato-protection and exercise to reduce body weight, no established medication exists for NAFLD treatment. Thus, the effect of a candidate substance, dansameum (DSE), using an in vitro and NAFLD mouse model (that is, apolipoprotein E-Knockout mice), were investigated. The molecular pathways for lipogenesis and inflammation were evaluated using Nile staining, Western blotting, reverse transcription-polymerase chain reaction, and immunohistochemistry. It was shown that DSE significantly ameliorated the production of lipogenesis-related factors,

including liver X receptor- α , peroxisome proliferator-activated receptor- γ , sterol regulatory element binding protein-1, fatty acid synthase, acetyl-CoA carboxylase 1, and CD36. In addition, DSE significantly reduced the production of inflammation factors, including interleukin-1 β , interleukin-6, and nuclear factor kappa B. Furthermore, DSE significantly reduced the phosphorylation of c-Jun amino terminal kinase. Taken together, this suggests that DSE may be a functional food candidate for regulating NAFLD, based on its effects.

Keywords Nonalcoholic fatty liver disease · Lipogenesis · Inflammation · Steatosis · Traditional Chinese medicine · Functional foods

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10068-019-00579-8>) contains supplementary material, which is available to authorized users.

✉ Jin Hong Cheon
cheonjh-pnu@naver.com

Sang Hyun Ahn
dongana@hanmail.net

Kang Pa Lee
gostop48@konkuk.ac.kr

Kibong Kim
kkb2630@gmail.com

Jun-Yong Choi
kmd@pusan.ac.kr

Sun-Young Park
sue-7@hanmail.net

¹ Department of Anatomy, College of Korean Medicine, Semyung University, Semyung-ro 65, Jecheon-si, Chungcheongbuk-do 27136, Republic of Korea

² Department of Medical Science, School of Medicine, Konkuk University, Neungdong-ro 120, Gwangjin-gu, Seoul-si 05029, Republic of Korea

³ Department of Korean Pediatrics, School of Korean Medicine, Pusan National University, Pusandaehak-ro 47, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do 50612, Republic of Korea

⁴ Department of Internal Medicine, School of Korean Medicine, Pusan National University, Pusandaehak-ro 47, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do 50612, Republic of Korea

⁵ Department of Physiology, College of Korean Medicine, Semyung University, Semyung-ro 65, Jecheon-si, Chungcheongbuk-do 27136, Republic of Korea

⁶ Biomedical Research Institute, Pusan National University Hospital, Gudeok-ro 179, Seo-gu, Busan-si 49241, Republic of Korea

⁷ Department of Korean Pediatrics, Hospital of Korean Medicine, Pusan National University, 20 Geumo-ro, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do 50612, Republic of Korea

Abbreviations

NALFD	Non-alcoholic fatty liver disease
TCM	Traditional Chinese medicine
DSE	Dansameum
HF	High-fat
IL	Interleukin
TG	Triglycerides
LXR- α	Liver X receptor- α
SREBP-1	Sterol regulatory element binding protein-1
ACC1	Acetyl-CoA carboxylase 1
FAS	Fatty acid synthase
SCD	Stearoyl-CoA desaturase
NF- κ B	Nuclear factor kappa B
TNF- α	Tumor necrosis factor α
JNK	c-Jun amino terminal kinase
PA	Palmitic acid
ApoE ^{-/-}	Apolipoprotein E knock-out mice

Introduction

The major functions of the liver in fat metabolism can be classified into generating energy sources, converting cholesterol into bile acid, and storing triglycerides (TG) in fat tissue. However, the abnormal accumulation of TG in the liver (steatosis) is potentially associated with the development of hepatitis and cirrhosis (Bruha et al., 2012; Gao and Bataller, 2011; Nseir et al., 2014). Various side effects from antiarrhythmic, antiviral, steroid, and cytotoxic drugs can contribute to the development of nonalcoholic fatty liver disease (NAFLD) (Tolman and Dalpiaz, 2007; Willebrords et al., 2015). However, the incidence of NAFLD is most commonly associated with metabolic syndromes, such as diabetes, hypertriglyceridemia, and obesity (Paschos and Paletas, 2009). The most commonly used animal models in NAFLD studies are high-fat diets and apolipoprotein deficient (ApoE^{-/-}) mice (Van Herck et al., 2017). The clinical characteristics of NAFLD are excessive TG accumulation and inflammation in the liver (Liu et al., 2010). The main mechanism of lipogenesis is regulated through liver X receptor- α (LXR- α); and the well-known mediators of TG accumulation include sterol regulatory element binding protein-1 (SREBP-1), acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD). However, fat metabolism inevitably produces reactive oxygen species that trigger inflammatory pathways involving nuclear factor kappa B (NF- κ B), tumor necrosis factor α (TNF- α), and c-Jun amino terminal kinase (JNK), which leads to hepatic inflammation (Koo, 2013; Seki et al., 2012). Thus, recent studies regarding NAFLD have focused on inhibition of the LXR- α -related pathway for lipogenesis and inflammation.

However, few studies regarding NAFLD have identified medicines with clear efficacy, and the clinical guidelines focus on preventing disease progression using existing hepato-protective medicines and lifestyle modification (Fabbrini et al., 2010). Nevertheless, some studies have indicated that natural products can have hepato-protective effects, which may make them useful for treating NAFLD (Chen et al., 2017; Park et al., 2015).

Recent reports indicated that consumers prefer functional foods above all for their health benefits in order to prevent a variety of diseases including lifestyle diseases (Hasler, 2002). This preference, related to the development of functional foods based on Korean traditional medicine (KTM), has been gaining increasing attention (Park and Kim, 2014). A functional food candidate, dansameum (DSE), is a decoction that is created using *Salvia miltiorrhiza* root, *Amomum xanthioides* fruit, and *Santalum album lignum*. This herbal medicine, a KTM, has been reported with beneficial effects on blood circulation, oxidative stress, and inflammation (Jeong et al., 2011). Therefore, this present study examined the hepato-protective effects of DSE in mice that received a high-fat (HF) diet and in palmitic acid (PA)-induced HepG2 cells.

Materials and methods

Preparation of DSE

A crude decoction was prepared using *Salvia miltiorrhiza* root (40 g), *Amomum xanthioides* fruit (4 g), and *Santalum album lignum* (4 g) in 500 mL of sterile deionized water, which was heated at 100 °C for 3 h. The decoction product was concentrated and lyophilized by freeze-drying at -60 °C using a Dongbang VFDL03-50 freeze-dryer (Dongbang Hitech incorporation, Seongdong-gu, Seoul, Korea).

Cell culture

HepG2 cells (a human hepatoma cell line) were purchased from the American Type Culture Collection (Rockville, MD, USA). The cells were maintained at 37 °C and 5% CO₂ in Dulbecco's modified Eagle's medium containing 200 mg/L of L-glutamine (Hyclone, Logan, UT, USA), 10% (v/v) heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 100 U/mL penicillin (Invitrogen), and 100 µg/mL of streptomycin (Invitrogen).

Nile red staining

The HepG2 cells were induced using 0.5 mM PA in the presence or absence of DSE (100 µg/mL and 200 µg/mL).

After incubation, the cells were fixed using 4% paraformaldehyde for 15 min at room temperature. After washing using phosphate-buffered saline, intracellular neutral lipids were stained using Nile red (100 ng/mL) for 5 min at room temperature. Images were acquired using an Olympus FV-1000 confocal laser scanning microscope (Olympus Corporation, Hachioji-shi, Tokyo, Japan).

Reverse transcription-polymerase chain reaction

Total RNA were extracted from the HepG2 cells using TRIzol reagent (Invitrogen). First-strand cDNA were synthesized using the Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's protocol. The expressions of target genes for reverse transcription-polymerase chain reaction (RT-PCR) were normalized to that of glyceraldehyde-3-phosphate dehydrogenase. The PCR was performed using specific primers for LXR- α (sense, 5'-ACAACCTGGGCATGATCGAGA-3'; antisense, 5'-AAGC CGGGTAGCTGTTTAGC-3'), PPAR- γ (sense, 5'-AGAG CCTTCCAACCTCCCTCA-3'; antisense, 5'-CAAGGCATT TCTGAAACCGA-3'), SREBP-1 (sense, 5'-CTGAACTGT GTGACCCAGCC-3'; antisense, 5'-TCTCAGACTCCTGC AGCACC-3'), ACC1 (sense, 5'-GGAACAGTGTGCGGTG AACAC-3'; antisense, 5'-GTGGCTCCACAGACGATGAG-3'), FASN (sense, 5'-GACGTCTGCAAGCCCAAGTA-3'; antisense, 5'-CATCGTCTCCACCAAATGC-3'), SCD1 (sense, 5'-GCCCCCTACTTGGGAAGACG-3'; antisense, 5'-GTGGCTCCACAGACGATGAG-3'), CD36 (sense, 5'-TCAGCAAATGCAAAGAAGGG-3'; antisense, 5'-CACC AATGGTCCCAGTCTCA-3'), IL-1 β (sense, 5'-TTCTTT CCCTTCATCTTTGA-3'; antisense, 5'-ACCACTTGTTG CTCCATATC-3'), IL-6 (sense, 5'-ACTCACCTCTTCAG AACGAA-3'; antisense, 5'-CTCAAACCTCCAAAAGACC AG-3'), and glyceraldehyde-3-phosphate dehydrogenase (sense, 5'-AAC CTG CCA AAT ATG ATG AC-3'; antisense, 5'-ATA CCA GGA AAT GAG CTT GA-3').

Western blot analysis

Cells were cultured and induced using 0.5 mM PA in the presence or absence of DSE (100 μ g/mL and 200 μ g/mL). The cell pellets were subsequently resuspended in a lysis buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 150 mM NaCl, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and a protease inhibitor cocktail), and the proteins were separated in 10% reducing SDS-PAGE gels before being transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA) using 20% methanol, 25 mM Tris, and 192 mM glycine. The membranes were blocked using 5% non-fat dry milk and incubated overnight with the primary antibodies at 4 °C before washing. The

membranes were then incubated for 1 h with horseradish-peroxidase conjugated secondary antibodies, subjected to further wash steps, and then developed using an enhanced chemiluminescence system (SuperSignal® West Femto; Thermo Scientific, Vernon Hills, IL, USA).

Animal care

Apolipoprotein E knockout (ApoE^{-/-}) mice (6-week-old male, 18–20 g) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and used for the experiment, after confirmation by genotyping method (Supplementary data). All procedures involving animals were approved by the Institutional Animal Care and Use Committee at Dongguk University (2014-010), and the experimental protocols complied with the NIH Guide for the Care and Use of Laboratory Animals. All mice were housed individually in stainless steel cages with ad libitum access to water and food. The ApoE^{-/-} mice were randomized into three groups: a control group that received the AIN 93G stock diet, a NAFLD-induced group that received a HF diet (60% fat, 20% carbohydrates, and 20% protein) for 12 weeks, and a DSE treatment group that received DSE during the 12 weeks of the HF diet. The DSE was administered orally every day at a dose of 240 mg/kg (dissolved in 50 μ L of normal saline) for 12 weeks. All mice were anesthetized using an intraperitoneal injection of sodium pentobarbital (25 μ g/10 g body weight).

Immunohistochemistry

The mice were perfused and fixed using 10% formalin for 4 min, and then the liver specimens were prepared according to the routine procedures for cryosectioning. The 5- μ m sections were placed in 0.3% hydrogen peroxide for 3 min to block endogenous peroxidase activity, and then blocked using 5% goat serum for 1 h. The tissues were subsequently incubated for 72 h at 4 °C in 3% goat serum containing the various primary antibodies. Antibodies to LXR, SREBP-1, CD36, 8-oxoguanine (8-OXG), PPAR- γ , TNF- α , NF- κ B, phosphorylated NF- κ B, IL-1 β , IL-6, β -actin, and lamin A/C were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies to JNK and phosphorylated JNK were obtained from Cell Signaling Technology (Danvers, MA, USA). The tissues were subsequently washed 3 times using phosphate-buffered saline, and the Vectastain ABC Kit was used to induce the avidin-biotin complex interaction, according to the manufacturer's instructions. Immunostaining analysis was performed using a substrate solution of 0.05% DAB, and the slides were counterstained using hematoxylin. All sections were evaluated using a light microscope (Olympus

Fig. 1 Effects of Dansameum (DSE) on the accumulation of intracellular lipids. (A) HepG2 cells were pretreated using DSE for 16 h, and then incubated with 0.5 mM palmitic acid (PA) for 8 h before staining using Nile red. (B) Statistical results obtained from

panel (A). Intracellular lipid droplets were evaluated using fluorescence microscopy (magnification $\times 400$). Data are presented as mean $\pm$ standard error ($n = 6$). * $p < 0.05$ versus the PA-stimulated response in the absence of DSE treatment. (Color figure online)

Fig. 2 Effects of Dansameum (DSE) on lipogenesis pathways in HepG2 cells. HepG2 cells were pretreated using DSE for 16 h, and then stimulated using 0.5 mM palmitic acid (PA) for 8 h. RT-PCR and gel electrophoresis were used to evaluate mRNA expression levels. (A) The mRNA expressions of lipogenesis-related receptors: liver X receptor- α (LXR- α) and peroxisome proliferator-activated receptor gamma (PPAR- γ). (B and C) The mRNA expressions of LXR- α and PPAR- γ , relatively to PA-stimulated cells without DSE

pretreatment ($n = 6$). (D) The mRNA expressions of lipogenesis-related transcription factors: sterol regulatory element binding protein-1 (SREBP-1), acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD)-1, and CD36. (E–I) The protein expressions of SREBP-1, ACC1, FAS, SCD-1, and CD36, relative to PA-stimulated cells without DSE pretreatment ($n = 6$). Data are presented as mean $\pm$ standard error. * $p < 0.05$ versus the PA-stimulated response in the absence of DSE treatment

Fig. 3 Protective effect of Dansameum (DSE) on lipogenesis pathways in the liver of ApoE^{-/-} mice that received a high-fat (HF) diet. Photomicrographs of the untreated ApoE^{-/-} mice, mice that received only the HF diet for 12 weeks, and mice that received 240 mg/kg of DSE at 12 weeks after the HF diet. (A) Hematoxylin and eosin staining (bar = 100 μm), the red lines indicates the inflammation and damaged areas. (B) Liver tissue sections that were stained using Oil

Red O (bar = 100 μm). (C) The graph indicates the intensity of Oil Red O stain from panel (B). (D) Immunohistochemical staining for LXR, PPAR-γ, SREBP-1, and CD36. (E–H) The expressions of LXR-α, PPAR-γ, SREBP-1, and CD36 in ApoE^{-/-} mice (n = 6). Data are presented as mean ± standard error. **p* < 0.05 versus the HF-diet response in the absence of DSE treatment in ApoE^{-/-} mice

BX50; Olympus Co. Ltd., Hachioji-shi, Tokyo, Japan) at × 200 magnification.

Statistical analysis

The statistical data were expressed as mean ± standard error, based on at least three independent experiments. All data were analyzed using GraphPad Prism software (Prism 4.00 for Windows; GraphPad, San Diego, CA, USA), based on Student's *t* test for inter-group differences and one-way analysis of variance with Tukey's test for multiple comparisons. *p* values of < 0.05 were considered statistically significant.

Results and discussion

DSE inhibits PA-induced cellular steatosis

NAFLD is characterized by chronic inflammation and hepatic steatosis that is caused by excessive TG accumulation (Liu et al., 2010). Experimental design refers to previous study (Park et al., 2015). Therefore, we investigated whether DSE regulates steatosis in vitro using Nile staining of PA-induced steatosis in HepG2 cells. That the PA caused the formation of intracellular lipid droplets (indicated in red) are shown in Fig. 1. However, DSE (100 μg/mL and 200 μg/mL) significantly reduced the intensity of the Nile staining, which indicates that it suppressed the cellular steatosis.

Fig. 4 Effects of Dansameum (DSE) on the phosphorylation of c-Jun amino terminal kinase (JNK) in HepG2 cells. **(A)** A total of 1×10^5 HepG2 cells were pretreated using DSE for 16 h, and then incubated with 0.5 mM palmitic acid (PA) for 8 h. Whole-cell lysates were prepared and protein expressions were determined using Western

blotting. **(B)** The relative expressions of phosphorylated JNK, relative to the untreated group (n = 6). Data are presented as mean $\pm$ standard error. * $p < 0.05$ versus the PA-stimulated response in the absence of DSE treatment

DSE suppresses the expressions of lipogenesis-related factors in PA-induced HepG2 cells and HF diet-fed ApoE^{-/-} mice

Immunohistochemistry and RT-PCR were used to investigate whether DSE regulated lipogenesis-related receptors and factors in PA-induced HepG2 cells and HF diet-fed ApoE^{-/-} mice. Total RNA were isolated from HepG2 cells that were or were not pretreated using DSE (100 μg/mL and 200 μg/mL) for 16 h, and then stimulated using PA (0.5 mM) for an additional 8 h. That DSE decreased the mRNA expressions of PA-induced LXR-α and PPAR-γ, as well as the mRNA expressions of lipogenesis-related factors, such as SREBP1, ACC1, FAS, SCD1, and CD36, are shown in Fig. 2. Compared to normal fed ApoE^{-/-} mice, the livers of HF diet-fed ApoE^{-/-} mice exhibited increased inflammation, fat accumulation, and protein expressions of LXR, PPAR-γ, SREBP1, and CD36 (Fig. 3). However, DSE treatment (240 mg/kg) decreased the protein expressions of these lipogenesis related-factors, compared to the HF diet-fed ApoE^{-/-} mice. Fat accumulation may be modulated by the LXR-α receptor, which leads to increased expression of various transcription factors, such as SREBP1c, ACC1, FAS, and SCD1 (Kim et al., 2015). Thus, an LXR agonist has been shown to inhibit the expression of these transcription factors in PA-induced HepG2 cells (Naura et al., 2009). In ApoE^{-/-} mice, the NAFLD-related inflammation and steatosis were induced by the 12-week HF diet (Ress and Kaser, 2016), and the present study revealed that DSE had potentially pharmacological effects on PA-induced HepG2 cells and the livers of HF diet-fed ApoE^{-/-} mice.

Although recent studies have examined natural products for treating NAFLD, there were no available prescription drugs. Interestingly, we observed that DSE affected the

genetic and protein expressions of lipogenesis-related and inflammation factors in PA stimulated-HepG2 cells and in the livers of HF diet-fed ApoE^{-/-} mice. For example, DSE affected the expression of LXR-α, which plays a central role in regulating cytoplasmic transcription factors (e.g., SREBP-1c, AAC, FAS, and SCD1) that are involved in de novo lipogenesis (Guo et al., 2015; Kim et al., 2015; Schultz et al., 2000). Therefore, we suggest that beverages or tea containing DSE may be helpful in inhibiting fat accumulation.

Based on our results, consuming DSE during exercise may have a combined effect on fat accumulation. However, this approach must be balanced with the potential use of fat as an energy source. In addition, although we confirmed the inhibitory effect of DSE on steatosis, we did not examine the activation of carbohydrate mechanisms and signaling pathways in the liver during exercise. Thus, further studies are needed to understand the potential effects of DSE consumption during exercise.

DSE modulates the phosphorylation of JNK, the expressions of inflammatory cytokines and NF-κB activation in PA-induced HepG2 cells and in HF diet-fed ApoE^{-/-} mice

The JNK pathway is critical for free fatty acid-mediated signal transduction, and JNK activation is implicated in NAFL (Papa et al., 2009). We examined whether DSE regulated JNK phosphorylation in PA-induced HepG2 cells and in HF diet-fed ApoE^{-/-} mice. That DSE pretreatment suppressed the phosphorylation of JNK in vitro is shown in Fig. 4. Immunohistochemistry and RT-PCR were used to assess the inhibitory effect of DSE on the steatosis-induced inflammatory response. DSE (100 μg/mL and 200 μg/mL) inhibited the overexpression of pro-inflammatory

Fig. 5 Effects of Dansameum (DSE) on the expressions of pro-inflammatory cytokines and NF- κ B activation in HepG2 cells. HepG2 cells were pretreated using DSE for 16 h, and then incubated with 0.5 mM palmitic acid (PA) for 8 h. **(A)** The mRNA expressions of IL-1 β and IL-6 were determined using RT-PCR. **(B and C)** The protein expressions of IL-1 β and IL-6, relative to PA-stimulated cells without DSE pretreatment ($n = 6$). **(D and F)** Whole-cell lysates and nuclear

fractions were prepared to evaluate protein expressions using Western blot analysis. **(E and G)** The phosphorylation of NF- κ B in whole cells and the nucleus, relative to PA-stimulated cells without DSE pretreatment ($n = 6$). Data are presented as mean $\pm$ standard error. * $p < 0.05$ versus the PA-stimulated response in the absence of DSE treatment

cytokines, such as IL-1 β and IL-6, in PA-induced HepG2 cells (Fig. 5). In addition, DSE inhibited the cytoplasmic phosphorylation and nuclear translocation of NF- κ B. That DSE treatment significantly inhibited the over-expression of phosphorylated JNK, IL-1 β , IL-6, TNF- α , and NF- κ B in HF diet-fed ApoE $^{-/-}$ mice are shown in Fig. 6.

We suggest that medical treatments of NAFLD must target at least two of its complex pathways. First, they must block TG accumulation in the liver, as excessive TG accumulation plays a role in chronic inflammation and subsequent liver cell death (Brenner et al., 2013; Fabbrini et al., 2010). Numerous studies have indicated that obesity is associated with NAFLD (Lara-Castro and Garvey,

2008), and the major pathogenic mechanism in liver disease involves insulin resistance that is driven by excessive TG accumulation (Birkenfeld and Shulman, 2014). Although insulin secreted from the pancreas after meals normally increases lipogenesis, as well as reduces lipolysis and oxidation of fatty acids in the mitochondria, insulin resistance can lead to elevated levels of insulin and subsequent increase in the risk of diabetes and metabolic syndrome (Ayala et al., 2014; Yki-Järvinen, 2005). Therefore, we suggest that NAFLD therapy must aim at preventing or inhibiting TG accumulation in the liver. Second, we believe that NAFLD therapy must help limit the reactive oxygen species production in the liver, despite the fact that this production is inevitable during lipid

Fig. 6 Effects of Dansameum (DSE) on JNK, pro-inflammatory cytokines, and NF-κB activation in ApoE^{-/-} mice that received a high-fat (HF) diet. Photomicrographs of the untreated ApoE^{-/-} mice, mice that received only the HF diet for 12 weeks, and mice that received 240 mg/kg of DSE at 12 weeks after the HF diet. **(A)** the ROS marker indicates 8-oxoguanine (8-OXG), (bar = 100 μm) **(B)** The graph indicates the intensity of ROS stain from panel **(A)**.

(C) JNK expression in mouse liver tissues (bar = 100 μm). **(D)** The relative expressions of phosphorylated JNK in ApoE^{-/-} mice (n = 6). **(E)** Immunohistochemical staining for LXR-α, PPAR-γ, SREBP-1, and CD36. **(F–I)** The expressions of LXR-α, PPAR-γ, SREBP-1, and CD36 in ApoE^{-/-} mice (n = 6). Data are presented as mean ± standard error. **p* < 0.05 versus the HF-diet response in the absence of DSE treatment in ApoE^{-/-} mice

metabolism. Furthermore, excessive oxidative stress can trigger the JNK pathway, which is associated with inflammation in hepatocytes (Higuchi et al., 2008). Therefore, we speculate that multi-faceted medical treatments are needed to prevent TG accumulation and oxidative stress during the treatment of NAFLD. Furthermore, DSE ameliorated the PA-stimulated expressions of JNK, TNF-α, IL-1β, and IL-6, as well as the molecular expressions of JNK, pro-inflammation cytokines, and NF-κB.

The reason for NAFLD is that oxidative stress induces JNK pathway and increases the expression of TNF-α, IL-1β, and IL-6 (Turner et al., 2007). Recent studies have shown various biological activities of DSE, including that of inhibiting toxicity as well as an anti-atherosclerotic

activity (Jeong et al., 2011; Park et al., 2018). In particular, that DSE inhibited the overexpression of oxidative stress is shown in Fig. 6. Therefore, we speculate that DSE has a strong anti-oxidative and various biological effects.

In conclusion, DSE regulated the molecular levels of lipogenesis-related factors, such as LXR-α, SREBP-1, ACC1, and SCD-1. In addition, DSE regulated the inflammatory response in PA-induced HepG2 cells and HF diet-fed ApoE^{-/-} mice, by controlling the expressions of IL-1β, IL-6, TNF-α, and NF-κB, as well as the phosphorylation of JNK. Based on these results, we suggest that DSE could help improve NAFLD by inhibiting TG accumulation, as well as its antioxidant effects on the anti-inflammatory response.

Acknowledgements This paper was supported by the Research Program of Pusan National University hospital.

Authors' contributions SHA and KPL are contributed equally, to the study conception and experimental design, collected data, and performed analyses. KBK and JYC performed the histological examination of the liver, SYP studied ROS-related factors and was a major contributor in writing the manuscript JHC participated in the study conception. All authors read and approved the final manuscript.

Funding This study was supported by Biomedical Research Institute Grant (2016-10), Pusan National University Hospital.

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interests.

Availability of data and material All data generated or analyzed during this study are included in this published article.

Ethics approval All procedures involving animals were approved by the Institutional Animal Care and Use Committee at Dongguk University (2014-010).

References

- Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell. Longev* 2014: 360438 (2014)
- Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. *Hepatology* 59: 713–723 (2014)
- Brenner C, Galluzzi L, Kepp O, Kroemer G. Decoding cell death signals in liver inflammation. *J. Hepatol* 59: 583–594 (2013)
- Bruha R, Dvorak K, Pettryl J. Alcoholic liver disease. *World J. Hepatol* 4: 81–90 (2012)
- Chen Q, Wang T, Li J, Wang S, Qiu F, Yu H, Zhang Y, Wang T. Effects of natural products on fructose-induced nonalcoholic fatty liver disease (NAFLD). *Nutrients* (2017). <https://doi.org/10.3390/nu9020096>.
- Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 51: 679–689 (2010)
- Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 141: 1572–1585 (2011)
- Guo R, Liong EC, So KF, Fung ML, Tipoe GL. Beneficial mechanisms of aerobic exercise on hepatic lipid metabolism in non-alcoholic fatty liver disease. *Hepatobiliary Pancreat. Dis. Int.* 14: 139–144 (2015)
- Hasler CM. Functional foods: benefits, concerns and challenges—a position paper from the American council on science and health. *J. Nutr.* 132: 3772–3781 (2002)
- Higuchi N, Kato M, Shundo Y, Tajiri H, Tanaka M, Yamashita N, Kohjima M, Kotoh K, Nakamura M, Takayanagi R, Enjoji M. Liver X receptor in cooperation with SREBP-1c is a major lipid synthesis regulator in nonalcoholic fatty liver disease. *Hepatol. Res.* 38: 1122–1129 (2008)
- Jeong HJ, Choi Y, Kim MH, Kang IC, Lee JH, Park C, Park R, Kim HM. Rosmarinic acid, active component of Dansam-Eum attenuates ototoxicity of cochlear hair cells through blockage of caspase-1 activity. *PLoS ONE*. 6: e18815 (2011)
- Kim DI, Park MJ, Lim SK, Park JI, Yoon KC, Han HJ, Gustafsson JA, Lim JH, Park SH (2015) PRMT3 regulates hepatic lipogenesis through direct interaction with LXR α . *Diabetes* 64: 60–71.
- Koo SH. Nonalcoholic fatty liver disease: molecular mechanisms for the hepatic steatosis. *Clin. Mol. Hepatol.* 19: 210–215 (2013)
- Lara-Castro C, Garvey WT. Intracellular lipid accumulation in liver and muscle and the insulin resistance syndrome. *Endocrinol. Metab. Clin. North Am.* 37: 841–856 (2008)
- Liu Q, Bengmark S, Qu S. The role of hepatic fat accumulation in pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Lipids Health Dis.* 9: 42 (2010)
- Naura AS, Hans CP, Zerfaoui M, Errami Y, Ju J, Kim H, Matrougui K, Kim JG, Boulares AH (2009) High-fat diet induces lung remodeling in ApoE-deficient mice: an association with an increase in circulatory and lung inflammatory factors. *Lab. Invest.* 89: 1243–1251.
- Nseir W, Hellou E, Assy N. Role of diet and lifestyle changes in nonalcoholic fatty liver disease. *World J. Gastroenterol.* 20: 9338–9344 (2014)
- Papa S, Bubici C, Zazzeroni F, Franzoso G. Mechanisms of liver disease: cross-talk between the NF-kappaB and JNK pathways. *Biol. Chem.* 390: 965–976 (2009)
- Park H, Kim HS. Korean traditional natural herbs and plants as immune enhancing, antidiabetic, chemopreventive, and antioxidative agents: a narrative review and perspective. *J. Med. Food.* 17: 21–27 (2014)
- Park H, Hwang YH, Kim DG, Jeon J, Ma JY. Hepatoprotective effect of herb formula KIOM2012H against nonalcoholic fatty liver disease. *Nutrients* 7: 2440–2455 (2015)
- Park KS, Ahn SH, Lee KP, Park SY, Cheon JH, Choi JY, Kim K (2018) The natural compound dansameum reduces foam cell formation by downregulating CD36 and peroxisome proliferator-activated receptor-gamma; Expression. *Pharmacogn. Mag.* 13(Suppl 4): S868–S874
- Paschos P, Paletas K. Non alcoholic fatty liver disease and metabolic syndrome. *Hippokratia* 13: 9–19 (2009)
- Ress C, Kaser S. Mechanisms of intrahepatic triglyceride accumulation. *World J. Gastroenterol.* 22: 1664–1673 (2016)
- Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, Lustig KD, Shan B. Role of LXRs in control of lipogenesis. *Genes. Dev.* 14: 2831–2838 (2000)
- Seki E, Brenner DA, Karin M. A liver full of JNK: signaling in regulation of cell function and disease pathogenesis, and clinical approaches. *Gastroenterology* 143: 307–320 (2012)
- Tolman KG, Dalpiaz AS. Treatment of non-alcoholic fatty liver disease. *Ther. Clin. Risk Manag.* 3: 1153–1163 (2007)
- Turner NA, Mughal RS, Warburton P, O'Regan DJ, Ball SG, Porter KE. Mechanism of TNF α -induced IL-1 α , IL-1 β and IL-6 expression in human cardiac fibroblasts: effects of statins and thiazolidinediones. *Cardiovasc. Res.* 76: 81–90 (2007)
- Van Herck MA, Vonghia L, Francque SM. Animal models of nonalcoholic fatty liver disease—a starter's guide. *Nutrients*. 9: pii: E1072 (2017)
- Willebrords J, Pereira IV, Maes M, Crespo Yanguas S, Colle I, Van Den Bossche B, Da Silva TC, de Oliveira CP, Andraus W, Alves VA, Cogliati B, Vinken M. Strategies, models and biomarkers in experimental non-alcoholic fatty liver disease research. *Prog. Lipid Res.* 59: 106–125 (2015)
- Yki-Järvinen H. Fat in the liver and insulin resistance. *Ann. Med.* 37: 347–356 (2005)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.