Biochemical and Biophysical Research Communications 588 (2022) 154–160

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Hepatoprotective effects of gemigliptin and empagliflozin in a murine model of diet-induced non-alcoholic fatty liver disease

Nami Lee [a, 1], Yu Jung Heo [a, 1], Sung-E Choi [b], Ja Young Jeon [a], Seung Jin Han [a], Dae Jung Kim [a], Yup Kang [b], Kwan Woo Lee [a], Hae Jin Kim [a, *]

[a] Department of Endocrinology and Metabolism, Ajou University School of Medicine, 164 Worldcup-ro, Yeoungtong-gu, Suwon, 16499, Republic of Korea
[b] Department of Physiology, Ajou University School of Medicine, 164 Worldcup-ro, Yeoungtong-gu, Suwon, 16499, Republic of Korea

ARTICLE INFO

Article history:
Received 26 October 2021
Received in revised form
14 December 2021
Accepted 18 December 2021
Available online 20 December 2021

Keywords:
Non-alcoholic fatty liver disease
Inflammation
Oxidative stress
Fibrosis
Dipeptidyl peptidase-4 inhibitors
Sodium-glucose cotransporter 2 inhibitors

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) includes a broad spectrum of liver diseases characterized by steatosis, inflammation, and fibrosis. This study aimed to investigate the potential of dipeptidyl peptidase-4 inhibitors and sodium-glucose cotransporter 2 inhibitors in alleviating the progression of NAFLD.

The NAFLD model was generated by feeding male C57BL/6J mice a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD) for 7 weeks. After 2 weeks of CDAHFD feeding, the NAFLD model mice were assigned to four groups, namely (i) VEHICLE, (ii) gemigliptin (GEMI), (iii) empagliflozin (EMPA), and (iv) GEMI + EMPA. For the next 5 weeks, mice received the vehicle or the drug based upon the group to which they belonged. Thereafter, the triglyceride concentration, extent of fibrosis, and the expression of genes encoding inflammatory cytokines, chemokines, and antioxidant enzymes were analyzed in the livers of mice. The NAFLD activity score and hepatic fibrosis grade were assessed via hematoxylin and eosin and Sirius Red staining of the liver tissue samples.

All mice belonging to the GEMI, EMPA, and GEMI + EMPA groups showed improvements in the accumulation of liver triglycerides and the expression of inflammatory cytokines and chemokines. Additionally, the oxidative stress was reduced due to inhibition of the c-Jun N-terminal kinase pathway and upregulation of the antioxidant enzymes. Furthermore, in these three groups, the galectin-3 and interleukin 33-induced activity of tumor necrosis factor-α was inhibited, thereby preventing the progression of liver fibrosis.

These findings suggest that the GEMI, EMPA, and GEMI + EMPA treatments ameliorate hepatic steatosis, inflammation, oxidative stress, and fibrosis in CDAHFD-induced NAFLD mouse models.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder that progresses from simple steatosis to non-alcoholic steatohepatitis (NASH), with a global prevalence of significantly increased to 25% [1]. Incidentally, NAFLD is a chronic inflammatory disease that is characterized by an increase of free fatty acid (FFA) content due to the onset of obesity [2]. Moreover, insulin resistance can induce endoplasmic reticulum stress and the activation of inflammatory cell. Inflammatory cytokines and chemokines, including transforming growth factor β (TGF-β), activate the hepatic stellate cells, resulting in hepatic fibrosis. Unfortunately, there is no definite treatment option for NAFLD that is more effective than weight loss [3,4].

Dipeptidyl peptidase-4 inhibitors (DPP-4i) function as glucose-lowering agents by inhibiting DPP-4, an enzyme that breaks down incretins [5]. Previously, certain *in vitro* and *in vivo* studies have demonstrated that some DPP-4i, such as anagliptin [6,7], gemigliptin [8], and sitagliptin [9,10], can exert anti-inflammatory effects and thereby attenuate NAFLD progression.

Sodium-glucose cotransporter 2 inhibitors (SGLT2i) are another class of antidiabetic drugs that can improve hyperglycemia by increasing glucose excretion via urine, independent of insulin action [11]. In fact, certain SGLT2i, such as canagliflozin [12] and dapagliflozin [13,14], have reportedly shown positive effects with

* Corresponding author.
E-mail address: jinkim@ajou.ac.kr (H.J. Kim).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2021.12.065

Abbreviations

NAFLD	non-alcoholic fatty liver disease
CDAHFD	choline-deficient, L-amino acid-defined, high-fat diet
GEMI	gemigliptin
EMPA	empagliflozin
JNK	c-Jun N-terminal kinase
NASH	non-alcoholic steatohepatitis
FFA	free fatty acid
TGF	transforming growth factor
DPP-4i	dipeptidyl peptidase-4 inhibitors
SGLT2i	sodium-glucose cotransporter 2 inhibitors
MKK	mitogen-activated protein kinase kinase
TG	triglyceride
MDA	malondialdehyde
NAS	NAFLD activity score
TNF	tumor necrosis factor
IL	interleukin
IFN	interferon
MCP-1	monocyte chemoattractant protein-1
CCL	C–C motif chemokine ligand
CXCL	C-X-C motif chemokine ligand
SOD	superoxide dismutase
GPx-1	glutathione peroxidase-1
α-SMA	α-smooth muscle actin
COL1α1	collagen type 1 α1
LECT-2	leukocyte cell-derived chemotaxin 2

respect to NAFLD treatment in animal models.

Previously, we reported that gemigliptin and empagliflozin can, individually or in combination, exhibit anti-inflammatory effects by inhibiting the consecutive activities of mitogen-activated protein kinase kinase (MKK) 4, 7/c-Jun N-terminal kinase (JNK) and Janus kinase 2 (JAK2)/signal transducer and activator of transcription (STAT) 1, 3 pathways *in vitro* [15]. In the present study, we attempted to determine whether DPP-4i and SGLT2i can ameliorate the progression of NAFLD, such as fat accumulation, inflammation, and fibrosis, in a diet-induced NAFLD mouse model.

2. Materials and methods

2.1. Animal groups and treatment

Seven-week-old male C57BL/6J mice (OrientBio Inc., Sungnam, Republic of Korea) were fed either standard chow diet ("CONTROL" group; n = 8) or a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD), which consisted of 60 kcal% fat (mostly from palm oil), 0.1% methionine, and no added choline (A19053002, Research Diets Inc., New Brunswick, NJ, USA). After 2 weeks, mice fed CDAHFD were randomly divided into four groups (n = 8 in each group) according to their drug treatment. These included (i) VEHICLE: CDAHFD-fed mice with vehicle treatment for 5 weeks; (ii) GEMI: CDAHFD-fed mice with gemigliptin (LG Life Sciences Ltd., Seoul, Republic of Korea) treatment for 5 weeks (30 μg/g per day by oral gavage) [16]; (iii) EMPA: CDAHFD-fed mice with empagliflozin (Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany) treatment for 5 weeks (10 μg/g per day by oral gavage) [17]; (iv) GEMI + EMPA: CDAHFD-fed mice with gemigliptin and empagliflozin co-treatment for 5 weeks (Supplementary Fig. 1). Mice were housed at a temperature of 22 °C with a 12 h light/dark cycle and fed *ad libitum*. Finally, blood samples were collected from the hearts of 14-week-old mice, and the mice were sacrificed for liver harvesting. All treatments and animal care were conducted in accordance with the Ajou Institutional Animal Care & Use Committee (IACUC) guidelines, and the experimental protocol was approved by the Ajou IACUC (Approval No. 2019-0013).

2.2. Biochemical analysis of liver samples

Tissue triglycerides (TGs) were extracted using the Folch extraction method [18]; subsequently, the total TG amount was detected using the EZ-Triglyceride Quantification Assay Kit (DOGEN, Seoul, Korea) according to the manufacturer's instructions. The amount of TG in each group was calculated using standard curve. The malondialdehyde (MDA) concentrations of liver samples were measured using the OxiSelect MDA Adduct Competitive ELISA kit (Cell Biolabs, Inc, San Diego, CA, USA) according to the manufacturer's instructions.

2.3. Histopathological analysis of liver samples

Mouse livers were isolated, fixed in 4% formalin, and embedded in paraffin. Histochemical staining was performed with hematoxylin and eosin (H&E) and Sirius Red. The NAFLD activity score (NAS), which has been designed by the NASH Clinical Research Network, and the hepatic fibrosis grade were analyzed from H&E- and Sirius Red-stained tissue, respectively. Immunohistochemical staining was performed by incubating the tissue samples with a primary antibody against α-smooth muscle actin (αSMA; Abcam, Cambridge, UK, 1:500), followed by horseradish peroxidase-conjugated anti-rabbit secondary antibody (Dako, Glostrup, Denmark).

2.4. Western blot assay

Total protein was extracted from whole liver using RIPA buffer and a protease inhibitor cocktail (Roche Applied Science, Mannheim, Germany). The proteins were separated using 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene-difluoride membranes (Millipore, Billerica, MA, USA). Subsequently, the membranes were blocked using 5% (w/v) bovine serum albumin for 30 min, and incubated overnight with primary antibodies, including those against phosphor-JNK (Cell Signaling Technology, Danvers, MA, USA), total JNK, and β-actin (Santa Cruz Biotech, Santa Cruz, CA) at 4 °C. Thereafter, the membranes were incubated with secondary antibodies (horseradish peroxidase-conjugated anti-mouse IgG and anti-rabbit IgG; Bethyl Laboratories, Montgomery, TX, USA). Finally, the blots were visualized and analyzed using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

2.5. RNA isolation and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted from liver tissues using the RNAiso Plus reagent (TaKaRa Bio, Otsu, Japan). The quantity and purity of RNA were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the extracted RNAs (1 μg) were converted to complementary DNAs (cDNAs) using the PrimeScript™ 1st strand cDNA synthesis kit

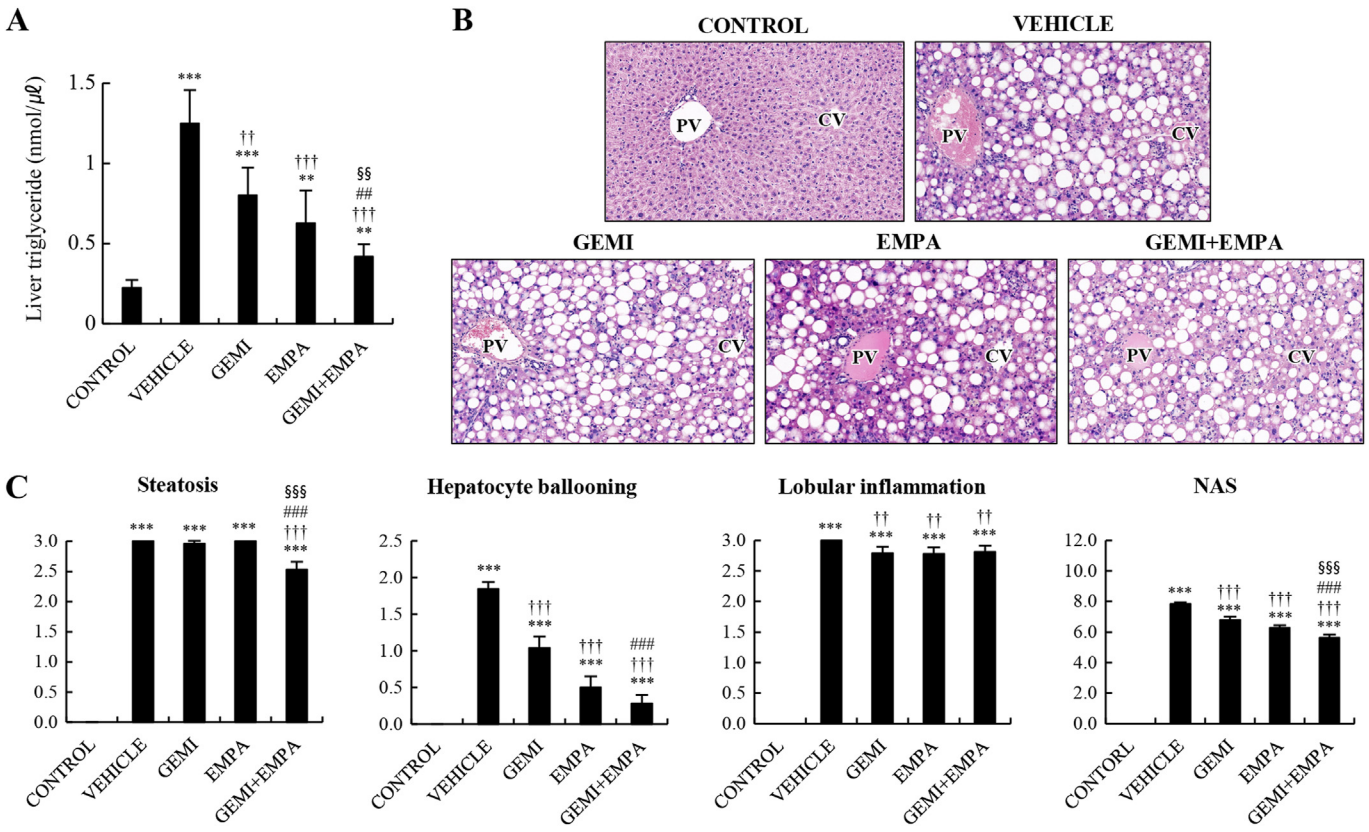

Fig. 1. Effects of gemigliptin and empagliflozin on hepatic steatosis and non-alcoholic fatty liver disease (NAFLD) activity score (NAS) in a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD)-induced NAFLD mouse model. (A) Hepatic triglyceride content. (B) Hematoxylin and eosin staining of liver sections (20 ×). (C) Grading of hepatic steatosis, hepatocyte ballooning, lobular inflammation, and NAS.
*P < 0.05, **P < 0.01, and ***P < 0.001 in comparisons of the CONTROL group with the VEHICLE, GEMI, EMPA, or GEMI + EMPA treatment groups. †P < 0.05, ††P < 0.01, and †††P < 0.001 in comparisons of the VEHICLE group with the GEMI, EMPA, or GEMI + EMPA treatment groups. $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, and $^{\#\#\#}$P < 0.001 in comparison between the GEMI and GEMI + EMPA treatment groups. §P < 0.05, §§P < 0.01, and §§§P < 0.001 in comparison between the EMPA and GEMI + EMPA treatment group.
CONTROL: vehicle treated standard chow-diet group; VEHICLE: vehicle treated CDAHFD-diet group; GEMI: gemigliptin treated CDAHFD-diet group; EMPA: empagliflozin treated CDAHFD-diet group; GEMI + EMPA: gemigliptin and empagliflozin co-treated CDAHFD-diet group; PV: portal vein; CV: central vein.

according to the manufacturer's instructions. Thereafter, the cDNAs were subjected to qRT-PCR amplification, according to the instructions of the SYBR Green Master Mix using a TaKaRa TP-815 instrument. The primer sets used for amplification are listed in Supplementary Table 1. The amplification of the gene encoding glyceraldehyde 3-phosphate dehydrogenase was used as a reference standard to normalize the target signal. Amplification specificity was controlled via a melting curve analysis.

2.6. Enzyme-linked immunosorbent assay (ELISA)

The plasma concentrations of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and interferon-ɣ (IFN-ɣ) were quantified using an ELISA DuoSet kit (R & D System, Minneapolis, MN, USA) according to the manufacturer's instructions.

2.7. Statistical analysis

All experiments were conducted in triplicate, and data are presented as mean ± standard deviation (SD). The results were analyzed using Student's *t*-test, and the statistical significance was set at P value ≤ 0.05.

3. Results

3.1. Gemigliptin and empagliflozin reduce hepatic steatosis and NAS in CDAHFD-induced NAFLD mice

We observed that the body weight of the GEMI + EMPA group mice was lower than that of the VEHICLE group mice (Supplementary Fig. 2A). Additionally, the hepatic TG concentrations in all the three treatment groups of mice were lower than those of the VEHICLE group, with further reduction in the case of GEMI + EMPA treatment (Fig. 1A). The plasma TG levels exhibited a decrease only in the EMPA treatment group, and none of GEMI, EMPA, or GEMI + EMPA treatment had any effect on the plasma FFA levels (Supplementary Fig. 2B). Furthermore, plasma alanine aminotransferase and aspartate aminotransferase levels that had increased due to the CDAHFD showed improvements only with the EMPA treatment. Lipid droplets, hepatocyte ballooning, and inflammatory cell infiltration were observed in H&E-stained liver sections of all the CDAHFD-fed mice (Fig. 1B and C). Hepatic steatosis scores decreased only in GEMI + EMPA-treated mice, whereas hepatocellular ballooning and lobular inflammation scores decreased in all the three treatment groups. The NAS was also reduced in all treatment groups, with a further decrease in GEMI + EMPA-treated mice (mean ± SD: VEHICLE 7.8 ± 0.09, GEMI 6.8 ± 0.21, EMPA 6.3 ± 0.17, and GEMI + EMPA 5.6 ± 0.23).

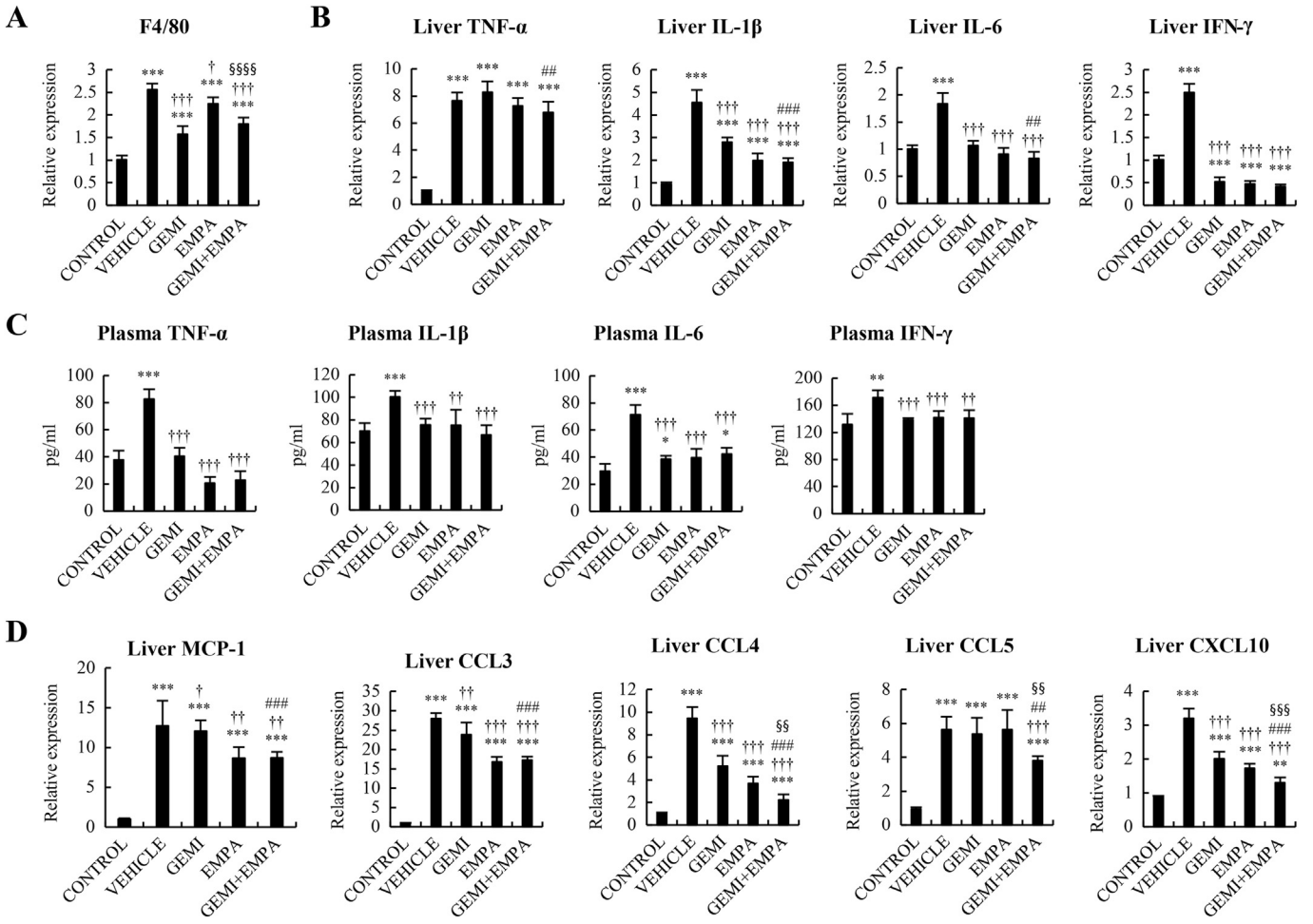

Fig. 2. Effects of gemigliptin and empagliflozin on hepatic inflammation in a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD)-induced non-alcoholic fatty liver disease (NAFLD) mouse model. (A) Relative mRNA expression of macrophage surface marker F4/80, as determined using qRT-PCR analysis. (B) Expression of pro-inflammatory cytokine genes such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6 and interferon-γ (IFN-γ) in liver, as assessed using qRT-PCR. (C) Pro-inflammatory cytokine concentrations in plasma, determined using ELISA. (D) mRNA expression of pro-inflammatory chemokines, such as monocyte chemoattractant protein-1 (MCP-1), C–C motif chemokine ligand (CCL)3, CCL4, CCL5, and C-X-C motif chemokine ligand (CXCL10), in liver, as determined using qRT-PCR.
*P < 0.05, **P < 0.01, and ***P < 0.001 in comparisons of the CONTROL group with the VEHICLE, GEMI, EMPA, or GEMI + EMPA treatment groups. †P < 0.05, ††P < 0.01, and †††P < 0.001 in comparisons of the VEHICLE group with the GEMI, EMPA, or GEMI + EMPA treatment groups. $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, and $^{\#\#\#}$P < 0.001 in comparison between the GEMI and GEMI + EMPA treatment groups. §P < 0.05, §§P < 0.01, and §§§P < 0.001 in comparison between the EMPA and GEMI + EMPA treatment group.
CONTROL: vehicle treated standard chow-diet group; VEHICLE: vehicle treated CDAHFD-diet group; GEMI: gemigliptin treated CDAHFD-diet group; EMPA: empagliflozin treated CDAHFD-diet group; GEMI + EMPA: gemigliptin and empagliflozin co-treated CDAHFD-diet group; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay.

3.2. Gemigliptin and empagliflozin attenuate macrophage activation as well as the expression of hepatic and systemic inflammatory cytokines in CDAHFD-induced NAFLD mice

Expression of the gene encoding the liver macrophage surface marker F4/80 was increased in the VEHICLE group mice, but it was decreased in all the three treatment groups (Fig. 2A). Moreover, except of TNF-α, all other hepatic pro-inflammatory cytokine genes, such as the ones encoding IL-1β, IL-6, and IFN-ɣ were remarkably downregulated in the GEMI, EMPA, and GEMI + EMPA treatment groups (Fig. 2B). On the contrary, the plasma levels of cytokines, including TNF-α, were decreased in all the three treatment groups as compared to those in the VEHICLE group (Fig. 2C). Furthermore, we founded that the mRNA expression of inflammatory chemokines, such as monocyte chemoattractant protein-1 (MCP-1), C–C motif chemokine ligand (CCL)3, CCL4, and C-X-C motif chemokine ligand (CXCL)10, was significantly downregulated in the livers of mice in all the three treatment groups (Fig. 2D). Among them, the GEMI + EMPA-treated mice exhibited an additional suppression of CCL4 and CXCL10 gene expression as compared to that in the GEMI- and EMPA-treated groups. Incidentally, CCL5 expression was inhibited only in the GEMI + EMPA-treated mice.

3.3. Gemigliptin and empagliflozin suppress hepatic oxidative stress via inhibition of JNK phosphorylation in CDAHFD-induced NAFLD mice

Next, we investigated the expression of antioxidant enzymes to assess the effects of gemigliptin and empagliflozin on oxidative stress, which, in turn induces NASH progression. The mice in the VEHICLE group exhibited a marked suppression in the mRNA expression of enzymatic antioxidants in the liver as compared to that in the CONTROL group (Fig. 3A). Moreover, the catalase gene expression increased simultaneously in all the three treatment groups, but the gene encoding superoxide dismutase (SOD) 2 was upregulated only in the GEMI + EMPA treatment group.

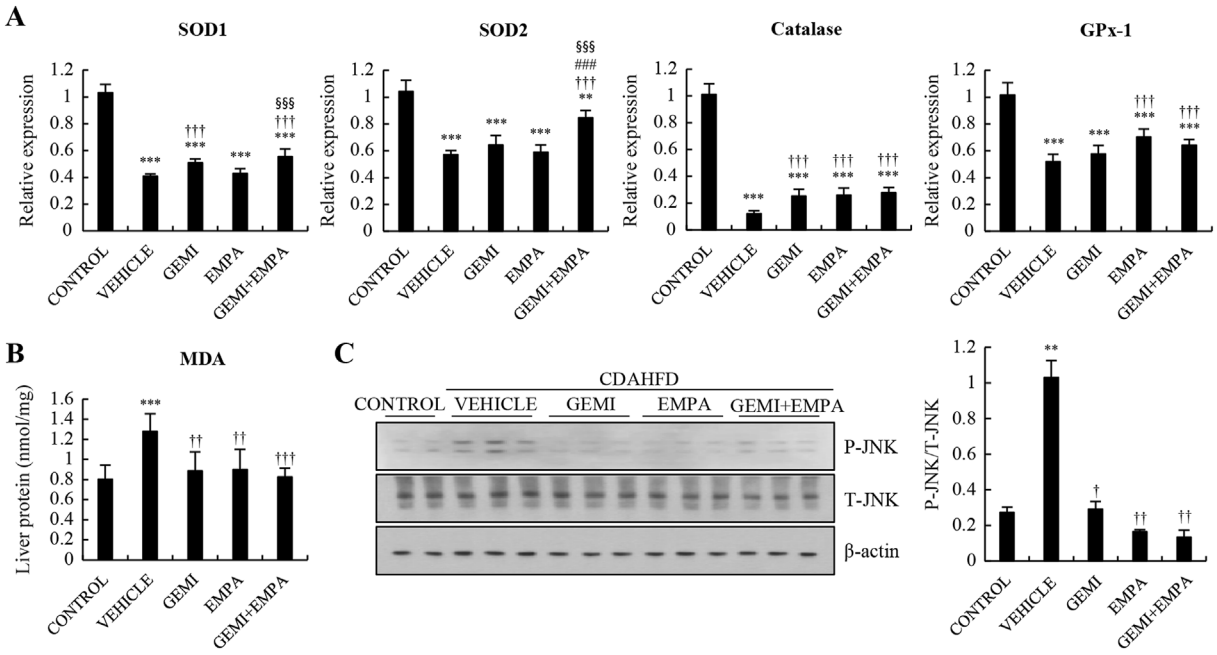

Fig. 3. Effects of gemigliptin and empagliflozin on oxidative stress in a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD)-induced non-alcoholic fatty liver disease (NAFLD) mouse model. (A) Relative mRNA expression of antioxidant enzymes in liver, as determined using qRT-PCR. (B) Liver malondialdehyde (MDA) concentrations determined using ELISA. (C) Representative hepatic expression of c-Jun N-terminal kinase (JNK) and p-JNK determined using western blot.
*P < 0.05, **P < 0.01, and ***P < 0.001 in comparisons of the CONTROL group with the VEHICLE, GEMI, EMPA, or GEMI + EMPA treatment groups. †P < 0.05, ††P < 0.01, and †††P < 0.001 in comparisons of the VEHICLE group with the GEMI, EMPA, or GEMI + EMPA treatment groups. #P < 0.05, ##P < 0.01, and ###P < 0.001 in comparison between the GEMI and GEMI + EMPA treatment groups. §P < 0.05, §§P < 0.01, and §§§P < 0.001 in comparison between the EMPA and GEMI + EMPA treatment group.
CONTROL: vehicle treated standard chow-diet group; VEHICLE: vehicle treated CDAHFD-diet group; GEMI: gemigliptin treated CDAHFD-diet group; EMPA: empagliflozin treated CDAHFD-diet group; GEMI + EMPA: gemigliptin and empagliflozin co-treated CDAHFD-diet group; SOD: superoxide dismutase; GPx-1: glutathione peroxidase-1; p-JNK: phosphorylated JNK; t-JNK: total JNK; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay.

Incidentally, the mRNA expression of SOD1 and glutathione peroxidase-1 (GPx-1) increased in the GEMI- and EMPA-treated mice, respectively. We also observed a reduction in the levels of the end product of lipid peroxidation, MDA, which is consistent with antioxidant effects of gemigliptin and empagliflozin (Fig. 3B). Additionally, JNK phosphorylation, which is known to be associated with oxidative stress, was significantly increased in the VEHICLE, but was inhibited in the GEMI, EMPA, and GEMI + EMPA treatment groups (Fig. 3C).

3.4. Gemigliptin and empagliflozin reduce hepatic fibrosis progression in CDAHFD-induced NAFLD mice

We performed Sirius Red staining and α-SMA immunohistochemical staining to investigate NASH progression in all liver samples (Supplementary Fig. 2C, Fig. 4A). Fibrotic connective tissue accumulation in the VEHICLE group was significantly higher as compared to that in the CONTROL group. The mean fibrosis grade of the VEHICLE group was 2.6 ± 0.36, while that of GEMI, EMPA, and GEMI + EMPA groups decreased to 1.8 ± 0.31, 1.9 ± 0.29, and 1.6 ± 0.25, respectively (Fig. 4B). Pro-fibrotic genes, such as the ones encoding collagen type 1 α1 (COL1α1), smooth muscle and aorta actin α-2, and tissue inhibitor matrix metalloproteinase 1, were overexpressed in the VEHICLE group (Fig. 4C). However, this overexpression was suppressed in the GEMI, EMPA, and GEMI + EMPA treatment groups. Moreover, TGF-β mRNA expression, a key fibrosis mediator, was suppressed by downregulation of the galectin-3 gene and a decrease in IL-33 secretion in the GEMI, EMPA, and GEMI + EMPA groups (Fig. 4D).

4. Discussion

In the present study, we demonstrated the anti-inflammatory and antioxidant effects of gemigliptin and empagliflozin in a CDAHFD-induced NAFLD mouse model that were ultimately able to alleviate hepatic fibrosis by downregulating galectin-3 expression and reducing IL-33 secretion.

Oxidative stress and inflammation are important regulatory factors in NASH progression [19,20]. Incidentally, CCL5 directly induces hepatic steatosis in the early stages of NAFLD [21], while CCL3, CCL4, and CXCL10 concentrations increase in NASH [22]. In particular, CXCL10 plays an important role in NAFLD progression associated with inflammation, oxidative stress, and fibrosis [23].

The overexpression of DPP-4 induces hepatic steatosis via superoxide generation [24]. Interestingly, a DPP4i, sitagliptin, reportedly improved hepatic steatosis by increasing insulin sensitivity [10] and reducing the biological activity of inflammatory cytokines and chemokines [9] in a diet-induced NAFLD model. Another study reported that gemigliptin alleviates lipid accumulation by downregulating leukocyte cell-derived chemotaxin 2 (LECT2) expression in an AMP-activated protein kinase-dependent manner [8]. The LECT2 is a hepatokine that is upregulated by obesity and NASH progression through activated M1 macrophages via the MKK4/JNK pathway [25]. We demonstrated that gemigliptin can reduce hepatic TG concentrations, downregulate CCL4 and CXCL10, which are major chemotactic factors in macrophages, and enhance the expression of genes encoding SOD1 and catalase, which are important antioxidants in the progression of NAFLD.

Incidentally, SGLT2 is expressed primarily in the proximal tubules of the kidney [26]. Previously, a study reported that SGLT2 is

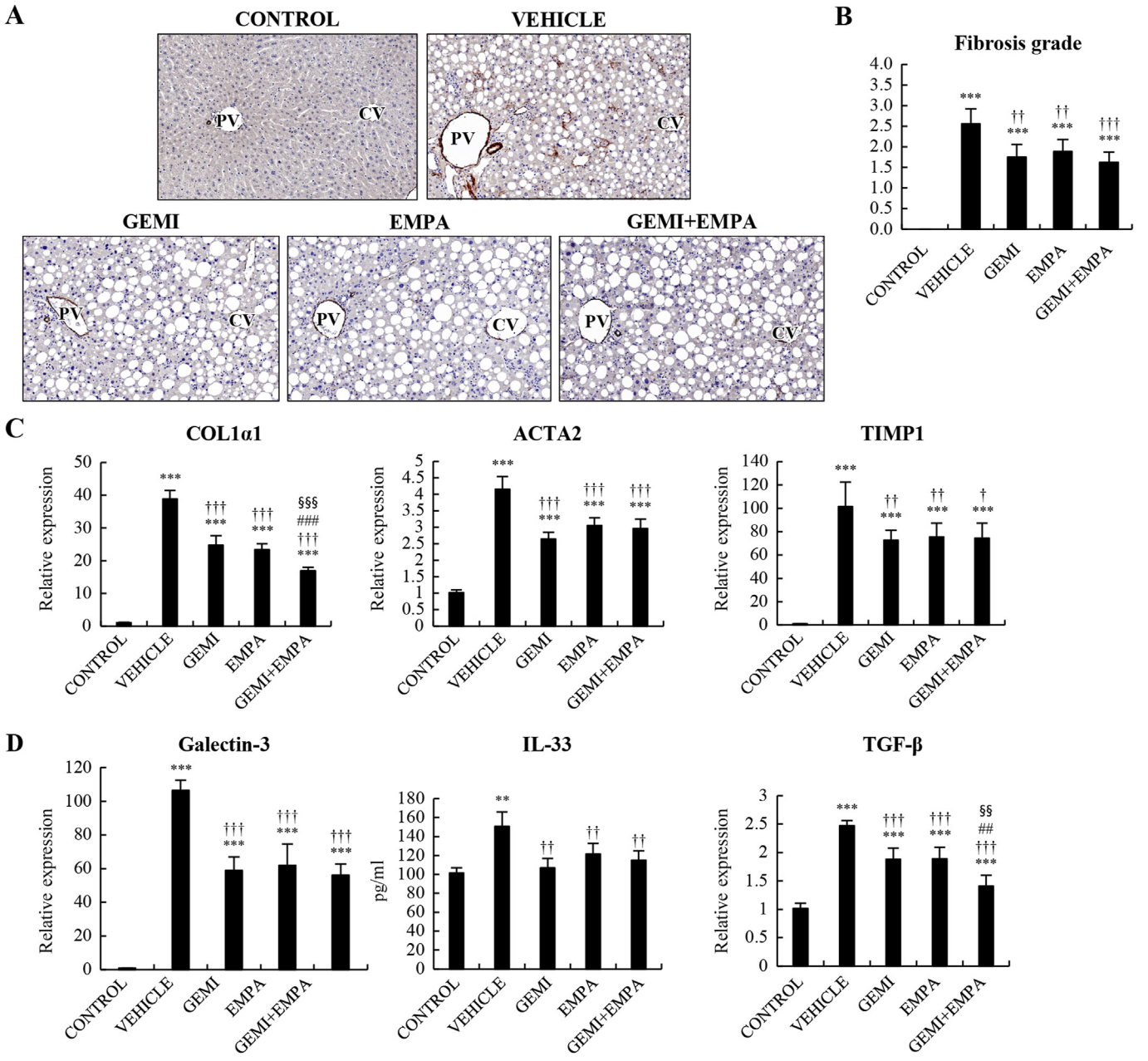

Fig. 4. Effects of gemigliptin and empagliflozin on hepatic fibrosis in a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD)-induced non-alcoholic fatty liver disease (NAFLD) mouse model. (A) Immunohistochemical staining of liver sections for α-smooth muscle actin (α-SMA, 20 ×). (B) Fibrosis grade. (C) Expression of pro-fibrotic genes encoding collagen type 1 α1 (COL1α1), smooth muscle and aorta actin α-2 (ACTA2), and tissue inhibitor matrix metalloproteinase 1 (TIMP1), as determined using qRT-PCR analysis. (E) Hepatic galectin-3 and transforming growth factor β (TGF-β) mRNA expression determined using qRT-PCR, and interleukin (IL)-33 concentrations determined using ELISA. *P < 0.05, **P < 0.01, and ***P < 0.001 in comparisons of the CONTROL group with the VEHICLE, GEMI, EMPA, or GEMI + EMPA treatment groups. †P < 0.05, ††P < 0.01, and †††P < 0.001 in comparisons of the VEHICLE group with the GEMI, EMPA, or GEMI + EMPA treatment groups. $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, and $^{\#\#\#}$P < 0.001 in comparison between the GEMI and GEMI + EMPA treatment groups. §P < 0.05, §§P < 0.01, and §§§P < 0.001 in comparison between the EMPA and GEMI + EMPA treatment group.
CONTROL: vehicle treated standard chow-diet group; VEHICLE: vehicle treated CDAHFD-diet group; GEMI: gemigliptin treated CDAHFD-diet group; EMPA: empagliflozin treated CDAHFD-diet group; GEMI + EMPA: gemigliptin and empagliflozin co-treated CDAHFD-diet group; PV: portal vein; CV: central vein; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay.

partially expressed by macrophages and hepatocytes [27]. Dapagliflozin, an SGLT2i, can diminished the activity of reactive oxygen species–NOD-like receptor family pyrin domain containing 3 inflammasome, thereby reducing hepatic TG and FFA levels as well as hepatic steatosis and ballooning scores [14]. Canagliflozin can reduce serum TG levels and hepatic fat accumulation by inhibiting prostaglandin E2 in obese diabetic NAFLD mice [12]. We demonstrated that empagliflozin reduces hepatic TG levels, reduces the gene expression of inflammatory cytokines and chemokines, such as CCL3, CCL4, and CXCL10, and ameliorates oxidative stress by enhancing the expression of catalase, but not SOD1.

Galectin-3 is a fibrogenic regulator expressed in hepatic resident macrophages and IL-33 is another profibrotic modulator secreted by hepatocytes [28]. Galectin-3 regulates the IL-33/IL-33 receptor axis and promotes hepatic fibrosis. Since TGF-β requires intracellular galectin-3 to activate myofibroblasts and produce procollagen,

inhibition of galectin-3 and IL-33 induces a decrease in TGF-β expression [29]. To our knowledge, this study is the first to demonstrate that gemigliptin and empagliflozin can suppress galectin-3 expression as well as decrease IL-33 production, thereby reducing the expression of genes encoding TGF-β and COL1α1.

In this study, the gemigliptin and empagliflozin co-treatment showed notable improvement in some parameters of hepatic steatosis (CCL5 and hepatic TG), oxidative stress (SOD2), and fibrosis (TGF-β and COL1α1).

5. Conclusions

The DPP-4i and SGLT2i can alleviate hepatic steatosis, oxidative stress, inflammation, and fibrosis in CDAHFD-induced NAFLD mice. Therefore, DPP-4i and SGLT2i are expected to have a beneficial effect in arresting the progression of NAFLD.

Author's contributions

HJK performed the conceptualization and supervised. NL and YJH contributed the experiments, performed the data curation, and wrote the original draft. SEC, JYJ, SJH, DJK, YK, and KWL reviewed the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Hae Jin Kim reports financial support was provided by National Research Foundation of Korea (NRF).

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) [NRF-2020R1F1A1074019]. We would like to thank Editage (www.editage.co.kr) for editing and reviewing this manuscript for English language.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2021.12.065.

References

[1] F. Negro, Natural history of NASH and HCC, Liver Int. 40 (Suppl 1) (2020) 72–76.
[2] J.K. Dowman, J.W. Tomlinson, P.N. Newsome, Pathogenesis of non-alcoholic fatty liver disease, QJM 103 (2) (2010) 71–83.
[3] Yumi Ando, J.H. Jou, Nonalcoholic fatty liver disease and recent guideline updates, Clinical Liver Disease 17 (2021) 23–28.
[4] E. Vilar-Gomez, Y. Martinez-Perez, L. Calzadilla-Bertot, et al., Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis, Gastroenterology 149 (2) (2015) 367–378, e5; quiz e14-15.
[5] N.A. Thornberry, B. Gallwitz, Mechanism of action of inhibitors of dipeptidyl-peptidase-4 (DPP-4), Best Pract. Res. Clin. Endocrinol. Metabol. 23 (4) (2009) 479–486.
[6] M. Kawakubo, M. Tanaka, K. Ochi, et al., Dipeptidyl peptidase-4 inhibition prevents nonalcoholic steatohepatitis-associated liver fibrosis and tumor development in mice independently of its anti-diabetic effects, Sci. Rep. 10 (1) (2020) 983.
[7] T. Shinjo, Y. Nakatsu, M. Iwashita, et al., DPP-IV inhibitor anagliptin exerts anti-inflammatory effects on macrophages, adipocytes, and mouse livers by suppressing NF-kappaB activation, Am. J. Physiol. Endocrinol. Metab. 309 (3) (2015) E214–E223.
[8] H.J. Hwang, T.W. Jung, B.H. Kim, et al., A dipeptidyl peptidase-IV inhibitor improves hepatic steatosis and insulin resistance by AMPK-dependent and JNK-dependent inhibition of LECT2 expression, Biochem. Pharmacol. 98 (1) (2015) 157–166.
[9] J. Shirakawa, H. Fujii, K. Ohnuma, et al., Diet-induced adipose tissue inflammation and liver steatosis are prevented by DPP-4 inhibition in diabetic mice, Diabetes 60 (4) (2011) 1246–1257.
[10] S.B. Akaslan, C.K. Degertekin, G. Yilmaz, et al., Effects of sitagliptin on nonalcoholic fatty liver disease in diet-induced obese rats, Metab. Syndr. Relat. Disord. 11 (4) (2013) 243–250.
[11] A.A. Tahrani, A.H. Barnett, C.J. Bailey, SGLT inhibitors in management of diabetes, Lancet Diabetes Endocrinol. 1 (2) (2013) 140–151.
[12] K. Yoshino, T. Hosooka, M. Shinohara, et al., Canagliflozin ameliorates hepatic fat deposition in obese diabetic mice: role of prostaglandin E2, Biochem. Biophys. Res. Commun. 557 (2021) 62–68.
[13] M.K. ElMahdy, M.G. Helal, T.M. Ebrahim, Potential anti-inflammatory effect of dapagliflozin in HCHF diet- induced fatty liver degeneration through inhibition of TNF-alpha, IL-1beta, and IL-18 in rat liver, Int. Immunopharm. 86 (2020) 106730.
[14] W. Leng, M. Wu, H. Pan, et al., The SGLT2 inhibitor dapagliflozin attenuates the activity of ROS-NLRP3 inflammasome axis in steatohepatitis with diabetes mellitus, Ann. Transl. Med. 7 (18) (2019) 429.
[15] N. Lee, Y.J. Heo, S.E. Choi, et al., Anti-inflammatory effects of empagliflozin and gemigliptin on LPS-stimulated macrophage via the IKK/NF-kappaB, MKK7/JNK, and JAK2/STAT1 signalling pathways, J Immunol Res 2021 (2021) 9944880.
[16] E. Jung, J. Kim, S.H. Kim, et al., Gemigliptin, a novel dipeptidyl peptidase-4 inhibitor, exhibits potent anti-glycation properties in vitro and in vivo, Eur. J. Pharmacol. 744 (2014) 98–102.
[17] M. Kern, N. Kloting, M. Mark, et al., The SGLT2 inhibitor empagliflozin improves insulin sensitivity in db/db mice both as monotherapy and in combination with linagliptin, Metabolism 65 (2) (2016) 114–123.
[18] J. Folch, M. Lees, G.H.S. Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1) (1957) 497–509.
[19] E. Kathirvel, P. Chen, K. Morgan, et al., Oxidative stress and regulation of antioxidant enzymes in cytochrome P4502E1 transgenic mouse model of nonalcoholic fatty liver, J. Gastroenterol. Hepatol. 25 (6) (2010) 1136–1143.
[20] G.C. Farrell, D. van Rooyen, L. Gan, et al., NASH is an inflammatory disorder: pathogenic, prognostic and therapeutic implications, Gut Liver 6 (2) (2012) 149–171.
[21] B.M. Kim, A.M. Abdelfattah, R. Vasan, et al., Hepatic stellate cells secrete Ccl5 to induce hepatocyte steatosis, Sci. Rep. 8 (1) (2018) 7499.
[22] X. Pan, A. Chiwanda Kaminga, A. Liu, et al., Chemokines in non-alcoholic fatty liver disease: a systematic review and Network meta-analysis, Front. Immunol. 11 (2020) 1802.
[23] Z. Xu, X. Zhang, J. Lau, et al., C-X-C motif chemokine 10 in non-alcoholic steatohepatitis: role as a pro-inflammatory factor and clinical implication, Expet Rev. Mol. Med. 18 (2016) e16.
[24] Z. Long, M. Cao, S. Su, et al., Inhibition of hepatocyte nuclear factor 1b induces hepatic steatosis through DPP4/NOX1-mediated regulation of superoxide, Free Radic. Biol. Med. 113 (2017) 71–83.
[25] N. Takata, K.A. Ishii, H. Takayama, et al., LECT2 as a hepatokine links liver steatosis to inflammation via activating tissue macrophages in NASH, Sci. Rep. 11 (1) (2021) 555.
[26] A. Bołdys, B. Okopień, Inhibitors of type 2 sodium glucose co-transporters – a new strategy for diabetes treatment, Pharmacol. Rep. 61 (2009) 778–784.
[27] C. Xu, W. Wang, J. Zhong, et al., Canagliflozin exerts anti-inflammatory effects by inhibiting intracellular glucose metabolism and promoting autophagy in immune cells, Biochem. Pharmacol. 152 (2018) 45–59.
[28] N. Pejnovic, I. Jeftic, N. Jovicic, et al., Galectin-3 and IL-33/ST2 axis roles and interplay in diet-induced steatohepatitis, World J. Gastroenterol. 22 (44) (2016) 9706–9717.
[29] Neil C. Henderson, Alison C. Mackinnon, Sarah L. Farnworth, et al., Galectin-3 regulates myofibroblast activation and hepatic fibrosis, Proc. Natl. Acad. Sci. Unit. States Am. 103 (2006) 5060–5065.