Wild Bitter Melon Extract Regulates LPS-Induced Hepatic Stellate Cell Activation, Inflammation, Endoplasmic Reticulum Stress, and Ferroptosis

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The activation of hepatic stellate cells (HSCs) is a key component of liver fibrosis. Two antifibrosis pathways have been identified, the reversion to quiescent-type HSCs and the clearance of HSCs through apoptosis. Lipopolysaccharide- (LPS-) induced HSC activation and proliferation have been associated with the development of liver fibrosis. We determined the pharmacological effects of wild bitter melon (WM) on HSC activation following LPS treatment and investigated whether WM treatment affected cell death pathways under LPS-treated conditions, including ferroptosis. WM treatment caused cell death, both with and without LPS treatment. WM treatment caused reactive oxygen species (ROS) accumulation without LPS treatment and reversed the decrease in lipid ROS production in HSCs after LPS treatment. We examined the effects of WM treatment on fibrosis, endoplasmic reticulum (ER) stress, inflammation, and ferroptosis in LPS-activated HSCs. The western blotting analysis revealed that the WM treatment of LPS-activated HSCs induced the downregulation of the connective tissue growth factor (CTGF), α-smooth muscle actin (α-SMA), integrin-β1, phospho-JNK (p-JNK), glutathione peroxidase 4 (GPX4), and cystine/glutamate transporter (SLC7A11) and the upregulation of CCAAT enhancer-binding protein homologous protein (CHOP). These results support WM as an antifibrotic agent that may represent a potential therapeutic solution for the management of liver fibrosis.

1. Introduction

Chronic liver fibrosis is a health problem, characterized by severe morbidity and significant mortality [1–3]. The underlying physiology of chronic liver fibrosis has been associated with the rapid activation and transdifferentiation of quiescent HSCs fibrogenic myofibroblast-like cells following liver injury or the development of liver fibrosis [4, 5], resulting in cell proliferation, migration, extracellular matrix (ECM) accumulation [6], contraction, chemotaxis, and inflammatory signaling [7]. ECM accumulation has been associated with the increased expression of α-smooth muscle actin (α-SMA), type I and III collagens, and tissue inhibitor of metalloproteinase-1 (TIMP-1), following the development of liver fibrosis [5, 8–10]. The contraction of HSCs has been proposed to mediate fibrosis by regulating sinusoidal blood flow and ECM remodeling [11]. Recent studies have shown that HSCs are activated by external signals, contributing to liver inflammation or liver injury by producing inflammatory cytokines and directing T lymphocytes into the parenchyma [12]. Multiple cellular and molecular signaling pathways are involved in the regulation of HSC activation: (1) the release of mitogenic (transforming growth factor-alpha (TGF-α) [13]), platelet-derived growth factor
2. Materials and Methods

2.1. Reagents. WM extract (WM) was purchased from License Biotech, Co., Ltd. (Taipei, Taiwan). The total phenolic extract (TPE) was obtained as described by Huang et al. [54].

2.2. Antibodies. The following antibodies were used for immunofluorescence staining and Western blotting: rabbit polyclonal antibodies against CHOP (#A0854, 1:1000 dilution, Abclonal, MA, USA), p-JNK (#AP0808, 1:1000 dilution, Abclonal, MA, USA), JNK (#A4867, 1:1000 dilution, Abclonal, MA, USA), CTGF (#A11456, 1:1000 dilution, Abclonal, MA, USA), α-SMA (#A1011, 1:1000 dilution, Abclonal, MA, USA), integrin-β1 (#A11060, 1:1000 dilution, Abclonal, MA, USA), GPX4 (#A1933, 1:1000 dilution, Abclonal, MA, USA), SLC7A11 (#A13685, 1:1000 dilution, Abclonal, MA, USA), and β-actin (#AC026, 1:5000 dilution, Abclonal, MA, USA).

2.3. Cell Culture. HSC-T6, a rat HSC cell line, was purchased from Millipore (MA, USA). HSC-T6 cells were cultured at 37°C in Dulbecco’s minimum essential medium (DMEM; Gibco, NY, USA), supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin, and 2.5 µg/ml amphotericin B), in a humidified atmosphere containing 5% CO₂. The culture medium was replaced every other day. Once the cells reached 70–80% confluency, they were trypsinized and seeded into 6-well or 24-well plastic dishes for further experiments.

2.4. Analysis of Cell Viability. Cell viability was measured using WST-1 assay. Cells were seeded at a density of 5 × 10⁴ cells/ml in 24-well plates and cultured in phenol red-free DMEM, containing 0.5% heat-inactivated FBS, for 24 h. Cells were then incubated with 20 µg/ml of WM or 10 µg/ml of LPS, as indicated, for 24 h. WST-1 reagent was then added to the medium and incubated at 37°C for 2 h. The absorbance was measured at 450 nm using a microplate reader (Thermo Labsystems, Waltham, MA, USA).

2.5. Western Blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 10% acrylamide gels, with 20 µg of protein loaded into each lane. After electrophoresis, the proteins were transferred from the gel to a polyvinylidene fluoride (PVDF) membrane, at 350 mA for 2 hours, and the membrane was then blocked with 5% nonfat milk for 1 hour. The membranes were incubated with primary antibodies, diluted 1:1,000 in 5% nonfat milk, overnight at 4°C. Membranes were washed in TBST buffer (20 mM Tris-HCl, pH 7.4, 137 mM NaCl, and 0.1% Tween-20) 3 times, for 10 minutes each time, incubated with secondary antibodies conjugated to horseradish peroxidase (HRP), at 1:10,000 dilution, for 1 hour at room temperature, washed again, and stained with a Western HRP substrate. Protein bands were visualized on X-ray film using an enhanced chemiluminescence system (Kodak).
2.6. Lipid ROS Detection. Cells were incubated with 2 µM C11-BODIPY 581/591 (Thermo Fisher Scientific), in culture medium for 1 h and then washed with phosphate-buffered saline. After trypsinization, cells were collected and used for flow cytometry (BD Biosciences, San Jose, CA, USA), using an excitation wavelength of 488 nm and an emission wavelength of 517–527 nm.

2.7. Statistical Analysis. Continuous data were expressed as the mean ± standard error of the mean. Statistical differences among means from different groups were determined by one-way or a two-way analysis of variance, followed by a Bonferroni post hoc test for continuous variables. P values <0.05 were considered significant differences.

3. Results

3.1. WM Treatment Caused ROS Accumulation and Cell Death. The acceleration ROS accumulation has been shown to disrupt redox homeostasis and cause severe damage in cancer cells, resulting in cancer cell death via the activation of apoptosis, autophagic cell death, and necroptosis [55]. The induction of apoptosis on HSCs via the stimulation of ROS accumulation represents a potential strategy for addressing liver fibrosis [56]. Our results showed that WM treatment induced ROS overproduction in HSCs relative to untreated cells (Figures 1(a) and 1(b)). Decreased HSC viability was detected after treatment with 20 µg/ml WM for 24 h compared with untreated cells (Figure 1(c)). These results indicated that WM treatment induced ROS accumulation and cell death.

3.2. WM Treatment Resulted in Lipid ROS Accumulation and Cell Death in LPS-Activated HSCs. LPS is a well-known activator of HSCs and LPS treatment results in the activation of a proinflammatory, myofibroblast phenotype [57]. ROS-induced lipid peroxidation and lipid ROS accumulation has been reported to play critical roles in cell death pathways, including apoptosis, autophagy, and ferroptosis [58]. As shown in Figure 2, the results indicated that lipid ROS accumulation decreased in LPS-activated HSCs compared with untreated HSCs (Figures 2(a) and 2(b), column 1 vs. column 2 but increased after the WM treatment of LPS-activated HSCs (WM treatment in LPS-activated HSCs, Figures 2(a) and 2(b), column 2 vs. column 4). In contrast, cell viability significantly decreased after WM treatment in quiescent HSCs compared with untreated cells (Figure 2(c), column 1 vs. column 3). Interestingly, WM treatment caused cell death in LPS-activated HSCs (Figure 2(c), column 2 vs. column 4). Therefore, we proposed that WM treatment resulted in lipid ROS accumulation and cell death in LPS-activated HSCs.

3.3. WM Treatment Enhanced ER Stress, Attenuated Inflammation, and Triggered Ferroptosis in LPS-Activated HSCs. CHOP plays a critical role in ER stress-induced apoptosis [59]. Oyadomari and Mori demonstrated that when severe ER stress conditions persist, apoptotic signaling pathways become activated, resulting in the induction of CHOP [60]. Our results showed that CHOP expression levels decreased in LPS-activated HSCs (Figures 3(a) and 3(b), column 1 vs. column 2) but increased following the WM treatment of LPS-activated HSCs (WM treatment in LPS-activated HSCs, Figures 3(a) and 3(b), column 2 vs. column 4). Additionally, JNK is a well-known regulator of the inflammatory response [61]. As shown in Figures 3(a) and 3(b), the expression levels of p-JNK increased in LPS-activated HSCs, compared with quiescent HSCs (Figures 3(a) and 3(b), column 1 vs. column 2) and decreased after the WM treatment of LPS-activated HSCs (WM treatment in LPS-activated HSCs, Figures 3(a) and 3(b), column 2 vs. column 4).

Ferroptosis is a newly identified cell death pathway, which occurs in an iron-dependent manner and is characterized by iron accumulation and lipid peroxidation during the cell death process [62]. SLC7A11 is a key regulator of the antioxidant system $\text{Xc}^-$, which mediates the exchange of cysteine and glutamate, and is widely distributed in the phospholipid bilayer [63]. GPX4 (glutathione peroxidase 4) activity decreases with increasing system $\text{Xc}^-$ activity, resulting in decreased antioxidant capacity, lipid ROS accumulation, and ultimately oxidative damage and ferroptosis [64]. Friedmann Angeli et al. reported that knockout of GPX4 caused cell death via ferroptosis, both in vitro and in vivo [64]. In the present study, the results showed that the expression levels of GPX4 and SLC7A11 increased in LPS-activated HSCs, compared with untreated HSCs (Figures 3(c) and 3(d), column 1 vs. column 2), but decreased after the WM treatment of LPS-activated HSCs (WM treatment in LPS-activated HSCs, Figures 3(c) and 3(d), column 2 vs. column 4). Altogether, these results indicated that WM treatment sensitized LPS-activated HSCs ER stress, attenuated inflammation, and triggered ferroptosis.

3.4. WM Treatment Has Antifibrotic Effects on LPS-Activated HSCs. Activated HSCs are well-known as potential therapeutic targets in liver fibrosis [65]. We investigated whether WM treatments have any antifibrotic effects in LPS-activated HSCs. As shown in Figures 4(a) and 4(b), the expression levels of CTGF, $\alpha$-SMA, and integrin-$\beta$1 increased in LPS-activated HSCs, compared with untreated HSCs (Figures 4(a) and 4(b), column 1 vs. column 2), but decreased after WM treatment (WM treatment in LPS-activated HSCs, Figures 4(a) and 4(b), column 2 vs. column 4). Therefore, we suggested that WM treatment has great potential for use to treat and prevent liver fibrosis through effects on activated HSCs.

4. Discussion

Activated HSCs play major roles in the pathogenesis of liver fibrosis [66]. Growing evidence has suggested that the induction of HSC cell death and the inhibition of HSC growth may represent potential strategies for the treatment and/or prevention of liver fibrosis [9, 33, 67–70]. Furthermore, natural fruits may be used as additional therapeutic
Figure 1: WM treatment induced ROS production and decreased cell viability. (a) Cells treated with (WM+) or without (WM−) 20 µg/ml WM for 24 h. The levels of intracellular ROS were determined using DCF-DA, and fluorescence was detected using FACS Calibur analysis. Control samples refer to cells without DCF-DA. (b) ROS levels are expressed as the mean fluorescence intensity. (c) Cells treated with either the vehicle (0 µg/ml WM) or WM (20 µg/ml) for 24 h. After the incubation period, cell viability was determined using WST-1 assay. All data are presented as the mean ± SD. *p < 0.05, **p < 0.01, n = 3.

Figure 2: Continued.
Figure 2: WM treatment reversed the decrease in lipid ROS production and increased cell viability in LPS-activated HSCs. (a) Changes in cellular lipid ROS levels, associated with the indicated conditions in HSC-T6 cells. (b) ROS levels are expressed as mean fluorescence intensity. (c) Cells cultured using the indicated conditions, for 24 h. After the incubation period, cell viability was determined using WST-1 assay. Controls refer cells without 2 μM C11-BODIPY 581/591. LPS 0 indicates cells without LPS treatment. LPS 10 indicates cells treated with 10 μg/ml LPS. WM 20 indicates cells treated with 20 μg/ml WM. All data are presented as the mean ± SD. *p < 0.05, n = 3.

Figure 3: Continued.
approaches to inhibit hepatic fibrogenesis. To our knowledge, this study demonstrated, for the first time, that an extract from the natural fruit WM could attenuate LPS-induced HSC activation via the regulation of ER stress and ferroptosis. However, the pharmacological effects of WM treatments in HSCs remain unclear. Our results indicated that WM treatment caused ROS accumulation, lipid ROS accumulation, and cell death in LPS-activated HSCs (Figures 1 and 2). WM treatment also increased ER stress-induced apoptosis and attenuated inflammation and ferroptosis in LPS-activated HSCs (Figure 3). We also detected the effects of WM treatment on the expression of the following proteins: α-SMA, a critical marker of HSC activation [71]; CTGF, a maker of liver fibrosis [72, 73]; and integrin-β1, a hallmark of hepatic fibrosis [74]. As shown in Figure 4, WM treatment decreased the expression levels of these proteins (Figure 4). Therefore, these data demonstrated that WM treatment may protect against liver fibrosis via HSC inactivation or death.

Astaxanthin was shown to inhibit liver fibrosis via HSC inactivation and the decreased formation of ECM in carbon tetrachloride (CCL₄) and bile duct ligation mouse models [75]. Similar results were also reported for treatments with curcumin [76], blueberry [77], silymarin [78], 3, 5-diethoxy-3’-hydroxyresveratrol [79], quercetin [80], epigallocatechin-3-gallate [81], coffee [82], and vitamins [83]. Additionally, Kuo et al. suggested that the marine extract from a gorgonian coral Pinnigorgia sp. (Pin) could induce apoptosis in HSC-T6 cells via ROS-ERK/JNK-caspase-3 signaling and may exhibit therapeutic potential for the clearance of HSCs [84]. Other studies have reported similar results [85–87]. These studies further strengthen the evidence for the use of bioactive food components and natural products with potential antifibrotic effects in therapeutic approaches designed to slow or reverse the development of liver fibrosis.

Huang et al. reported that the quantitative high-performance liquid chromatography analysis of WM TPE revealed gallic, chlorogenic, caffeic, ferulic, and cinnamic acids, myricetin, quercetin, luteolin, apigenin, and thymol and that WMTPE displayed an anti-inflammatory response against Propionibacterium acnes-induced skin inflammation, in vivo [54]. Chen et al. showed that gallic acids attenuated dimethylnitrosamine-induced fibrosis via the regulation of Smad phosphorylation [88]. Chlorogenic acids protect against CCL₄-induced liver fibrosis through the suppression of oxidative stress in the liver and HSCs [89]. Caffeic and ferulic acids have been shown to prevent liver damage and ameliorate liver fibrosis in CCL₄-treated rats [90, 91]. Wang et al. demonstrated that trans-cinnamic acid has antibesity effects in oleic acid- (OA-) induced HepG2 cells and high-fat diet- (HFD-) fed mice [92]; however, the role played by trans-cinnamic acid in HSCs remains unclear. Myricetin modulated the polarization of macrophages via...
the inhibition of TREM-1-TLR2/4-MyD88 signaling molecules in macrophages and attenuated liver inflammation and fibrosis in a choline-deficient, L-amino acid-defined, high-fat diet-induced nonalcoholic steatohepatitis model [93]. Quercetin caused decreased oxidative stress and inflammation and prevented liver fibrosis via the induction of HSC apoptosis [94]. Li et al. speculated that luteolin exhibits antifibrotic effects in HSCs and liver fibrosis by targeting the AKT/mTOR/p70S6K and TGFβ/Smad signaling pathways in CCl₄, dimethylnitrosamine, and bile duct ligation induced animal models of fibrosis and rat HSCs and HSC-T6 cells [95]. A computational approach indicated that apigenin was predicted to have antifibrotic activity [96]. Thymol significantly ameliorated liver injury due to endotoxicity in gastric ulcer rat models [97]; however, the role played by thymol in liver fibrosis remains uncertain.

**Figure 4:** WM treatment attenuated fibrosis in LPS-activated HSCs. (a) Changes in the expression levels of CTGF, α-SMA, and integrin-β1. β-Actin was used as an internal control. (b) Quantitative evaluation of the levels of specific proteins, assessed by ImageJ. All data are presented as the mean ± SD. n = 3, *p < 0.05, **p < 0.01.
5. Conclusions

In summary, the present study demonstrated that the pre-treatment of HSCs with WM prevented LPS-induced HSC-T6 cell activation (as demonstrated by CTGF, α-SMA, and integrin-β1 levels) and inflammation (as indicated by p-JNK levels). WM treatment caused ROS/lipid ROS overproduction, cell death, ER stress activation (as indicated by CHOP expression), and ferroptosis (as indicated by GPX4 and SLC7A11 expression) in LPS-activated HSC-T6 cells (Figure 5). These novel findings deepen our understanding of the mechanistic actions underlying WM treatments. Because WM showed potential antifibrotic effects in activated HSCs, further in vivo studies should be performed to determine the potential effects of WM treatment on various liver fibrosis models.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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References

[1] P. A. Bonis, S. L. Friedman, and M. M. Kaplan, "Is liver fibrosis reversible?" New England Journal of Medicine, vol. 344, pp. 452–454, 2001.

[2] S. L. Friedman, "Hepatic fibrosis-overview," Toxicology, vol. 254, pp. 120–129, 2008.

[3] S. L. Friedman, "Mechanisms of hepatic fibrogenesis," Gastroenterology, vol. 134, pp. 1655–1669, 2008.

[4] S. L. Friedman, "Hepatic stellate cells: protein, multifunctional, and enigmatic cells of the liver," Physiological Reviews, vol. 88, pp. 125–172, 2008.

[5] W. Hou and W. K. Syn, "Role of metabolism in hepatic stellate cell activation and fibrogenesis," Frontiers in Cell and Developmental Biology, vol. 6, p. 150, 2018.

[6] R. Bataller and D. A. Brenner, "Liver fibrosis," Journal of Clinical Investigation, vol. 115, pp. 209–218, 2005.

[7] T. Tsuchida and S. L. Friedman, "Mechanisms of hepatic stellate cell activation," Nature Reviews Gastroenterology & Hepatology, vol. 14, pp. 397–411, 2017.

[8] F. R. Murphy, R. Issa, X. Zhou et al., "Inhibition of apoptosis of activated hepatic stellate cells by tissue inhibitor of metalloproteinase-1 is mediated via effects on matrix metalloproteinase inhibition," Journal of Biological Chemistry, vol. 277, no. 13, pp. 11069–11076, 2002.

[9] S. L. Friedman, "Evolving challenges in hepatic fibrosis," Nature Reviews Gastroenterology & Hepatology, vol. 7, pp. 425–436, 2010.

[10] P. Bedossa and V. Paradis, "Liver extracellular matrix in health and disease," Journal of Pathology, vol. 200, pp. 504–515, 2003.

[11] R. K. Soon Jr and H. F. Yee Jr., "Stellate cell contraction: role, regulation, and potential therapeutic target," Clinical Liver Disease, vol. 12, pp. 791–803, 2008.

[12] T. Fujita and S. Narumiya, "Roles of hepatic stellate cells in liver inflammation: a new perspective," Inflammation and Regeneration, vol. 36, p. 1, 2016.

[13] H. Tsukamoto, "Cytokine regulation of hepatic stellate cells in liver fibrosis," Alcoholicism: Clinical and Experimental Research, vol. 23, pp. 911–916, 1999.

[14] L. Wong, G. Yasamaki, R. J. Johnson, and S. L. Friedman, "Induction of beta-platelet-derived growth factor receptor in rat hepatic lipocytes during cellular activation in vivo and in culture," Journal of Clinical Investigation, vol. 94, pp. 1563–1569, 1994.

[15] M. Pinzani, "PDGF and signal transduction in hepatic stellate cells," Frontiers in Bioscience, vol. 7, pp. d1720–d1726, 2002.

[16] R. G. Gillies, K. Wallace, and Y. P. Han, "Interleukin-1 participates in the progression from liver injury to fibrosis," American Journal of Physiology-Gastrointestinal and Liver Physiology, vol. 296, pp. GI324–GI331, 2009.

[17] H. L. Reeves and S. L. Friedman, "Activation of hepatic stellate cells-a key issue in liver fibrosis," Frontiers in Bioscience, vol. 7, pp. d808–d826, 2002.

[18] B. Dewidar, C. Meyer, S. Dooley, and A. N. Meindl-Beinker, "TGF-beta in hepatic stellate cell activation and liver fibrogenesis-updated 2019," Cells, vol. 8, 2019.

[19] M. H. Chou, Y. H. Huang, T. M. Lin et al., "Selective activation of toll-like receptor 7 in activated hepatic stellate cells may modulate their profibrogenic phenotype," Biochemical Journal, vol. 447, pp. 25–34, 2012.

[20] H. Liu, X. Pan, H. Cao et al., "IL-32gamma promotes integrin alphavbeta6 expression through the activation of NF-kappaB in HSCs," Experimental and Therapeutic Medicine, vol. 14, pp. 3880–3886, 2017.

[21] S. W. Beaven, K. Wroblewski, J. Wang et al., "Liver X receptor signaling is a determinant of stellate cell activation and susceptibility to fibrotic liver disease," Gastroenterology, vol. 140, pp. 1052–1062, 2011.

[22] T. Li, A. L. Eheim, S. Klein et al., "Novel role of nuclear receptor rev-erba in hepatic stellate cell activation: potential therapeutic target for liver injury," Hepatology, vol. 59, no. 6, pp. 2383–2396, 2014.

[23] K. Palumbo-Zerr, P. Zerr, A. Distler et al., "Orphan nuclear receptor NR4A1 regulates transforming growth factor-beta signaling and fibrosis," Nature Medicine, vol. 21, pp. 150–158, 2015.

[24] A. Duran, E. D. Hernandez, M. Reina-Campos et al., "p62/SQSTM1 by binding to vitamin D receptor inhibits hepatic stellate cell activity, fibrosis, and liver cancer," Cancer Cell, vol. 30, pp. 595–609, 2016.

[25] Y. H. Li, D. H. Choi, E. H. Lee, S. R. Seo, S. Lee, and E.-H. Cho, "Sirtuin 3 (SIRT3) regulates α-smooth muscle actin (α-SMA) production through the succinate dehydrogenase-G protein-coupled receptor 91 (GPR91) pathway in hepatic stellate cells," Journal of Biological Chemistry, vol. 291, no. 19, pp. 10277–10292, 2016.

[26] Y. H. Li, S. H. Woo, D. H. Choi, and E. H. Cho, "Succinate causes alpha-SMA production through GPR91 activation in hepatic stellate cells," Biochemical and Biophysical Research Communications, vol. 463, pp. 853–858, 2015.

[27] L. F. Thoen, E. L. Guimaraes, L. Dolle et al., "A role for autophagy during hepatic stellate cell activation," Journal of Hepatology, vol. 55, pp. 1353–1360, 2011.

[28] V. Hernández-Gea and S. L. Friedman, "Autophagy fuels tissue fibrogenesis," Autophagy, vol. 8, no. 5, pp. 849–850, 2012.

[29] V. Hernandez-Gea, Z. Ghiassi-Nejad, R. Rozenfeld et al., "Autophagy releases lipid that promotes fibrogenesis by activated hepatic stellate cells in mice and in human tissues," Gastroenterology, vol. 142, pp. 938–946, 2012.

[30] V. Hernández-Gea, M. Hilscher, R. Rozenfeld et al., "Endoplasmic reticulum stress induces fibrogenic activity in hepatic stellate cells through autophagy," Journal of Hepatology, vol. 59, no. 1, pp. 98–104, 2013.

[31] Q. Ou, Y. Weng, S. Wang et al., "Silybin alleviates hepatic steatosis and fibrosis in NASH mice by inhibiting oxidative stress and involvement with the NF-kappaB pathway," Digestive Diseases and Sciences, vol. 63, pp. 3398–3408, 2018.

[32] J. Li, Y. R. Zhao, and Z. Tian, "Roles of hepatic stellate cells in acute liver failure: from the perspective of inflammation and fibrosis," World Journal of Hepatology, vol. 11, pp. 412–420, 2019.

[33] J. P. Pradere, J. Klawe, S. De Minicis et al., "Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice," Hepatology, vol. 58, pp. 1461–1473, 2013.

[34] A. Wehr, C. Baecx, F. Heymann et al., "Chemokine receptor CXCR6-dependent hepatic NK T cell accumulation promotes inflammation and liver fibrosis," Journal of Immunology, vol. 190, pp. 5226–5236, 2013.
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[35] J. A. Fallowfield, “Therapeutic targets in liver fibrosis,” *American Journal of Physiology: Gastrointestinal and Liver Physiology*, vol. 300, pp. G709–G715, 2011.

[36] S. L. Friedman, “Preface,” *Clinics in Liver Disease*, vol. 12, no. 4, pp. xiii–xiv, 2008.

[37] H. N. Fan, H. J. Wang, C. R. Yang-Dan et al., “Protective effects of hydrogen sulfide on oxidative stress and fibrosis in hepatic stellate cells,” *Molecular Medicine Reports*, vol. 7, pp. 247–253, 2013.

[38] J. Saengsai, S. Kongtunjanphuk, N. Yoswatthana, T. Kummalue, and W. Jiratcharryakul, “Antibacterial and antiproliferative activities of plumerin, an iridoid isolated from Momordica charantia vine,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 823178, 10 pages, 2015.

[39] T. H. Tsai, W. C. Huang, H. T. Ying et al., “Wild bitter melon leaf extract inhibits Porphyromonas gingivalis-induced inflammation: identification of active compounds through bioassay-guided isolation,” *Molecules*, vol. 21, p. 454, 2016.

[40] T. M. H. Pham, D.-H. Ngo, D.-N. Ngo, and T. S. Vo, “Investigation of biological activities of wild bitter melon (Momordica charantia linn. Var. Abbreviata ser.)” *Biomolecules*, vol. 9, no. 6, p. 211, 2019.

[41] C. Hsu, T. H. Tsai, Y. Y. Li, W. H. Wu, C. J. Huang, and P. J. Tsai, “Wild bitter melon (Momordica charantia Linn. Var. abbreviata Ser.) extract and its bioactive components suppress Propionibacterium acnes-induced inflammation,” *Food Chemistry*, vol. 135, pp. 976–984, 2012.

[42] J. R. Weng, L. Y. Bai, C. F. Chiu, J. L. Hu, S. J. Chiu, and C. Y. Wu, “Cucurbitane triterpenoid from Momordica charantia induces apoptosis and autophagy in breast cancer cells, in part, through peroxisome proliferator-activated receptor gamma activation,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 935675, 12 pages, 2013.

[43] E. F. Fang, C. Z. Zhang, W. P. Fong, and T. B. Ng, “RNase MC2: a new Momordica charantia ribonuclease that induces apoptosis in breast cancer cells associated with activation of MAPKs and induction of caspase pathways,” *Apoptosis*, vol. 17, pp. 377–387, 2012.

[44] R. B. Ray, A. Raychoudhuri, R. Steele, and P. Nerurkar, “Bitter melon (Momordica charantia) extract inhibits breast cancer cell proliferation by modulating cell cycle regulatory genes and promotes apoptosis,” *Cancer Research*, vol. 70, pp. 1925–1931, 2010.

[45] Y. Li, L. Yin, L. Zheng et al., “Application of high-speed counter-current chromatography coupled with a reverse micelle solvent system to separate three proteins from Momordica charantia,” *Journal of Chromatography. B. Analytical Technologies in the Biomedical and Life Sciences*, vol. 895–896, pp. 77–82, 2012.

[46] D. Kwatra, A. Venugopal, D. Standing et al., “Bitter melon extracts enhance the activity of chemotherapeutic agents through the modulation of multiple drug resistance,” *Journal of Pharmaceutical Sciences*, vol. 102, pp. 4444–4454, 2013.

[47] M. Kaur, G. Deep, A. K. Jain et al., “Bitter melon juice activates cellular energy sensor AMP-activated protein kinase causing apoptotic death of human pancreatic carcinoma cells,” *Carcinogenesis*, vol. 34, pp. 1585–1592, 2013.

[48] E. F. Fang, C. Z. Zhang, J. H. Wong, J. Y. Shen, C. H. Li, and T. B. Ng, “The MAP30 protein from bitter gourd (Momordica charantia) seeds promotes apoptosis in liver cancer cells in vitro and in vivo,” *Cancer Letters*, vol. 324, pp. 66–74, 2012.

[49] C. Z. Zhang, E. F. Fang, H. T. Zhang, L. L. Liu, and J. P. Yun, “Momordica charantia lectin exhibits antitumor activity towards hepatocellular carcinoma,” *Investigational New Drugs*, vol. 33, pp. 1–11, 2015.

[50] P. Pitchakarn, S. Suzuki, K. Ogawa et al., “Induction of G1 arrest and apoptosis in androgen-dependent human prostate cancer by Kuguacin J, a triterpenoid from Momordica charantia leaf,” *Cancer Letters*, vol. 306, pp. 142–150, 2011.

[51] S. D. Xiong, K. Yu, X. H. Liu et al., “Ribosome-inactivating proteins isolated from dietary bitter melon induce apoptosis and inhibit histone deacetylase-1 selectively in premalignant and malignant prostate cancer cells,” *International Journal of Cancer*, vol. 125, pp. 774–782, 2009.

[52] P. Pitchakarn, S. Ohnuna, K. Pintha, W. Pompimon, S. V. Ambudkar, and P. Limtrakul, “Kuguacin J isolated from Momordica charantia leaves inhibits P-glycoprotein (ABCB1)-mediated multidrug resistance,” *Journal of Nutritional Biochemistry*, vol. 23, pp. 76–84, 2012.

[53] M. Bortolotti, D. Mercatelli, and L. Polito, “Momordica charantia, a nutraceutical approach for inflammatory related diseases,” *Frontiers in Pharmacology*, vol. 10, p. 486, 2019.

[54] W. C. Huang, T. H. Tsai, C. J. Huang et al., “Inhibitory effects of wild bitter melon leaf extract on Propionibacterium acnes-induced skin inflammation in mice and cytokine production in vitro,” *Food Functions*, vol. 6, pp. 2550–2560, 2015.

[55] S. J. Kim, H. S. Kim, and Y. R. Seo, “Understanding of ROS-inducing strategy in anticancer therapy,” *Oxidative Medicine and Cellular Longevity*, vol. 2019, Article ID 5381692, 12 pages, 2019.

[56] A. M. Brunati, M. A. Pagano, A. Bindoli, and M. P. Rigobello, “Thiol redox systems and protein kinases in hepatic stellate cell regulatory processes,” *Free Radical Research*, vol. 44, pp. 363–378, 2010.

[57] F. J. Bohanon, X. Wang, C. Ding et al., “Oridonin inhibits hepatic stellate cell proliferation and fibrogenesis,” *Journal of Surgical Research*, vol. 190, pp. 55–63, 2014.

[58] L. J. Su, J. H. Zhang, H. Gomez et al., “Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and Ferroptosis,” *Oxidative Medicine and Cellular Longevity*, vol. 2019, Article ID 5080843, 13 pages, 2019.

[59] H. Hu, M. Tian, C. Ding, and S. Yu, “The C/EBP homologous protein (CHOP) transcription factor functions in endoplasmic reticulum stress-induced apoptosis and microbial infection,” *Frontiers in Immunology*, vol. 9, p. 3083, 2018.

[60] S. Oyadomari and M. Mori, “Roles of CHOP/GADD153 in endoplasmic reticulum stress,” *Cell Death & Differentiation*, vol. 11, pp. 381–389, 2004.

[61] R. J. Davis, “Signal transduction by the JNK group of MAP kinases,” *Cell*, vol. 103, pp. 239–252, 2000.

[62] J. Li, F. Cao, H. L. Yin et al., “Ferroptosis: past, present and future,” *Cell Death and Disease*, vol. 11, p. 88, 2020.

[63] S. J. Dixon, K. M. Lemberg, M. R. Lamprecht et al., “Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice,” *Nature Cell Biology*, vol. 16, pp. 1180–1191, 2014.

[64] R. K. Moreira, “Hepatic stellate cells and liver fibrosis,” *Archives of Pathology & Laboratory Medicine*, vol. 131, pp. 1728–1734, 2007.

[65] J. S. Troeger, I. Mederacke, G. Y. Gwak et al., “Deactivation of hepatic stellate cells during liver fibrosis resolution in mice,” *Gastroenterology*, vol. 143, pp. 1073–1083, 2012.
[67] R. Franco and J. A. Cidlowski, “Glutathione efflux and cell death,” Antioxidants & Redox Signaling, vol. 17, pp. 1694–1713, 2012.

[68] K. Ray, “Hepatic stellate cells hold the key to liver fibrosis,” Nature Reviews Gastroenterology & Hepatology, vol. 11, no. 2, p. 74, 2014.

[69] J. E. Puche, Y. Saiman, and S. L. Friedman, “Hepatic stellate cells and liver fibrosis,” Comprehensive Physiology, vol. 3, pp. 1473–1492, 2013.

[70] L. M. Kuo, C. Y. Kuo, C. Y. Lin, M. F. Hung, J. J. Shen, and J. E. Puche, Y. Saiman, and S. L. Friedman, “Hepatic stellate cells and liver fibrosis,” Molecules, vol. 19, pp. 3327–3344, 2014.

[71] J. J. Tomasek, G. Gabbiani, B. Hinz, C. Chaponnier, and T. L. Hwang, “Intracellular glutathione depletion by oridonin leads to apoptosis in hepatic stellate cells,” Molecules, vol. 3, pp. 349–363, 2002.

[72] G. Huang and D. R. Brigstock, “Regulation of hepatic stellate cells by connective tissue growth factor,” Frontiers in Bioscience (Landmark Ed.), vol. 17, pp. 2495–2507, 2012.

[73] V. Paradis, D. Dargere, M. Vidaud et al., “Expression of connective tissue growth factor in experimental rat and human liver fibrosis,” Hepatology, vol. 30, pp. 968–976, 1999.

[74] G. Huang and D. R. Brigstock, “Integrin expression and function in the response of primary culture hepatic stellate cells to connective tissue growth factor (CCN2),” Journal of Cellular and Molecular Medicine, vol. 15, pp. 1087–1095, 2011.

[75] M. Shen, K. Chen, J. Lu et al., “Protective effect of astaxanthin on liver fibrosis through modulation of TGF-beta1 and autophagy,” Molecules, vol. 20, pp. 18767–18780, 2014.

[76] Y. Zhao, X. Ma, J. Wang et al., “Curcumin protects against CCl4-induced liver fibrosis in rats by inhibiting HIF-1alpha through an ERK-dependent pathway,” Molecules, vol. 19, pp. 393–395, 2014.

[77] Y. Wang, M. Cheng, B. Zhang, F. Nie, and H. Jiang, “Dietary supplementation of blueberry juice enhances hepatic expression of metallothionein and attenuates liver fibrosis in rats,” PLoS One, vol. 8, p. e58659, 2013.

[78] S. Gharbia, C. Balta, H. Herman et al., “Enhancement of silmryan anti-fibrotic effects by complexation with hydroxypropyl (HPBCD) and randomly methylated (RAMEB) β-cyclodextrins in a mouse model of liver fibrosis,” Frontiers in Pharmacology, vol. 9, pp. 883–888, 2018.

[79] P. J. Lee, H. J. Park, N. Cho, and H. P. Kim, “3, 5-Diethoxy-3′-Hydroxyresveratrol (DEHR) ameliorates liver fibrosis via Caveolin-1 activation in hepatic stellate cells and in a mouse model of bile duct ligation injury,” Molecules, vol. 23, 2018.

[80] X. Li, Q. Jin, Q. Yao et al., “The flavonoid quercetin ameliorates liver inflammation and fibrosis by regulating hepatic macrophages activation and polarization in mice,” Frontiers in Pharmacology, vol. 9, pp. 72, 2018.

[81] K. Shen, X. Feng, R. Su, H. Xie, L. Zhou, and S. Zheng, “Epigallocatechin 3-gallate ameliorates bile duct ligation induced liver injury in mice by modulation of mitochondrial oxidative stress and inflammation,” PLoS One, vol. 10, p. e0126278, 2015.

[82] M. Wadhawan and A. C. Anand, “Coffee and liver disease,” Journal of Clinical and Experimental Hepatology, vol. 6, pp. 40–46, 2016.

[83] M. Bae, Y. K. Park, and J. Y. Lee, “Food components with antifibrotic activity and implications in prevention of liver disease,” Journal of Nutritional Biochemistry, vol. 55, pp. 1–11, 2018.

[84] L. M. Kuo, P. J. Chen, P. J. Sung et al., “The bioactive extract of Pinnigorgia sp. induces apoptosis of hepatic stellate cells via ROS-ERK/JNK-caspase-3 signaling,” Marine Drugs, vol. 16, 2018.

[85] Y. Tao, F. Wang, Q. Guo et al., “Curcumin induces RIPK1/RIPK3 complex-dependent necroptosis via JNK1/2ROS signaling in hepatic stellate cells,” Redox Biology, vol. 19, pp. 375–387, 2018.

[86] Y. Y. Tao, X. C. Yan, T. Zhou, L. Shen, Z. L. Liu, and C. H. Liu, “Fuzheng Huayu recipe alleviates hepatic fibrosis via inhibiting TNF-alpha induced hepatocyte apoptosis,” BMC Complementary and Alternative Medicine, vol. 14, p. 449, 2014.

[87] Y. H. Yeh, C. Y. Liang, M. L. Chen et al., “Apoptotic effects of hsian-tsao (Mesona procumbens Hemsley) on hepatic stellate cells mediated by reactive oxygen species and ERK, JNK, and caspase-3 pathways,” Food Science & Nutrition, vol. 7, no. 5, pp. 1891–1898, 2019.

[88] Y. Chen, Z. Zhou, Q. Mo, G. Zhou, and Y. Wang, “Gallic acid attenuates dimethylnitrosamine-induced liver fibrosis by alteration of Smad phosphoisoform signaling in rats,” BioMed Research International, vol. 2018, Article ID 1682743, 14 pages, 2018.

[89] H. Shi, A. Shi, L. Dong et al., “Chlorogenic acid protects against liver fibrosis in vivo and in vitro through inhibition of oxidative stress,” Clinical Nutrition, vol. 35, pp. 1366–1373, 2016.

[90] N. Yang, S. Dang, J. Shi et al., “Caffeic acid phenethyl ester attenuates liver fibrosis via inhibition of TGF-beta1/Smad3 pathway and induction of autophagy pathway,” Biochemical and Biophysical Research Communications, vol. 486, pp. 22–28, 2017.

[91] M. Mu, S. Zuo, R. M. Wu et al., “Ferulic acid attenuates liver fibrosis and hepatic stellate cell activation via inhibition of TGF-beta1/Smad3 signaling pathway,” Drug Design, Development and Therapy, vol. 12, pp. 4107–4115, 2018.

[92] Z. Wang, S. Ge, S. Li, H. Lin, and S. Lin, “Anti-obesity effect of trans-cinnamic acid on HepG2 cells and HFD-fed mice,” Food and Chemical Toxicology, vol. 137, Article ID 111148, 2020.

[93] Q. Yao, S. Li, X. Li, F. Wang, and C. Tu, “Myricetin modulates macrophage polarization and mitigates liver inflammation and fibrosis in a murine model of nonalcoholic steatohepatitis,” Frontiers in Medicine (Lausanne), vol. 7, p. 71, 2020.

[94] L. D. Hernández-Ortega, B. E. Alcántar-Díaz, L. A. Ruiz-Corzo et al., “Quercetin improves hepatic fibrosis reducing hepatic stellate cells and regulating pro-fibrogenic/anti-fibrogenic molecules balance,” Journal of Gastroenterology and Hepatology, vol. 27, no. 12, pp. 1865–1872, 2012.

[95] J. Li, X. Li, W. Xu et al., “Antifibrotic effects of luteolin on hepatic stellate cells and liver fibrosis by targeting AKT/mTOR/p70S6K and TGFbeta/Smad signalling pathways,” Liver International, vol. 35, pp. 1222–1233, 2015.

[96] D. F. Hicks, N. Goossens, A. Blas-Garcia et al., “Transcriptome-based repurposing of apigenin as a potential anti-fibrotic agent targeting hepatic stellate cells,” Science Reports, vol. 7, p. 42563, 2017.

[97] F. Geyikoglu, E. G. Yilmaz, H. S. Ero et al., “Hepatoprotective and antimicrobial effects of the Myrtaceae family,” Food and Bioprocess Technology, vol. 7, pp. 980–981, 2018.