The Traditional Chinese Medicine Formula FTZ Protects against Cardiac Fibrosis by Suppressing the TGFβ1-Smad2/3 Pathway

Yue Zhang,1 Dongwei Wang,1 Kaili Wu,1 Xiaoqi Shao,1 Hongtao Diao,1 Zhiying Wang,1 Mengxian Sun,1 Xueying Huang,1 Yun Li,1 Xinyuan Tang,1 Meiling Yan,1 and Jiao Guo2,3,4,5

1Center for Drug Research and Development, Guangdong Pharmaceutical University, Guangzhou 510006, China
2Guangdong Metabolic Diseases Research Center of Integrated Chinese and Western Medicine, Guangzhou 510006, China
3Key Laboratory of Glucolipid Metabolic Disorder, Ministry of Education of China, Beijing, China
4Institute of Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou 510006, China
5Guangdong TCM Key Laboratory for Metabolic Diseases, Guangzhou 510006, China

Correspondence should be addressed to Jiao Guo; gyguoyz@163.com

Received 10 December 2021; Revised 9 March 2022; Accepted 30 March 2022; Published 19 April 2022

Academic Editor: Longfei Yang

Copyright © 2022 Yue Zhang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Fu fang Zhen Zhu Tiao Zhi (FTZ) is a patented preparation of Chinese herbal medicine that has been used as a natural medicine to treat several chronic diseases including cardiovascular disease. However, its effects on cardiac fibrosis remain unclear. Therefore, this study was designed to investigate the effects and potential mechanisms of FTZ in treating cardiac fibrosis.

Methods. FTZ was administered to mice by oral gavage daily at a dosage of 1.2 g/kg or 2.4 g/kg of body weight for 7 weeks after a transverse aorta constriction (TAC) surgery. Doppler echocardiography, hematoxylin and eosin staining, and Masson’s trichrome staining were used to assess the effect of FTZ on the cardiac structure and function of mice that had undergone TAC. EdU and wound-healing assays were performed to measure the proliferative and migratory abilities of cardiac fibroblasts. Western blotting and qRT-PCR were used to determine the expression of TGFβ1, Col1A2, Col3, and α-SMA proteins and mRNA levels.

Results. FTZ treatment reduced collagen synthesis, attenuated cardiac fibrosis, and improved cardiac function in mice subjected to TAC. Moreover, FTZ treatment prevented the proliferation and migration of cardiac fibroblasts and reduced Ang-II-induced collagen synthesis. Furthermore, FTZ downregulated the expression of TGFβ1, p-smad2, and p-smad3 and inhibited the TGFβ1-Smad2/3 pathway in the setting of cardiac fibrosis.

Conclusion. FTZ alleviated the proliferation and migration of cardiac fibroblasts and suppressed collagen synthesis via the TGFβ1-Smad2/3 pathway during the progression of cardiac fibrosis. These findings indicated the therapeutic potential of FTZ in treating cardiac fibrosis.

1. Introduction

Cardiovascular disease continues to be the leading cause of death worldwide [1]. Heart failure (HF) is the common clinical manifestation of the advanced stages of many cardiac diseases. Several factors including cardiac fibrosis contribute to HF. As an intricate progression, cardiac fibrosis is characterized by adverse cardiac structural remodeling, which may eventually lead to HF [2, 3].

Fu fang Zhen Zhu Tiao Zhi (FTZ) is an effective traditional Chinese herbal preparation predominantly composed of eight Chinese herbs with definite curative effects and without obvious toxic or side effects. It has been used for over 10 years in a clinical setting to treat nonalcoholic fatty liver disease [4], atherosclerosis, diabetes [5, 6], aging [7, 8], and disorders of glucose and lipid metabolism. Results from our preliminary in vivo studies demonstrated that FTZ could ameliorate several pathological processes such as inflammation, abnormal blood coagulation, endothelial dysfunction, and the formation of atherosclerotic plaques. In addition, FTZ can regulate glucose and lipid metabolism and reduce oxidative stress in rodent models [9–11]. More importantly, a recent study reported that FTZ could ameliorate diabetic cardiomyopathy by inhibiting inflammation.
and cardiac fibrosis [12]; however, its mechanism in inhibiting cardiac fibrosis was unclear. Given the universality of FTZ, some of the individual herbs in FTZ including glossy privet fruit [13], Atractylodes [14], Coptis [15, 16], and pseudoginseng [17, 18] have been traditionally used to treat fibrosis. Thus, in this study, we examined the effects of FTZ on the heart and explored the therapeutic effect of FTZ in cardiac fibrosis.

Several studies suggest that chronic hypertension might lead to cardiac pressure overload, which contributes to the progression of cardiac fibrosis. Besides, it has been reported that hormones and growth factors, such as angiotensin II (Ang-II) and transforming growth factor-β1, could promote the activation of cardiac fibroblasts (CFs), causing an increase in α-SMA positive cells. Excess activation of CFs might eventually cause cardiac fibrosis and dysfunction due to the secretion of abundant extracellular matrix (ECM) [19, 20]. During this process, Ang-II elevates TGFβ1 expression, which subsequently mediates the phosphorylation of Smad2 and Smad3. Activated TGFβ1-Smad2/3 signaling upregulates the levels of various targets including Col1A2 and Col3. These findings suggest that suppressing the TGFβ1-Smad2/3 pathway might help inhibit the activated CFs and alleviate cardiac fibrosis [21, 22].

In this study, we demonstrated that FTZ could not only improve cardiac dysfunction but also ameliorate cardiac fibrosis. Additionally, we performed a series of experiments using cardiac-fibrotic models and found that FTZ was effective in treating cardiac fibrosis, and its molecular mechanism involved regulation of the TGFβ1-Smad2/3 pathway.

2. Methods and Materials

2.1. Preparation of FTZ. Eight kinds of Chinese medicinal herbs in FTZ (Citri sarcodactylis fructus, Ligustri lucidi fructus, Salviae miltiorrhizae radix et rhizoma, Notoginseng radix et rhizoma, Coptidis rhizoma, Atractylodis macrocephalae rhizoma, Circii japonici herba et radix, and Eucommiae cortex) were purchased from Zhixin Chinese Herbal Medicine Co. Ltd. (Guangzhou, China) and identified by Professor Wei He and Senior Lecturer Li Yong, Guangdong Pharmaceutical University. The preparation of FTZ was consistent with the protocol described previously [23]. And the quality control of FTZ was performed by UPLC-MS/MS as previously reported [24]. The FTZ used in this study was from the First Affiliated Hospital of Guangdong Pharmaceutical University.

2.2. Animals and Treatment. Male C57BL/6 mice (6–8 weeks old) weighing 20–22 g were purchased from Changzhou Cavens Laboratory Animal Co. Ltd., China. Mice were housed in cages, provided a chow diet, and subjected to a 12 h light/12 h dark cycle in a room maintained at standard conditions (temperature 25 ± 1°C; humidity 55 ± 5%). All experiments were approved by the Animal Research Ethics Committee of Guangdong Pharmaceutical University. Protocols used for transverse aorta constriction (TAC) and sham operation were from previously reported studies [25]. Briefly, a TAC operation was performed for the partial ligation of the transverse aorta using a 6-0 suture that was banded over a 27-gauge needle. Mice were randomly assigned to 5 groups. A sham operation was performed on one of the groups of mice and TAC on the others. On day 7 after the operation, among the TAC mice, a quarter of the mice were administered captopril (intragastrically, 10 mg/kg/day), and the others were treated with either a low or a high dose (intragastrically, 1.2 g/kg/day or 2.4 g/kg/day) of FTZ or the vehicle for 7 weeks. FTZ dose was selected based on that used for humans clinically. Captopril dosage was determined based on that used in previous studies [2].

2.3. Echocardiography. After 7 weeks of drug administration, echocardiography was conducted using a Vevo 2100 system (VisualSonics, Canada) with a high-frequency (30 MHz) MS-400 transducer. Mice were anesthetized under isoflurane inhalation (1%). Cardiac indices were measured and calculated using computer algorithms. All measured cardiac indices are presented as the mean of 3 consecutive cardiac cycles.

2.4. Histopathology and Immunohistochemistry. Cardiac tissues were fixed in phosphate-buffered saline with 4% paraformaldehyde for 24 h at room temperature. Next, tissues were embedded in paraffin, and 4 μm-thick sections were prepared for histopathology experiments. Hematoxylin and eosin (H&E), Masson’s trichrome, and Sirius red staining were used following the manufacturers’ instructions to observe the changes in cardiac morphology and determine the extent of fibrosis. In addition, cardiac tissues were stained with α-smooth muscle actin (α-SMA; Proteintech, 1:2,000) or TGFβ1 (Proteintech, 1:500) antibodies at the same region of every heart slice. The α-SMA and TGFβ1 positive area and the fibrotic area were quantified by calculating the percentage of collagen staining using ImageJ analysis.

2.5. Cell Culture. CFs were isolated from 1–3 day old neonatal mice, cultured in Dulbecco’s modified Eagle medium (supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin) at 37°C in an atmosphere of 5% CO₂ and 95% air. CFs were stimulated with FTZ (50 μg/mL or 100 μg/mL) and Ang-II (100 nM). The concentrations of FTZ and Ang-II were determined by referring to previous studies [11, 26, 27].

2.6. Wound-Healing Assay. Cells were cultured overnight in six-well plates until they reached 95% confluence. Wound-healing assay was conducted by creating a scratch wound with a 0.1–0.20 μL pipette tip. Next, CFs were stimulated with Ang-II (100 nM) or/and FTZ (50 μg/mL or 100 μg/mL). Changes in scratches were observed at 24 and 48 h, and photographs were captured.
2.7. Cell-Proliferation Assay. After 24 h of treatment with FTZ and Ang-II, 10 μL of cell counting kit-8 (CCK-8) reagent was added to each well and incubated in the culture medium at 37°C for 3 h. The optical density of each well was obtained at 450 nm.

2.8. EdU-Proliferation Assay. After drug treatment, CFs were incubated with 10 μM EdU at 37°C for 3 h. Next, cells were fixed in 4% paraformaldehyde and treated with 0.3% Triton X-100 for 15 min, respectively, and stained according to the manufacturers’ instructions. Cell proliferation was observed, and images were photographed using a fluorescence microscope (Olympus Optics, Tokyo, Japan). The cell proliferation was calculated using ImageJ software.

2.9. Western Blotting. Total proteins were isolated from CFs using RIPA lysis buffer supplemented with a protease/phosphatase inhibitor. Proteins were fractionated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose (NC) membranes. After blocking with 5% nonfat milk or BSA, the NC membranes were incubated with the corresponding antibodies of the target proteins. The antibodies included p-smad2 (AbSci, 1:500), smad2 (Proteintech, 1:1,000), p-smad3 (AbSci, 1:500), smad3 (Proteintech, 1:1,000), TGFβ1 (Proteintech, 1:1,000), Col1A2 (Proteintech, 1:2,000), Col3 (Proteintech, 1:500), α-SMA (Proteintech, 1:20,000), and β-actin (Proteintech, 1:2,000). After overnight incubation at 4°C, the NC membranes were incubated with the secondary antibody (1:8,000) for 50 min at room temperature. The protein bands were scanned and analyzed using the Odyssey Imaging System.

2.10. Reverse Transcription and Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR). Total RNA was extracted from CFs or cardiac tissues using TRIzol following the manufacturer’s protocol and then reverse-transcribed to obtain cDNA. qRT-PCR was performed to detect mRNA levels of target genes using SYBR Green Real-Time PCR Master Mix. The 2−ΔΔCT method was used to present the changing levels of target genes, which were normalized to β-actin mRNA.

3.3. FTZ Inhibits CF Proliferation and Migration In Vitro. Some studies have shown that inhibiting proliferation, migration, and collagen deposition in CFs can prevent or even reverse cardiac fibrosis [20, 28]. To determine whether FTZ had a direct effect on CFs with Ang-II, we conducted a wound-healing assay and found that FTZ could inhibit Ang-II-induced CF migration (Figure 3(a)). EdU and CCK-8 assays showed that FTZ could significantly reduce the proliferative ability of CFs. Compared with the CFs treated with Ang-II, those treated with FTZ showed a marked decrease in proliferation with any effect on cell viability (Figures 3(b)–3(d) and S3). In addition, the weakened apoptosis program and abnormal apoptosis mechanism of cardiac fibroblasts are the main reasons for the further development of cardiac fibrosis [29]. As shown in Figure S4, FTZ could restore the normal apoptosis program. These results suggested that FTZ could inhibit abnormal proliferation and migration in CFs.
3.4. FTZ Reduces Collagen Synthesis In Vitro. Under pathological conditions, the abnormal migration and proliferation of CFs could promote excessive collagen secretion. Furthermore, abnormal accumulation of collagen can reduce myocardial compliance and increase myocardial hardness, which eventually lead to cardiac dysfunction during systole and diastole [19, 30]. This outcome is characterized by elevated levels of Col1A2, Col3, α-SMA, and CTGF. Therefore, changes in the expression of these fibrosis-related genes were determined. We found that FTZ could significantly inhibit the mRNA levels of Col1A2, Col3, α-SMA, and CTGF after induction with Ang-II (Figures 4(a)–4(d)). Consistently, the protein expression of Col1A2, Col3, and α-SMA was significantly decreased in CFs treated with FTZ compared with those treated with Ang-II (Figures 4(e)–4(h)). Furthermore, we evaluated the protein expression level of matrix metalloproteinases (MMPs), and we found that FTZ could reduce the expression of MMP1 and MMP2 (Figure S5). Overall, our findings suggested that FTZ could reduce collagen synthesis in CFs.

3.5. FTZ Attenuates Cardiac Fibrosis by Downregulating the TGFβ1-Smad2/3 Pathway. TGFβ1-Smad2/3 signaling participates in the progression of cardiac fibrosis and is considered a classical pathway in regulating cardiac fibrosis [21]. In this study, we determined the markers of the TGFβ1-Smad2/3 signaling pathway to investigate the potential mechanisms of FTZ in preventing cardiac dysfunction and cardiac fibrosis. We found that the expression of TGFβ1, p-smad2, and p-smad3 increased significantly after Ang-II induction; however, their expression decreased considerably after FTZ treatment (Figures 5(a)–5(e)). Moreover, immunohistochemical staining showed that the TGFβ1 was obviously upregulated and distributed in the myocardial tissue in mice subjected to TAC (Figure 5(f)). To better clarify the mechanism, we used the TGFβ1 agonist; we found that TGFβ1 agonist reversed FTZ inhibition of cardiac fibroblast activation (Figure S6). These results indicated that FTZ was effective in inhibiting the TGFβ1-Smad2/3 pathway in mice with cardiac fibrosis.

**Figure 1:** FTZ improves cardiac dysfunction and alleviates cardiac hypertrophy in pressure overload-mediated cardiac dysfunction in mice (a) Representative M-mode echocardiography of the left ventricular chamber. FTZ-L and FTZ-H represent doses of 1.2 and 2.4 g/kg FTZ, respectively. CAPT represents the 10 mg/kg captopril group. (b–f) Echocardiographic assessment of LVIDd, LVIDs, EF%, FS%, and E/A; n = 8 per group. (g) HW/BW ratios in each group; n = 8 per group. Data are presented as the mean ± SEM. **P < 0.01 versus the sham group and # # P < 0.05 and **P < 0.01 versus the TAC group.
Cardiac fibrosis is a common pathological phenomenon in cardiovascular disease that is characterized by excessive ECM deposition [31]. Cardiac fibrosis destroys the normal structure of the heart muscle, leading to myocardial dysfunction, electrical activity and mechanical impairment, and acceleration of HF progression [32]. Although current treatment strategies can be used to improve the clinical symptoms of patients with HF, it is difficult to reverse the pathological process of cardiac fibrosis, and its severity is closely related to the long-term mortality of patients.

**Figure 2:** FTZ reduces cardiac fibrosis in pressure-overload mice. (a–d) H&E staining results of cardiac tissue are shown. Scale bars: 50 μm. n = 3 per group. Masson’s trichrome staining results of cardiac tissue are shown. Scale bars: 50 μm. n = 3 per group. Fibrosis of cardiac tissues stained with Sirius Red. Scale bars: 50 μm. n = 3 per group. Immunohistochemical detection of α-SMA. Scale bars, 50 μm. n = 3 per group. (e–g) RT-qPCR to determine Col1A2, Col3, and CTGF expression. n = 6 per group. Data are presented as the mean ± SEM. **P < 0.01 versus the sham group and ##P < 0.01 versus the TAC group.

### 4. Discussion

Cardiac fibrosis is a common pathological phenomenon in cardiovascular disease that is characterized by excessive ECM deposition [31]. Cardiac fibrosis destroys the normal structure of the heart muscle, leading to myocardial dysfunction, electrical activity and mechanical impairment, and acceleration of HF progression [32]. Although current treatment strategies can be used to improve the clinical symptoms of patients with HF, it is difficult to reverse the pathological process of cardiac fibrosis, and its severity is closely related to the long-term mortality of patients.
In recent years, traditional Chinese medicine has been widely used in many countries to treat various diseases including cardiovascular diseases [33, 34]. FTZ is a...
representative prescription of the “Tiaogan Qishu Huazhuo” theory, which is summarized based on the clinical practice of more than 10 years [35]. The liver plays a vital role as a regulator by coordinating with multiple organs. The function of the heart is to regulate blood flow and circulation. One of the main functions of the liver is blood storage (Xin

**Figure 4:** FTZ reduces collagen synthesis in vitro. (a)–(d) RT-qPCR to determine the expression of Col1A2, Col3, CTGF, and α-SMA. n = 5 per group. (e)–(h) Col1A2, Col3, and α-SMA expression were determined using western blotting. n = 5 per group. Data are presented as the mean ± SEM. *P < 0.05 and **P < 0.01 versus the control group and #P < 0.05 and ##P < 0.01 versus the Ang-II group.
In addition, the heart regulates spiritual activities and, via regulating the liver, opens the central system, dredges the "qi" of the whole body, and makes it flow smoothly (Xin Cang Shen Er Gan Zhu Shu Xie). Therefore, the relationship between the heart and the liver is mainly manifested in two aspects: blood circulation and storage and mental regulation. Abnormal liver function can affect the storage of blood, which in turn can affect cardiac function and lead to arrhythmias. The prophylactic use of FTZ could alleviate stress and pressure overload on the heart by regulating the liver as well as by improving the poor flow of "qi" and blood resulting from the blockage of blood flow, thereby improving cardiac function. Studies have reported that FTZ mainly affects lipid metabolism, glucose metabolism, and other metabolic pathways, and has significant efficacy in diabetes, atherosclerosis, nonalcoholic fatty liver disease, and the improvement of insulin resistance and other disorders of glucose and lipid metabolism [6, 23]. Among the components of FTZ, glossy privet fruit, Atractylodes, Coptis, and pseudoginseng have been reported to have therapeutic effects on fibrosis. Therefore, we speculated that FTZ might play an important role in the treatment of cardiac fibrosis. Our results showed that FTZ could significantly improve cardiac function and inhibit collagen deposition in mice subjected to TAC and also inhibit Ang-II-induced proliferation and migration of fibroblasts in vitro.

Cardiac fibrosis results from the continuous and repeated aggravation of myocardial ischemia and hypoxia caused by severe atherosclerotic stenosis of the coronary artery. Currently, there is no effective approach to curing cardiac fibrosis. Captopril is an angiotensin-converting enzyme inhibitor commonly used to treat hypertension, HF, and cardiac fibrosis [36, 37]; however, we found that FTZ might have more benefits than captopril. Patients with cardiac fibrosis often suffer from atherosclerosis, diabetes, and abnormal lipid metabolism, and FTZ has a therapeutic effect on these diseases. Therefore, FTZ shows potential for the treatment of cardiac fibrosis.

Under normal conditions, the synthesis and degradation of extracellular matrix in the myocardium are in a dynamic balance. Among them, matrix metalloproteinases are the important material basis to maintain this equilibrium state,
which are responsible for the ECM degradation [38]. In a pathological state, the dynamic balance of ECM is broken that results in ECM depositions. After ANG-II treatment, the collagens contents in cardiac fibroblasts were increased. In order to maintain a balance between the production and degradation of the extracellular matrix, the levels MMPs were also upregulated [39]. Our results showed that after ANG-II treatment, the MMPs expression in cardiac fibroblasts were increased to keep the balance of the extracellular matrix, whereas the symptoms of cardiac fibrosis were relieved by FTZ, and also, the protein expression levels of collagens and MMPs were significantly decreased.

TGFβ1 plays an important role in the progression of cardiac fibrosis; it can stimulate the proliferation of fibroblasts and induce the expression of growth factors involved in regulating cell proliferation, adhesion, and migration [40]. With higher levels of inflammatory cytokines, the degree of fibrosis is gradually aggravated. Moreover, the damage repair space is smaller, and thus, the process of cardiac fibrosis is irreversible. The Smad family plays a key role in the transduction of TGFβ1 signals from the cell surface receptors to the nucleus. TGFβ1 in CFs binds to receptors and activates smad2 and smad3, leading to ECM deposition and cardiac fibrosis [21, 41]. We found that the expression of TGFβ1, p-smad2, and p-smad3 was significantly increased in the Ang-II group than in the control group; however, the expression in the FTZ-treated group was significantly decreased compared with that in the model group, suggesting that FTZ may play a role in preventing fibrosis by affecting the TGFβ1-Smad2/3 signal transduction pathway (Figure 6).

Our study has some limitations, and further evaluations are required. For instance, we only investigated the regulatory effect of FTZ on the TGFβ1-Smad pathway. The detailed molecular mechanism regulated by FTZ was not completely elucidated in this study. Therefore, future investigations are needed to determine how FTZ induces the TGFβ1-Smad pathway.

5. Conclusions

Our results show that FTZ could significantly improve TAC-induced systolic and diastolic dysfunction and reduce cardiac fibrosis in mice, indicating that it could effectively limit myocardial remodeling. Our findings established a novel connection between FTZ and cardiac fibrosis, which enhanced our understanding of the potential of the derivatives of traditional Chinese medicine monomers as effective therapeutics in managing cardiac fibrosis.

Abbreviations

FTZ: Fu Fang Zhen Zhu Tiao Zhi
HF: Heart failure
Ang-II: Angiotensin II
TAC: Transverse aorta constriction
TGFβ1: Transforming growth factor-β1
CFs: Cardiac fibroblasts
EF: Ejection fraction
FS: Fractional shortening
E/A: Transmitral early (E) to atrial (A)
LVIDd: Left ventricular internal diameter at end-diastole
LVIDs: Left ventricular internal diameter at end-systole
HW/BW: Heart weight/body weight
α-SMA: α-smooth muscle actin
qRT-PCR: quantitative real-time PCR
ECM: Extracellular matrix
OD: Optical density
H&E: Hematoxylin and eosin
CCK: Cell counting kit
NC: Nitrocellulose.

Data Availability

The data used and analyzed during our study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors’ Contributions

J G and Y Z designed the study. DW W carried out experiments. XQ S and HT D participated in the study design and interpretation of results and drafted the manuscript. ZY W, KL W, MX S, XY H, Y L, XY T, and ML Y participated in the research projects. All authors have read and approved the final version of this manuscript. Yue Zhang and Dongwei Wang contributed equally to this work.

Acknowledgments

This study was supported by the Key Project of National Natural Science Foundation of China (81830113), the National Key R&D Plan, “Research on Modernization of Traditional Chinese Medicine” (2018YFC1704200), and Major Basic and Applied Basic Research Projects of the Guangdong Province of China (2019B030302005).

Supplementary Materials

Supplementary Figure 1: FTZ alleviates cardiac hypertrophy in pressure overload mice. Supplementary Figure 2: FTZ reduces cardiac fibrosis in pressure overload mice. Supplementary Figure 3: CCK-8 kits for cell viability detection. Supplementary Figure 4: effects of FTZ on cell apoptosis. Supplementary Figure 5: FTZ reduces matrix metalloproteinases in vitro. Supplementary Figure 6: TGFβ1 agonist reverses FTZ inhibition of cardiac fibroblast activation. Supplementary materials associated with this article can be found in the online version. (Supplementary Materials)

References

[1] D. Kesidou, P. A. da Costa Martins, L. J. de Windt, M. Brittan, A. Beqqali, and A. H. Baker, “Extracellular vesicle miRNAs in the promotion of cardiac neovascularisation,” Frontiers in Physiology, vol. 11, Article ID 579882, 2020.
[2] D. Xiao, Y. Zhang, R. Wang et al., “Emodin alleviates cardiac fibrosis by suppressing activation of cardiac fibroblasts via upregulating metastasis associated protein 3,” Acta Pharmaceutica Sinica B, vol. 9, no. 4, pp. 724–733, 2019.
[3] L. Jin, S. Sun, Y. Ryu et al., “Gallic acid improves cardiac dysfunction and fibrosis in pressure overload-induced heart failure,” Scientific Reports, vol. 8, no. 1, p. 9302, 2018.
[4] H. Wang, H. Tan, W. Zhan et al., “Molecular mechanism of fufang zhenzhu tiaozi capsule in the treatment of type 2 diabetes mellitus with nonalcoholic fatty liver disease based on network pharmacology and validation in minipigs,” Journal of Ethnopharmacology, vol. 274, Article ID 114056, 2021.
[5] J. Cai, J. Zhang, S. Li, Y. Lin, X. Xiao, and J. Guo, “Comprehensive chemical analysis of zhenshu tiaozi formula and its effect on ameliorating glucolipid metabolic disorders in diabetic rats,” Biomedicine & Pharmacotherapy, vol. 133, Article ID 110160, 2021.
[6] L. Song, D. Zhang, C. Guo et al., “The traditional Chinese medicine formula Fufang-zhenzhu-tiaozhi protects myocardia from injury in diabetic minipigs with coronary heart disease,” Biomedicine & Pharmacotherapy, vol. 137, Article ID 111343, 2021.
[7] D. Luo, K. Chen, J. Li et al., “Gut microbiota combined with metabolomics reveals the metabolic profile of the normal aging process and the anti-aging effect of Fufang zhenshu tiaozhi(FTZ) in mice,” Biomedicine & Pharmacotherapy, vol. 121, p. 109550, 2020.
[8] P. Shenghua, Z. Ziqin, T. Shuyu, Z. Huixia, R. Xianglu, and G. Jiao, “An integrated fecal microbiome and metabolome in the aged mice reveal anti-aging effects from the intestines and biochemical mechanism of FuFang zhenshu tiaozhi(FTZ),” Biomedicine & Pharmacotherapy, vol. 121, Article ID 109421, 2020.
[9] T. Li, R. Zhang, Y. Liu, Y. Yao, J. Guo, and Z. Zeng, “Fufang-zhenzhu-tiaozi capsule ameliorates rabbit’s iliac artery restenosis by regulating adiponectin signaling pathway,” Biomedicine & Pharmacotherapy, vol. 128, Article ID 110311, 2020.
[10] R. Zhang, T. Li, J. Guo et al., “Fufang-zhenzhu-tiaozhi capsule reduces restenosis via the downregulation of NF-kappaB and inflammatory factors in rabbits,” Lipids in Health and Disease, vol. 17, no. 1, p. 272, 2018.
[11] Y. Chen, X. He, X. Yuan et al., “NLRP3 inflammasome formation and activation in nonalcoholic steatohepatitis: therapeutic target for antimetabolic syndrome remedy FTZ,” Oxidative Medicine and Cellular Longevity, vol. 2018, Article ID 2901871, 2018.
[12] L. Wang, H. Wu, Y. Deng et al., “FTZ ameliorates diabetic cardiomyopathy by inhibiting inflammation and cardiac fibrosis in the streptozotocin-induced model,” Evidence-based Complementary and Alternative Medicine, vol. 2021, Article ID 5582567, 16 pages, 2021.
[13] S. Chu, H. Zhang, and L. Ding, “Efficiency of sophora flavescens-fructus ligustri lucidi drug pairs in the treatment of liver fibrosis based on the response surface method,” Evidence-based Complementary and Alternative Medicine, vol. 2019, Article ID 8609490, 9 pages, 2019.
Evidence-Based Complementary and Alternative Medicine

[14] X. H. Cui, H. L. Wang, R. Wu, P. A. Yao, K. Z. Wei, and J. P. Gao, “Effect of atractyloides macrocephala rhizoma on isoproterenol-induced ventricular remodeling in rats,” Molecular Medicine Reports, vol. 17, no. 2, pp. 2607–2613, 2018.

[15] J. S. Wu, R. Shi, X. Lu, Y. M. Ma, and N. N. Cheng, “Combination of active components of xixin decoction ameliorates renal fibrosis through the inhibition of NF-κB and TGF-β1/Smad pathways in db/db diabetic mice,” PLoS One, vol. 10, no. 3, Article ID e0122661, 2015.

[16] N. Wang, Y. Feng, F. Cheung et al., “A comparative study on the hepatoprotective action of bear bile and coptidis rhizoma aqueous extract on experimental liver fibrosis in rats,” BMC Complementary and Alternative Medicine, vol. 12, no. 1, p. 239, 2012.

[17] K. H. Lu, C. T. Liu, R. Raghu, and L. Y. Sheen, “Therapeutic potential of Chinese herbal medicines in alcoholic liver disease,” Journal of Traditional and Complementary Medicine, vol. 2, no. 2, pp. 115–122, 2012.

[18] X. S. Xie, C. Zuo, Z. Y. Zhang et al., “Investigate the effects of compound radix notoginseng on renal interstitial fibrosis and kidney-targeting treatment,” Sichuan Da Xue Xue Bao Yi Xue Ban, vol. 43, no. 1, pp. 28–33, 2012.

[19] M. Abonnenc, A. A. Nabeebaccus, U. Mayr et al., “Extra-cellular matrix secretion by cardiac fibroblasts: role of microRNA-29b and microRNA-30c,” Circulation Research, vol. 113, no. 10, pp. 1138–1147, 2013.

[20] X. Yuan, J. Pan, L. Wen et al., “MiR-590-3p regulates proliferation, migration and collagen synthesis of cardiac fibroblast by targeting ZEB1,” Journal of Cellular and Molecular Medicine, vol. 24, no. 1, pp. 227–237, 2020.

[21] H. Khalil, O. Kanisicak, V. Prasad et al., “Fibroblast-specific TGF-β-Smad2/3 signaling underlies cardiac fibrosis,” Journal of Clinical Investigation, vol. 127, no. 10, pp. 3770–3783, 2017.

[22] R. R. Ferreira, R. D. S. Abreu, G. Vilar-Pereira et al., “TGF-β inhibitor therapy decreases fibrosis and stimulates cardiac improvement in a pre-clinical study of chronic chagas’ heart disease,” PLoS Neglected Tropical Diseases, vol. 13, no. 7, Article ID e0070602, 2019.

[23] D. Luo, J. Li, K. Chen, X. Rong, and J. Guo, “Untargeted metabonomics reveals the protective effect of fufang zhenshu tiaozhi (FTZ) on aging-induced osteoporosis in mice,” Frontiers in Pharmacology, vol. 9, p. 1483, 2019.

[24] X. Zhong, J. Guo, L. Wang et al., “Analysis of the constituents in rat serum after oral administration of fufang zhenshu tiaozhi capsule by UPLC-Q-TOF-MS/MS,” Chromatography, vol. 75, no. 3-4, pp. 111–129, 2012.

[25] A. Bugyei-Twum, C. Ford, R. Civitarese et al., “Sirutuin 1 activation attenuates cardiac fibrosis in a rodent pressure overload model by modifying smad2/3 transactivation,” Cardiovascular Research, vol. 114, no. 12, pp. 1629–1641, 2018.

[26] Y. H. Zhang, Y. Q. Ding et al., “MicroRNA-99b-3p promotes angiotensin II-induced cardiac fibrosis in mice by targeting GSK-3β,” Acta Pharmacologica Sinica, vol. 42, no. 5, pp. 715–725, 2021.

[27] J. Lin, D. Wei, D. Xin, J. Pan, and M. Huang, “Ellagic acid inhibits proliferation and migration of cardiac fibroblasts by down-regulating expression of HDAC1,” Journal of Toxicological Sciences, vol. 44, no. 6, pp. 425–433, 2019.

[28] B. Tian, J. Liu, P. Bitterman, and R. J. Bache, “Angiotensin II modulates nitric oxide-induced cardiac fibroblast apoptosis by activation of AKT/PKB,” Journal of American College of Cardiology, vol. 28, no. 3, pp. H1105–H1112, 2003.

[29] A. Polyak, P. Kui, N. Morvay et al., “Long-term endurance training-induced cardiac adaptation in new rabbit and dog animal models of the human athlete’s heart,” Reviews in Cardiovascular Medicine, vol. 9, no. 4, pp. 135–142, 2018.

[30] Y. Zhou, L. Deng, D. Zhao et al., “MicroRNA-503 promotes angiotensin II-induced cardiac fibrosis by targeting apelin-13,” Journal of Cellular and Molecular Medicine, vol. 20, no. 3, pp. 495–505, 2016.

[31] H. Tao, J. J. Yang, K. H. Shi, and J. Li, “Wnt signaling pathway in cardiac fibrosis: new insights and directions,” Metabolism, vol. 65, no. 2, pp. 30–40, 2016.

[32] X. Jian, Y. Liu, Z. Zhao, L. Zhao, D. Wang, and Q. Liu, “The role of traditional Chinese medicine in the treatment of atherosclerosis through the regulation of macrophage activity,” Biomedicine & Pharmacotherapy, vol. 118, Article ID 109375, 2019.

[33] P. Hao, F. Jiang, J. Cheng, L. Ma, Y. Zhang, and Y. Zhao, “Traditional Chinese medicine for cardiovascular disease: evidence and potential mechanisms,” Journal of the American College of Cardiology, vol. 69, no. 24, pp. 2952–2966, 2017.

[34] J. Guo, “Research progress on prevention and treatment of glucolipid metabolic disease with integrated traditional Chinese and western medicine,” Chinese Journal of Integrative Medicine, vol. 23, no. 6, pp. 403–409, 2017.

[35] Y. Zhang, L. Zhang, X. Fan et al., “Captopril attenuates TGF-β-induced heart failure via inhibiting Wnt3a/β-catenin and Jak2/Stat3 pathways,” Biomedicine & Pharmacotherapy, vol. 113, Article ID 108780, 2019.

[36] M. Gallego, L. Espiña, L. Vegas, E. Echevarria, M. M. Iriarte, and O. Casis, “Spironolactone and captopril attenuates isoproterenol-induced cardiac remodelling in rats,” Pharmacological Research, vol. 44, no. 4, pp. 311–315, 2001.

[37] N. I. Medeiros, J. A. S. Gomes, and R. Correa-Oliveira, “Synergic and antagonistic relationship between MMP-2 and MMP-9 with fibrosis and inflammation in Chagas’ cardiomyopathy,” Parasite Immunology, vol. 39, no. 8, Article ID e12446, 2017.

[38] Q. Su and Y. Zhang, “Glaucocalyxina attenuates angiotensin II-induced cardiac fibrosis in cardiac fibroblasts,” Biochemical and Biophysical Research Communications, vol. 503, no. 3, pp. 1949–1954, 2018.

[39] A. Leask, “TGFβ, cardiac fibroblasts, and the fibrotic response,” Cardiovascular Research, vol. 74, no. 2, pp. 207–212, 2007.

[40] B. Arnó, F. Galli, U. Roostalu et al., “TNAP limits TGF-β-dependent cardiac and skeletal muscle fibrosis by inactivating the SMAD2/3 transcription factors,” Journal of Cell Science, vol. 132, no. 15, 2019.