The Herbal Composition Gangjihwan from *Ephedra intermedia*, *Lithospermum erythrorhizon* and *Rheum palmatum* Ameliorates Hepatic Inflammation and Fibrosis in Obese C57BL/6J Mice and HepG2 Cells

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It was demonstrated that Gangjihwan (DF), which is the herbal composition composed of *Ephedra intermedia*, *Lithospermum erythrorhizon*, and *Rheum palmatum*, inhibits obesity and hepatic steatosis in high fat diet (HFD)-fed obese mice. The aim of this study was to determine the effects of DF on visceral obesity, hepatic inflammation and fibrosis and the mechanism of actions involved in this process using *in vivo* and *in vitro* approaches. DF was extracted with water (DF-FW), 30% grain alcohol (DF-GA30), and 70% grain alcohol (DF-GA70). Administration of DF to HFD-fed control mice decreased visceral tissue mass and visceral adipocyte size without adverse effects. Visceral fat mass was decreased by DF-GA30 and DF-GA70, and visceral adipocyte size by all three DF extracts compared with obese control mice. Histological analysis revealed that three kinds of DF extracts reduced toluidine blue-stained mast cells and collagen accumulation in the liver, the extents of which were most eminent in DF-GA70-treated mice. DF-GA70 decreased the mRNA levels of the inflammation (*TNFα* and *VCAM-1*), fibrosis (*α-SMA*), and apoptosis (*caspase 3*) genes, but increasing the anti-apoptosis gene (*Bcl-2*) mRNA levels in the liver of obese control mice. Consistent with the *in vivo* data, GA-70 also altered the expression of inflammation genes (*TNFα* and *MCP-1*) in HepG2 cells. These results indicate that DF not only inhibits visceral obesity, but also ameliorates visceral obesity-induced hepatic inflammation and fibrosis and that this process may be mediated by regulating the hepatic expression of inflammatory and fibrogenic genes.

**Key Words:** Herbal medicine, Visceral obesity, Hepatic inflammation, Hepatic fibrosis

Nonalcoholic fatty liver disease (NAFLD) is a pathological condition that covers from lipid accumulation in the liver (simple steatosis) to nonalcoholic steatohepatitis (NASH), fibrosis, and irreversible cirrhosis. NAFLD is a main cause of liver disease worldwide, with the prevalence between 10 to 30%. The prevalence of NAFLD has risen drastically in parallel with a rapid increase in obesity (Charlton, 2004; Vuppalanchi and Chalasani, 2009). In Western society, the NAFLD prevalence is estimated to be 20–30%, whereas it rises to 57–74% among obese patients, increasing up to 90% in morbidly obese subjects (Angulo, 2002; Machado et al., 2006; Milic and Stimac, 2012). There is evidence showing that NAFLD prevalence in the Asian countries is also rapidly growing due to the surge in obesity (Park et al., 2006; Fan, 2013; Chan et al., 2013; Koh et al., 2016).

Visceral obesity is particularly associated with NAFLD (Van der Poorten et al., 2008). For example, NAFLD is more common among Asians than among Caucasians, because visceral obesity occurs more frequently among Asians than among Caucasians with similar body mass index (Petersen...
et al., 2006; Schwimmer et al., 2006). It is known that the area and amount of visceral adipose tissue are deeply associated with the severity of hepatic inflammation and fibrosis in NAFLD patients (Van der Poorten et al., 2008; Yu et al., 2015).

Despite the large numbers of drugs used to treat NAFLD and NASH, there is no current established therapy for NAFLD and NASH other than lifestyle modifications. Because NAFLD occurs primarily in obese patients, weight reduction through lifestyle intervention, bariatric surgery, and drugs is the first-line treatment for all NAFLD patients. Current potential medications for NAFLD include insulin sensitizers such as metformin and thiazolidinediones, lipid-lowering drugs, antioxidants, glucagon-like peptide 1 analogues, and omega-3 fatty acids. However, since long-term intake of these drugs increases the risk of cancer, vascular disease, and depression, there is an urgent need for effective and safe therapeutic prescriptions to overcome NAFLD. Recent studies show that medicinal plants inhibit obesity and improve metabolic disorders including hyperlipidemia, insulin resistance, and NAFLD (Jeong et al., 2008; Shin et al., 2010; Roh et al., 2015; Yoon et al., 2017). Gangjihwan (DF), which is the herbal composition composed of three medicinal plants *Ephedra intermedia* Schrenk et C. A. Mayer, *Lithospermum erythrorhizon* Siebold et Zuccarini, and *Rheum palmatum* L., has been used to treat obesity in local clinics in Korea, although the mechanism of its action remains poorly understood.

Therefore, the effects of DF on visceral obesity and visceral obesity-induced hepatic inflammation and fibrosis were examined in high fat diet (HFD)-induced obese C57BL/6J mice and HepG2 cells, and investigated the histological and molecular events involved in this process. The present results demonstrate that DF inhibits visceral obesity, infiltration of inflammatory cells into the liver, and hepatic collagen accumulation and that this process may be mediated by regulating the hepatic expression of inflammation and fibrosis genes. Thus, DF may attenuate visceral obesity-induced hepatic fibroinflammation.

DF was prepared from food-grade extracts of the three medicinal herbs (40% *Ephedra intermedia*, 40% *Lithospermum erythrorhizon*, and 20% *Rheum palmatum*; expressed as % dry weight of DF) (Hwalim, Busan, Korea). The proportions used in this study are same as those used to treat patients in local clinics of Korea. Briefly, dried herbs were individually powdered and extracted with water (DF-FW), 30% grain alcohol (DF-GA30), and 70% grain alcohol (DF-GA70). The extracts were freeze-dried under vacuum and mixed for DF production. The extraction yields were 22.7%, 26.33%, and 22.9% for DF-FW, DF-GA30, and DF-GA70, respectively.

Eight-week-old male wild-type C57BL/6J mice (n=7/group) were housed and bred under pathogen-free conditions with a standard 12-h light/dark cycle. Prior to the administration of special diets, mice were fed standard rodent chow and water *ad libitum*. Mice were randomly divided into five groups: Mice fed a low fat diet (LFD; 10 kcal% fat, Research Diets, New Brunswick, NJ, USA) and received water by oral gavage for 8 weeks (Normal). The second group fed a HFD (45 kcal% fat, Research Diets) and received water by oral gavage (Control). The third group fed a HFD and received DF-FW (250 mg/kg BW) by oral gavage (DF-FW). The fourth group fed a HFD and received DF-GA30 (250 mg/kg BW) by oral gavage (DF-GA30). The fifth group received a HFD and received DF-GA70 (250 mg/kg BW) by oral gavage (DF-GA70). After an 8 h fast on the last day of the study, the animals were sacrificed by cervical dislocation. Liver tissues were removed, weighed, snap-frozen in liquid nitrogen and stored at -80 °C until use. Portions of the Liver and visceral adipose tissues were prepared for histology. All animal experiments were approved by the Institutional Animal Care and Use Committee of Dongeui University (permit number: R2015-009) and followed National Research Council Guidelines.

Tissue specimens were fixed in 10% phosphate-buffered formalin for 1 day and embedded in paraffin. To examine the hepatic inflammation, fibrosis, and apoptosis, liver tissue sections (5 μm) were cut and stained with toluidine blue, Masson's trichrome, and 4',6-diamidino-2-phenylindole (DAPI), respectively. To measure the visceral adipocyte size, epididymal adipose tissue (5 μm) was stained with hematoxylin and eosin. Stained preparations were analyzed using an image analysis system (Media cybernetics, Bethesda, MD).
HepG2 cells, a well-differentiated human hepatoblastoma cell line, were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA). Cells were cultured for 2 days until reaching 70–80% confluency. Cells were treated with various concentrations of three DF extracts in the presence of a mixture of oleic acid and palmitoleic acid for 2 days. Oleic acid and palmitoleic acid were dissolved in ethanol and isopropanol at concentrations of 0.8 and 0.4 mM, respectively. Cell viability was detected by 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide disodium salt (XTT) assay using a Cell Proliferation Kit II (Roche, Basel, Switzerland).

Total cellular RNA from mouse liver tissue and HepG2 cells was prepared using Trizol reagent (Gibco-BRL, Grand Island, NY, USA). Total cellular RNA (2 μg) was reverse transcribed using Moloney murine leukemia virus reverse transcriptase to generate an antisense cDNA template. The genes of interest were amplified from the synthesized cDNA using AccuPower® GreenStar™ qPCR PreMix (Bioneer, Deajeon, Korea) on an Excycler™ 96 Real Time Quantitative Thermal Block machine (Bioneer). The PCR primers used

| Genes | Gene bank | Primer sequences |
|-------|-----------|------------------|
| Human |           |                  |
| MCP-1 | NM_002982.3 | Forward: 5'- CACTCACTCCACACACCAAGA-3'  
|       |           | Reverse: 5'- CAAAGACCCTCAACACATCC-3'   |
| TNFα  | NM_000594.3 | Forward: 5'- AGCCCATGTGTTAGCACAACC-3'  
|       |           | Reverse: 5'- GGAAGACCCCTCCAGATAG-3'    |
| Mouse |           |                  |
| α-SMA | NM_007392.3 | Forward: 5'- CTGGAGAGAGCAATCGAAGTC-3'  
|       |           | Reverse: 5'- CTGATCCCAATCTGATGAAAG-3'   |
| Bcl-2  | NM_009741.5 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
| Caspase 3 | NM_009810.3 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
| Caspase 8 | NM_00127926.1 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
| CD68  | NM_00129058.1 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
| Collagen α1 | NM_007742.4 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
| ICAM-1 | NM_010493.3 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
| MCP-1  | NM_011333.3 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
| TGFβ1  | NM_011577.2 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
| TNFα  | NM_001278601.1 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
| VCAM-1 | NM_011693.3 | Forward: 5'- TGGCTACCGTGCGATGTC-3'     
|       |           | Reverse: 5'- AAGCAGGCTGCTGCTGCT-3'     |
for gene expression analysis are shown in Table 1. PCR
was performed using the following conditions: denaturing
at 95°C for 5 min followed by 50 cycles of 95°C for 10 s,
60°C for 40 s, and 72°C for 10 s. PCR products were calcu-
lated as copies per μl using a standard curve, and the relative
expression levels were calculated as the ratio of target gene
cDNA to β-actin cDNA.

Values are expressed as mean ± standard deviation (SD).
Statistical analysis was performed using analysis of variance
followed by Turkey's post-hoc tests. Statistical significance
was defined as a P-value <0.05.

The prevalence of NAFLD has risen rapidly in parallel
with a dramatic increase in obesity (Charlton, 2004;
Vuppalanchi and Chalasani, 2009). Visceral obesity is par-
ticularly associated with the NAFLD development including
hepatic inflammation and fibrosis (Van der Poorten et al.,
2008; Yu et al., 2015). To determine the effect of DF on
visceral obesity, HFD-fed obese mice were treated with
DF-FW, DF-GA30, and DF-GA70 for 8 weeks. HFD-fed
control mice had much higher visceral fat mass and visceral
adipocyte size compared with LFD-fed normal mice. How-
ever, treatment of obese mice with DF-GA30 and DF-GA70
reduced visceral adipose tissue weight compared with control
mice (Fig. 1A). Visceral adipocyte size was also decreased
by all three DF extracts (Fig. 1B), suggesting that DF may
inhibit visceral obesity and visceral adipocyte hypertrophy.
It is known that larger adipocytes in visceral adipose tissue
secret free fatty acids and inflammatory cytokines such as
monocyte chemoattractant protein 1 (MCP-1) and tumor
necrosis factor α (TNFα) compared with smaller adipocytes,
leading to metabolic syndromes including insulin resistance,
type 2 diabetes, NAFLD, and cardiovascular disease (Xu et
al., 2003; Curat et al., 2004; Despres and Lemieux, 2006;
Jeong and Yoon, 2009). Thus, DF can regulate obesity and
adipocyte hypertrophy, and might be a novel therapeutic
approach to treat obesity and related metabolic diseases.

Visceral obesity promotes hepatic inflammation and fibrosis
in rodents and humans (Cai et al., 2005; Cancello et al.,
2006; Mulder et al., 2016; Van der Poorten et al., 2008; Yu
et al., 2015). To determine whether DF regulates hepatic
inflammation, liver sections were stained with toluidine blue
to detect mast cells. Control mice showed increased toluidine
blue-stained mast cells in the liver compared with normal
mice (Fig. 2A). Consistent with the changes in visceral fat
mass and adipocyte size, three kinds of DF extracts reduced
mast cells in the liver. Of the three DF extracts, DF-GA70
was most effective in decreasing the hepatic inflammation
in obese mice. Liver inflammation is increased by the ex-
pression in hepatocytes of inflammatory genes, such as
MCP-1, TNFα, CD68, intercellular adhesion molecule-1
(ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1)
(Cai et al., 2005; Chatzigeorgiou et al., 2014; Tilg and

Fig. 1. Regulation of visceral fat weight and visceral adipocyte
size by DF in HFD-fed obese mice. Adult male mice were fed an
LFD (normal), an HFD (control), or the HFD supplemented with
DF-FW, DF-GA30, and DF-GA70 for 8 weeks. (A) Modulation
of visceral fat weight by DF. (B) Regulation of visceral adipocyte
size by DF. The size of adipocytes in a fixed area (1,000,000 μm²)
was measured. #P<0.05 compared to the normal group, *P<0.05
compared to the control group.
Expression levels of these inflammatory genes were increased in HFD-fed control mice compared with LFD-fed normal mice (Fig. 2B). In contrast, DF-GA70 treatment decreased $TNF_\alpha$ and $VCAM-1$ mRNA levels in

Fig. 2. Inhibition of hepatic inflammation by DF in HFD-fed obese mice. Adult male mice were fed an LFD (normal), an HFD (control), or the HFD supplemented with DF-FW, DF-GA30, and DF-GA70 for 8 weeks. (A) Representative toluidine blue-stained sections of liver tissues (original magnification $\times$ 400). (B) Expression of genes involved in inflammation. All values are expressed as the mean ± SD. $#P<0.05$ compared to the normal group, *$P<0.05$ compared to the control group.
control mice. These results indicate that DF may inhibit visceral obesity-induced hepatic inflammation by downregulation of TNFα and VCAM-1.

Hepatic fibrosis is characterized by the excessive accumulation of extracellular matrix (ECM) proteins including collagen, which occurs in most chronic liver disease. Pathogenesis of hepatic fibrosis includes hepatic stellate cell (HSC) activation, and expression and accumulation of ECM proteins. Cytokines such as transforming growth factor β1 (TGFβ1) induce fibrosis by arresting activated HSC that produce ECM proteins (Saile et al., 2001). To determine whether DF regulates hepatic fibrosis, liver sections were stained Masson's trichrome. Control mice showed Masson's trichrome-stained hepatic collagen accumulation in the liver compared with normal mice (Fig. 3A). However, hepatic collagen levels were reduced in mice treated with all three kinds of DF extracts, and DF-GA70 treatment almost completely cleared hepatic collagen in obese mice. Hepatic expression of TGFβ1, α-SMA, and collagen α1 is closely linked to hepatic fibrosis (Bataller and Brenner, 2005). To evaluate whether the inhibitory effects of DF on hepatic fibrosis in obese mice were associated with alterations of the expression of fibrosis genes such as TGFβ1, α smooth muscle actin (α-SMA), and collagen α1. Alpha-SMA mRNA levels were

Fig. 3. Inhibition of hepatic fibrosis by DF in HFD-fed obese mice. Adult male mice were fed an LFD (normal), an HFD (control), or the HFD supplemented with DF-FW, DF-GA30, and DF-GA70 for 8 weeks. (A) Representative Masson's trichrome-stained sections of liver tissues (original magnification × 100). (B) Expression of genes involved in fibrosis. All values are expressed as the mean ± SD. #P<0.05 compared to the normal group, *P<0.05 compared to the control group.
decreased by all three DF extracts (Fig. 3B). These results suggest that DF inhibits hepatic inflammation by upregulation of α-SMA in HFD-fed obese mice.

NAFLD is accompanied by hepatocyte apoptosis (Cao et al., 2016). Apoptotic bodies stimulate hepatic inflammation and fibrosis, and the magnitude of apoptosis correlates with stage of fibrosis in patients with NASH (Feldstein et al., 2003). Inhibition of hepatocyte apoptosis with a caspase inhibitor reduced hepatic injury and fibrosis in mice with liver injury (Canbay et al., 2004). In the present study, DAPI-stained liver sections revealed that HFD-fed control mice did not exhibit increased hepatocyte apoptosis compared with normal mice, suggesting that mild hepatic inflammation and fibrosis following HFD feeding may be due to the low level of apoptotic hepatocytes in this mouse model (Fig. 4A). Expectedly, hepatocyte apoptosis was not affected by DF treatment. Although, analysis of the expression of apoptosis (caspase 3 and caspase 8) and anti-apoptosis (B cell lymphoma 2 (Bcl-2)) genes showed that HFD feeding increased caspase 3 mRNA levels with decreasing Bcl-2 mRNA levels in the liver (Fig. 4B). In contrast, DF-GA30 and DF-GA70 decreased caspase 3 mRNA levels, whereas DF-FW and DF-GA70 increased Bcl-2 mRNA levels in the liver. These results indicate that DF regulates apoptotic

![Image](image_url)

**Fig. 4. Modulation of hepatocyte apoptosis by DF in HFD-fed obese mice.** Adult male mice were fed an LFD (normal), an HFD (control), or the HFD supplemented with DF-FW, DF-GA30, and DF-GA70 for 8 weeks. (A) Representative DAPI-stained sections of liver tissues (original magnification × 100). (B) Expression of genes involved in apoptosis. All values are expressed as the mean ± SD. #P<0.05 compared to the normal group, *P<0.05 compared to the control group.
gene expression although hepatocyte apoptosis was not observed in the histological analysis.

It is known that an NAFLD cell model is induced by an overload of free fatty acids (Gomez-Lechon et al., 2007; Liang et al., 2015). The previous study demonstrated that treatment of HepG2 cells with excess free fatty acids induced steatosis and regulated the mRNA expression of lipid metabolism genes (Yoon et al., 2017). Thus, the expression of genes involved in inflammation was examined in HepG2 cells treated with a mixture of free fatty acids such as oleic acid and palmitoleic acid. Free fatty acids increased the mRNA levels of TNFα and MCP-1 (Fig. 5A). However, mRNA levels of these genes were decreased by DF-GA70.

In addition, the XTT assays showed that the viability of HepG2 cells was not affected by DFs at all concentrations used in this study (Fig. 5B). These results suggest that DF inhibits the expression of inflammatory genes in HepG2 cells, supporting an inhibitory role of DF on hepatic inflammation in vivo.

In conclusion, these results demonstrate that DF, a polyherbal drug composed of Ephedra intermedia, Lithospermum erythrorhizon, and Rheum palmatum, inhibits visceral obesity and ameliorates hepatic fibroinflammation by regulating the expression of inflammation and fibrosis genes in animal and cell models of human NAFLD. In addition, these inhibitory effects were most effective in mice treated with DF-GA70.

Fig. 5. Effects of DF on mRNA expression of inflammation genes in HepG2 cells. Cells were treated with various concentrations of DF-FW, DF-GA30, and DF-GA70 in the presence of fatty acids. Control cells were treated with fatty acids only. (A) Expression of inflammation genes. (B) Effects of DF on HepG2 cell viability by XTT assay. All values are expressed as the mean ± SD. *P<0.05 compared to the control group.
These results suggest that DF may be an effective drug for preventing and treating obesity and NAFLD. Further studies will be necessary to establish the experimental conditions (e.g. longer duration of DF treatment and higher doses of DF) equivalent to human hepatic inflammation, fibrosis, and apoptosis, and to identify active compounds of each individual herbs for their effects on obesity and NAFLD.

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CONFLICT OF INTEREST
None declared.

REFERENCES
Angulo P. Nonalcoholic fatty liver disease. The New England Journal of Medicine. 2002. 346: 1221-1231.
Bataller R, Brenner DA. Liver fibrosis. Journal of Clinical Investigation. 2005. 115: 209-218.
Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nature Medicine. 2005. 11: 183-190.
Canbay A, Feldstein A, Baskin-Bey E, Bronk SF, Gores GJ. The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse. Journal of Pharmacology and Experimental Therapeutics. 2004. 308: 1191-1196.
Cancellro R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, Cossu C, Basdevant A, Bar Hen A, Bedossa P, Guerre-Milo M, Clément K. Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. Diabetes. 2006. 55: 1554-1561.
Cao L, Quan XB, Zeng WJ, Yang XO, Wang MJ. Mechanism of hepatocyte apoptosis. Journal of Cell Death. 2016. 9: 19-29.
Chan W, Tan A, Vethakkan S, Tah P, Vijayananthan A, Goh K. Non-alcoholic fatty liver disease in diabetics—prevalence and predictive factors in a multiracial hospital clinic population in Malaysia. Journal of Gastroenterology and Hepatology. 2013. 28: 1375-1383.
Charlton M. Nonalcoholic fatty liver disease: a review of current understanding and future impact. Clinical Gastroenterology and Hepatology. 2004. 2: 1048-1058.
Chatzigeorgiou A, Chung KJ, Garcia-Martin R, Alexaki VI, Klotzsche-von Ameln A, Phielier J, Sprott D, Kanckowski W, Tzanavari T, Bdeir M, Bergmann S, Cartellieri M, Bachmann M, Nikolaopoulos P, Androutsellis-Theotokis A, Siegert G, Bornstein SR, Muder MH, Boon L, Karalis KP, Lugtens E, Chavakis T. Dual role of B7 costimulation in obesity-related nonalcoholic steatohepatitis and metabolic dysregulation. Hepatology. 2014. 60: 1196-1210.
Curat CA, Miranville A, Sengenes C, Diehl M, Tonus C, Busse R, Bouloumie A. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. Diabetes. 2004. 53: 1285-1292.
Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006. 444: 881-887.
Diehl AM. Hepatic complications of obesity. Gastroenterology Clinics of North America. 2005. 34: 45-61.
Fan J. Epidemiology of alcoholic and nonalcoholic fatty liver disease in China. Journal of Gastroenterology and Hepatology. 2013. 28: 11-17.
Feldstein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, Gores GJ. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology. 2003. 125: 437-443.
Gomez-Lechon MJ, Donato MT, Martinez-Romero A, Jimenez N, Castell JV, O’Connor JE. A human hepatocellular in vitro model to investigate steatosis. Chemico-Biological Interactions. 2007. 165: 106-116.
Jeong S, Chae K, Jung YS, Rho YH, Lee J, Ha J, Yoon KH, Kim GC, Cho KS, Shin SS, Yoon M. The Korean traditional medicine Gyeongshingangjeehwan inhibits obesity through the regulation of leptin and PPARalpha action in OLETF rats. Journal of Ethnopharmacology. 2008. 119: 245-251.
Jeong S, Yoon M. Fenofibrate inhibits adipocyte hypertrophy and insulin resistance by activating adipose PPARalpha in high fat diet-induced obese mice. Experimental & Molecular Medicine. 2009. 41: 397-405.
Koh JC, Loo WM, Goh KL, Sugano K, Chan WK, Chiu WY, Choi MG, Gongchanvit S, Lee WJ, Lee WJ, Lee YY, Lesmana LA, Li YM, Liu CJ, Matsuura B, Nakajima A, Ng EK, Sollano JD, Wong SK, Wong VW, Yang Y, Ho KY, Dan YY. Asian consensus on the relationship between obesity and gastrointestinal and liver diseases. Journal of Gastroenterology and Hepatology. 2016. 31: 1405-1413.
Liang H, Zhang L, Wang H, Tang J, Yang J, Wu C, Chen S. Inhibi-
tery effect of gardenoside on free fatty acid-induced steatosis in HepG2 hepatocytes. International Journal of Molecular Sciences. 2015. 16: 27749-27756.

Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. Journal of Hepatology. 2006. 45: 600-606.

Miliš S, Stimac D. Nonalcoholic fatty liver disease/steatohepatitis: epidemiology, pathogenesis, clinical presentation and treatment. Digestive Diseases. 2012. 30: 158-162.

Mulder P, Morrison MC, Wielinga PY, van Duyvenvoorde W, Kooistra T, Kleemann R. Surgical removal of inflamed epididymal white adipose tissue attenuates the development of non-alcoholic steatohepatitis in obesity. International Journal of Obesity. 2016. 40: 675-684.

Park SH, Jeon WK, Kim SH, Kim HJ, Park DI, Cho YK, Sung IK, Sohn CI, Keum DK, Kim BI. Prevalence and risk factors of non-alcoholic fatty liver disease among Korean adults. Journal of Gastroenterology and Hepatology. 2006. 21: 138-143.

Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla Man C, Cobelli C, Shulman GI. Increased prevalence of insulin resistance and non-alcoholic fatty liver disease in Asian-Indian men. Proceedings of the National Academy of Sciences. 2006. 103: 18273-18277.

Roh JS, Lee H, Woo S, Yoon M, Kim J, Park SD, Shin SS, Yoon M. Herbal composition Gambigyeongsinshinwan from Curcuma longa, Alnus japonica, and Massa Medicata Fermentata inhibits lipid accumulation in 3T3-L1 cells and regulates obesity in Otsuka Long-Evans Tokushima Fatty rats. Journal of Ethnopharmacology. 2015. 171: 287-294.

Saile B, Matthes N, El Armouche H, Neubauer K, Ramadori G. The bcl, NFκB and p53/p21WAF1 systems are involved in spontaneous apoptosis and in the anti-apoptotic effect of TGF-beta or TNF-alpha on activated hepatic stellate cells. European Journal of Cell Biology. 2001. 80: 554-561.

Schwimmer JB, Deutsch R, Kahem T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. Pediatrics. 2006. 118: 1388-1393.

Shin SS, Jung YS, Yoon KH, Choi S, Hong Y, Park D, Lee H, Seo BI, Lee HY, Yoon M. The Korean traditional medicine gyeongshingangjeheuangwan inhibits adipocyte hypertrophy and visceral adipose tissue accumulation by activating PPARα actions in rat white adipose tissues. Journal of Ethnopharmacology. 2010. 127: 47-54.

Tilg H, Moschen AR. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. Trends in Endocrinology and Metabolism. 2008. 19: 371-379.

Van der Poorten D, Milten KL, Hui J, Hodge A, Trenell MI, Kench JG, London R, Peduto T, Chisholm DJ, George J. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. Hepatology. 2008. 48: 449-457.

Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management. Hepatology. 2009. 49: 306-317.

Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. Journal of Clinical Investigation. 2003. 112: 1821-1830.

Yoon S, Kim J, Lee H, Lee H, Lim J, Yang H, Shin SS, Yoon M. The effects of herbal composition Gambigyeongsinshinwan on hepatic steatosis and inflammation in Otsuka Long-Evans Tokushima fatty rats and HepG2 cells. Journal of Ethnopharmacology. 2017. 195: 204-213.

Yu SJ, Kim W, Kim D, Yoon JH, Lee K, Kim JH, Cho EJ, Lee JH, Kim HY, Kim YJ, Kim CY. Visceral obesity predicts significant fibrosis in patients with nonalcoholic fatty liver disease. Medicine. 2015. 94: e2159.

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