Electroacupuncture improves myocardial fibrosis in heart failure rats by attenuating ECM collagen deposition through modulation of TGF-β1/Smads signaling pathway

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Author contributions
Wang WH, Zhang JJ and Zhou MQ contributed to the conception of the study. Wang WH, Zeng QL, Wu HS participated in the animal experiments. Wang WH, Zhang JJ, Wu HS contributed significantly to analysis and manuscript preparation. Wang WH and Zeng QL performed the data analyses and wrote the manuscript. Wu SB and Zhou MQ helped perform the analysis with constructive discussions.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
HF, heart failure; ECM, extracellular matrix; LAD, left anterior descending artery; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVFS, left ventricular fractional shortening; EA, electroacupuncture stimulation; IL-1β, interleukin-1β; cTn, cardiac troponin; NT-proBNP, N-terminal brain natriuretic peptide precursor; CVF, collagen volume fraction; IOD, integrated optical density; PSR, picrosirius red staining.

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Abstract
Background: To explore the effects of electroacupuncture on cardiac function and myocardial fibrosis in rat models of heart failure, and to elucidate the underlying mechanism of electroacupuncture in heart failure treatment. Methods: Healthy male Sprague-Dawley rats were allocated into three groups: Sham group, Model group, and electroacupuncture (Model + EA) group, with each group comprising 8 rats. The model underwent a procedure involving the ligation of the left anterior descending coronary artery to induce a model of heart failure. The Model + EA group was used for 7 consecutive days for electroacupuncture of bilateral Shenmen (HT7) and Tongli (HT5), once a day for 30 min each time. Left ventricular parameters in rats were assessed using a small-animal ultrasound machine to analyze changes in left ventricular end-diastolic volume, left ventricular end-systolic volume, and left ventricular ejection fraction. Serum interleukin-1β (IL-1β), cardiac troponin (cTn), and N-terminal brain natriuretic peptide precursor levels were measured using ELISA. Histopathological changes in rat myocardium were observed through HE staining, while collagen deposition in rat myocardial tissue was assessed using the Masson staining method. Picrosirius red staining, immunohistochemical staining, and RT-qPCR were utilized to distinguish between the various types of collagen deposition. The expression level of TGF-β1 and SMAD2/3/4/7 mRNA in rat myocardial tissues was determined using RT-qPCR. Additionally, western blot analysis was conducted to assess the protein expression levels of TGF-β1, SMAD3, and p-SMAD3 in rat myocardial tissues. Results: Compared with the Sham group, the left ventricular ejection fraction and left ventricular fractional shortening values of the Model group were significantly decreased (P < 0.01); the left ventricular end-diastolic volume and left ventricular end-systolic volume values were remarkably increased (P < 0.01); serum N-terminal brain natriuretic peptide precursor content was increased (P < 0.01); serum IL-1β and cTn levels were increased (P < 0.01); myocardial collagen volume fraction was increased (P < 0.01); and those of the expression of TGF-β1 and SMAD2/3/4 mRNA was increased (P < 0.01); the expression of SMAD7 mRNA was decreased (P < 0.01); the protein expression levels of TGF-β1, SMAD3, and p-SMAD3 were increased (P < 0.01); the protein expression level of SMAD7 was decreased (P < 0.01) in the Model group. Compared to the Model group, the expression levels of the proteins TGF-β1, SMAD3, and p-SMAD3 in myocardial tissue were found to be decreased (P < 0.01), and the expression level of the protein SMAD7 was found to be increased (P < 0.01) in the Model + EA group; the collagen volume fraction and deposition of type I/III collagen were decreased (P < 0.01) in the Model + EA group. Conclusion: Electroacupuncture alleviates myocardial fibrosis in rats with heart failure, and this effect is likely due to attribution to the modulation of the TGF-β1/Smads signaling pathway, which helps reduce collagen deposition in the extracellular matrix.

Keywords: heart failure; electroacupuncture; heart meridian of Hand-Shaoyin; collagen deposition; TGF-β1/Smads signaling pathway; myocardial fibrosis
The effectiveness of acupuncture in treating cardiovascular diseases has been validated in both clinical and relevant basic research. However, there is a scarcity of reports on acupuncture specifically for heart failure. Our study indicates that acupuncture directed at the HT7 and HT5 acupoints in heart failure may be linked to ameliorating myocardial fibrosis.

### Medical history of objective

HT7 and HT5 belong to the heart meridian of Hand-Shaoyin (it refers to the Heart Channel of the Hand-Shaoyin system, which is one of the twelve main meridians in the body according to TCM theory. This meridian is closely associated with the function and health of the heart), the former is the primary point, and the latter for collateral points, which are important for the treatment of cardiovascular disease selected acupoints. The Yellow Emperor's Inner Canon (Huang Di Nei Jing, the era of its compilation is generally believed to be between the Pre-Qin period and the Han Dynasty) says: "When the five viscera are diseased, they should be treated at the twelve primary points". Acupuncture at the primary points helps maintain vitality and resist pathogenic factors. In conjunction with the collateral points, it communicates with the surface and the interior and effectively treats the diseases associated with the heart meridian.

### Background

Heart failure (HF) signifies the advanced stage in the progression of cardiovascular disease. Over recent years, there has been a noticeable increase in its prevalence, which has been paralleled by higher instances of disability and mortality [1, 2]. Over recent years, its prevalence has been on the rise, accompanied by elevated rates of disability and mortality [3]. Fibrosis stands out as a primary contributor to mortality in developed nations. This fibrotic process involves the replacement of heart muscle cells with collagen-rich scars following myocardial infarction, as well as excessive fibrosis in conditions like hypertension, diabetes, rheumatic heart disease, and HF [4]. Consequently, the economic burden of fibrosis-related heart diseases is substantial for both individuals and society.

Myocardial fibrosis, a prominent pathological feature of HF, is characterized by the excessive buildup and remodeling of the extracellular matrix (ECM) to form fibrous scars. This process impairs normal myocardial systolic and diastolic functions, ultimately resulting in cardiac dysfunction [5]. Hence, specific targeted antifibrotic drugs, matrix metalloproteinase inhibitors, microRNA therapies, and transforming growth factor-β (TGF-β) inhibitors are frequently employed in clinical practice to decrease collagen deposition, facilitate fibrotic tissue repair, and ultimately achieve partial restoration of cardiac function [6–9]. These interventions aim to mitigate the advancement of HF.

Notably, the TGF-β1/Smads pathway is instrumental in the development of myocardial fibrosis. As it triggers, it stimulates differentiation to generate ECM proteins, thereby inducing fibrosis [10]. This pathway is recognized as a significant therapeutic target due to its involvement in fibrotic processes. However, challenges exist in translating preclinical findings, as direct TGF-β blockade, while effective in fibrosis reduction, may trigger uncontrolled inflammation or disrupt the signaling pathway, risking structural integrity and causing non-healing wounds or ventricular dilatation [11]. Therefore, it is crucial to overcome these problems and find a safer and more effective strategy to reduce TGF-β induced fibrosis.

Acupuncture, with its millennia-long history in traditional Chinese medicine, is widely acknowledged for its effectiveness in treating cardiovascular diseases. Clinical research has demonstrated that electroacupuncture targeting the heart meridian acupoints Shennen (HT7) and Tongli (HT5) can enhance cardiovascular function, improve electrocardiograms, and alleviate arrhythmias in patients with coronary artery disease [12]. Experimental findings suggest that electroacupuncture at HT7 and HT5 may confer cardioprotective effects by modulating sympathetic nervous system excitability, inducing autophagy, regulating intracellular homeostasis and metabolism, and inhibiting cell apoptosis [13–17]. Meanwhile, acupuncture has been shown to treat cardiovascular disease by reducing the inflammatory response [18, 19]. Given the clinical efficacy and safety of acupuncture and the fact that acupuncture improves cardiovascular disease with multi-target, multi-level, and multi-pathway characteristics, it presents a potential solution to the challenges posed by the TGF-β pathway.

However, whether electroacupuncture can improve HF cardiac function by modulating myocardial fibrosis is not clear. Hence, this study aims to explore the therapeutic mechanism of electroacupuncture in HF treatment by assessing its impact on cardiac function, myocardial injury markers, myocardial collagen composition, and the TGF-β1/Smads pathway in HF rat models.

### Materials and methods

#### Animals

50 male Sprague-Dawley rats, each weighing between 180–220 g, three-month-old were acquired from Liaoning Changsheng Bio-technology Co., Ltd. (Benxi, China, Certificate of Conformity No. SCXX (ZHE) 2019-0004). They were accommodated at the Animal Research and Experimentation Center of Anhui University of Traditional Chinese Medicine (clean grade), maintained under controlled conditions: temperature range of 22–24 °C, humidity levels of 52–56%, 12-h light-dark cycle, while having continuous access to food and water. The treatment of experimental animals in this study rigorously adheres to the Guiding Opinions on the Proper Treatment of Experimental Animals promulgated by the Ministry of Science and Technology in 2006. Furthermore, the protocols employed in this animal study have been approved by the Animal Ethics Committee of Anhui University of Traditional Chinese Medicine (Certificate No. AHUCM-Rats-2023173).

The animals were randomly allocated into three groups: Sham, Model, and electroacupuncture (Model + EA) group (Figure 1).

![Figure 1 Experimental procedure. ECG, Electrocardiogram; ECHO, Echocardiogram; EA, electroacupuncture stimulation.](https://www.tmrjournals.com/tmr)
Animal grouping and model establishment
Following one week of acclimatization, of the 50 rats, 8 out of the 50 Sprague-Dawley rats were randomly selected for sham operation, forming the Sham group. This procedure involved opening the chest, threading a surgical suture through the heart, and then closing the chest without ligating the left anterior descending artery (LAD). The great rats of mice were anesthetized with 2% isoflurane (1 L/min) via inhalation, positioned supine on the operating table, and prepared for surgery by shaving the left chest area and disinfecting the surgical site. A 2 cm incision was made adjacent to the left sternum edge to expose the 4th intercostal space. Using a curved-opening hemostatic forceps, the pericardium was punctured, and the heart was exteriorized and ligated with a 7-0 suture. The LAD was similarly ligated, causing the anterior wall of the left ventricle to turn pale below the tie line [20]. 2 rats died from pneumothorax during the modeling process, leaving 40 rats for further study. Electrocardiograms were immediately monitored post-operation, with ST-segment elevation and ejection fraction (LVEF, %) ≤ 45% on the 28th day as markers of successful modeling (Figure 2). 28 rats were tested to establish the HF model, achieving a success rate of 70%. Subsequently, These were then randomly allocated to the Model or Model + EA groups, with 14 rats in each.

Main reagents and apparatus
The following main experiment reagents were purchased for this study: HE stain set, Masson Trichrome Staining kit, and Wolf Scarlet stain (Wuhan Xavier Biotechnology Co., Ltd., Wuhan, China, lot number: G1003, G1006, G1018); Serum interleukin-1β (IL-1β), cardiac troponin (cTn), and N-terminal brain natriuretic peptide precursor (NT-proBNP) Enzyme-Linked Immunosorbent Assay kits (Jiangsu Enzyme Immunity Industrial Co., Ltd., Yancheng, China, lot numbers: MM-0047R1, MM-71961R2, MM-0329R1); collagen I antibody (Wuhan Xavier Biotechnology Co., Ltd., Wuhan, China, lot number: GB11022-3); collagen III antibody (Wuhan Sanying Biotechnology Co., Ltd., Wuhan, China, lot number: 22734-1-AP); total RNA extraction reagent (Takara, Japan, lot number: AN30733A); RIPA Histoicyte rapid lysate (Thermo Scientific, Waltham, MA, USA, lot number: 89900); TGF-β1, SMAD2/3/4/7, COL1A1, and COL3A1 primers (Shanghai Bioengineering Co., Ltd., Shanghai, China, lot number: AN70485A); TGF-β1 antibody (Abcam, Cambridge, UK, lot number: ab215715); SMAD3 antibody (Cell Signaling Technology, Boston, MA, USA, lot number: 9523); P-SMAD3 antibody (Cell Signaling Technology, Boston, MA, USA, lot number: 9520); SMAD7 antibody (Thermo Scientific, Waltham, MA, USA, lot number: PA5-46373); and GAPDH antibody (Proteintech, Chicago, IL, USA, lot number: 60004-1-Ig).

The following instruments were used for the study: small animal Anesthesia Machine (Shenzhen Reward Life Science and Technology Co., Ltd., Shenzhen, China, R500), Electronic Needle Therapy Instrument (Suzhou Medical Supplies Factory Co., Ltd., Suzhou, China, SDZ-III), PowerLab 16-Guide physiological recorder (Australia AD Instruments, Dunedin, Australia, ML870), small animal Ultrasound Imaging System (Suzhou Feiyinuo Technology Co., Ltd., Suzhou, China, VINNO6 LAB model), high-speed benchtop Refrigerated Centrifuge (Thermo Scientific, Waltham, MA, USA, ST-16R), Enzyme Labeling Instrument (Shenzhen Radiometer, Shenzhen, China, RT-600), High-Throughput Tissue Grinder (Ningbo Xinzi Bio-technology Co., Ltd., Ningbo, China, SCIENTZ-48), ultra-mini Spectrophotometer (Thermo Scientific, Waltham, MA, USA, Nanodrop One), fluorescent quantitative PCR instrument (Thermo Scientific, Waltham, MA, USA, ML870), quantitative PCR (Thermo Scientific, Waltham, MA, USA, QuantStudio 5), vertical Electrophoresis (Bio-RAD, Hercules, CA, USA, Mini Protein 3 Cell), Embedding Machine (Wuhan Junjie Electronics Co., Ltd., Wuhan, China, JJ-12J, JB-55), Pathology Sectioning Machine (Shanghai Leica Instruments Co., Ltd., Shanghai, China, RM2016), orthogonal optical microscope (Nikon, Tokyo, Japan, Nikon Eclipse E100), and polarized light microscope (Yucescope, Hefei, China, YP710).

Electroacupuncture treatment
After 28 days of modeling, rats were given 2% isoflurane (1 L/min) inhalation anesthesia, and according to the literature commonly used experimental animal acupuncture points positioning, with disposable sterile acupuncture needles (0.16 mm × 7 mm) directly into the rat bilateral HT7 and HT5 [21]. The two-needle handles on the same side were attached to the electroacupuncture device’s output, sparse-dense wave, current intensity 1 mA, frequency 2 Hz/15 Hz, continuous stimulation for 30 min/time, once/day, for 7 consecutive days to give electroacupuncture stimulation. No electroacupuncture stimulation was performed in the Sham and Model groups.

Detection indexes and methods
Cardiac ultrasound measurement and analysis. After completing 7 days of electroacupuncture stimulation treatment (EA) treatment, the rats were anesthetized with isoflurane and positioned supine on the examination plate and maintained at a heart rate of 480–550 beats/min. Using the MS-400 probe, long-axis B-mode sections of the heart were obtained, adjusting the probe angle to visualize the left ventricle and papillary muscle. M-mode images were obtained with the measurement line positioned at the left ventricle’s maximal lumen diameter, transecting the papillary muscle laterally. In each rat group, parameters including left ventricular end-diastolic volume (LVEDV, mL), end-systolic volume (LVESV, mL), ejection fraction (LVEF, %), and fractional shortening (LVFS, %) were assessed, with the averages calculated from a minimum of three successive cardiac cycles.

ELISA. After the completion of 7 days of EA treatment, the rats were administered an overdose of isoflurane for anesthesia, followed by blood collection from the abdominal aorta, culminating in euthanasia. The blood samples were left to stand in an upright position in a 4 °C.
freezer for 20 min before being centrifuged in a high-speed refrigerated centrifuge (4 °C, 3,000 rpm, 15 min). The supernatant obtained was preserved at −80 °C for subsequent analysis. The serum concentrations of IL-1β, cTn, and NT-proBNP were quantified through ELISA. Absorbance values were measured according to ELISA instructions to calculate the actual sample concentrations.

**Pathological tissue staining.** After collecting blood from the abdominal aorta, the heart tissue was quickly removed, washed in a pre-chilled 0.9% saline solution at 4 °C, and then cut into sections at the apical 5 mm. The upper segment was fixed in 4% paraformaldehyde, while the apical portion was rapidly frozen in liquid nitrogen, and then stored at −80 °C for further analysis. After 24 h of paraformaldehyde fixation, the heart tissue underwent standard dehydration, paraffin embedding, and sectioning into approximately 4 μm slices. HE, Masson, and picro Sirius red (PSR) staining techniques were employed to assess myocardial tissue morphology and fibrosis. Microscopic observations and images were captured at 400× magnification. The collagen volume fraction (CVF) was calculated using ImageJ 6.0 software based on Masson-stained sections, while differentiation between collagen type I (red) and type III (yellow-green) was performed on PSR-stained sections to determine the respective percentages in the myocardial tissue area.

**Immunohistochemical staining.** The paraffin sections were dewaxed to water, followed by antigen retrieval using citrate buffer. Subsequently, a 3% hydrogen peroxide solution was applied to block endogenous peroxidase, and 3% BSA was evenly distributed over the tissues in the histochemical circle. After overnight incubation with primary antibodies at 4 °C, secondary antibodies were applied. Following DAB color development, cell nuclei were stained, and the sections were dehydrated and sealed. The collagen expression level was quantified by calculating the integrated optical density (IOD) using ImageJ 6.0 software based on the sections.

**RT-qPCR.** Roughly 50 mg of myocardial tissue was harvested for total RNA extraction and subsequent assay of RNA purity. The extracted RNA was subjected to genomic DNA reaction and reverse transcription to generate cDNA. TGF-β1, SMAD2/3/4/7, COLIA1, and COL3A1 were utilized as internal references alongside GAPDH. The PCR reaction commenced with an initial denaturation at 95 °C for 5 min, followed by 40 cycles (95 °C for 10 s, 60 °C for 20 s, 72 °C for 20 s). Each sample was tested in triplicate, and relative gene expression levels were quantified using the 2^−ΔΔCt method. The specific primer sequences are listed in Table 1.

**Western blot.** Myocardial tissue was homogenized in RIPA tissue lysate at a 1:10 ratio, followed by lysis, and centrifugation for 10 min to collect the supernatant as the total protein solution. A quarter volume of protein loading buffer was added to this solution, which was then denatured in a boiling water bath for 15 min and subjected to electrophoresis and membrane transfer. The samples were then washed in TBST, and incubated in 5% skimmed milk at room temperature for 30 min. Primary antibodies (TGF-β1, SMAD3, SMAD7, p-SMAD3 at 1:1,000 dilution, and GAPDH at 1:30,000 dilution) were incubated at 4 °C overnight with gentle agitation. The secondary antibody, diluted in TBST at 1:5,000 ratio, was applied for 30 min at room temperature. After three TBST washes, the bands were scanned using an imaging system following the addition of a luminescent solution. GAPDH served as the internal reference. ImageJ software was used to determine the gray value, with GAPDH serving as the internal control, and the target protein’s expression was quantified relative to this control.

**Statistical analysis.** Data analysis was conducted using SPSS 17.0 software, while GraphPad Prism 9.4.0 was utilized for creating charts. Measurement data were presented as mean ± standard deviation. As this study was a randomized controlled trial, the data conformed to normal distribution and variance homogeneity, warranting the use of the t-test for two-group comparisons. For multiple-group comparisons, one-way ANOVA was employed if normal distribution and variance homogeneity were observed. Conversely, the Kruskal-Wallis test was applied when data did not follow a normal distribution or when variance was heterogeneous, with (P < 0.05) or (P < 0.01) indicating statistically significant differences.

**Results**

**Impact of electroacupuncture on cardiac performance, enzymes profiles, and biomarkers associated with HF in HF rats**

**Assessment of LVEF, LVFS, LVEDV, LVESV.** After HF, compared to the Sham group, the LVEF and LVFS values in the Model group decreased notably (P < 0.01), and the LVEDV and LVESV values increased significantly (P < 0.01); this indicates a marked decline in cardiac function post-HF. Compared to the Model group, the LVEF and LVFS values in the Model + EA group improved significantly (P < 0.01), and the LVEDV and LVESV values decreased correspondingly (P < 0.01) (Figure 3).

**Serum NT-proBNP levels.** After HF, compared to the Sham group, the serum NT-proBNP levels were significantly higher in the Model group (P < 0.01), suggesting that the degree of HF in rats was severe. Compared to the Model group, the serum NT-proBNP levels in the Model + EA group decreased (P < 0.01) (Figure 4).

**Serum IL-1β, cTn levels.** After HF, compared to the Sham group, serum IL-1β, and cTn levels were elevated in the Model group (P < 0.01), and after 7 days of electroacupuncture, both serum IL-1β and cTn levels in the Model + EA group decreased in comparison to the Model group (P < 0.01) (Figure 5).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
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<tbody>
<tr>
<td>TGF-β1</td>
<td>Forward primer: GACGGCAACACGCACATTCA</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: TTCCGTCCTTCTGTGTTCA</td>
</tr>
<tr>
<td>COLIA1</td>
<td>Forward primer: GACAGGCGAACACAGGTGACAGAG</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: GAGAGCCAGGGAGAGCAGGAG</td>
</tr>
<tr>
<td>COL3A1</td>
<td>Forward primer: GACAGATGCTGGTGCTGAGAG</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: CGGCTGAGAAGAAGTGGAGAAGGAG</td>
</tr>
<tr>
<td>SMAD2</td>
<td>Forward primer: GTGTCACATCTTGACATCCT</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: GTTCTCCACACTGGTCCCTT</td>
</tr>
<tr>
<td>SMAD3</td>
<td>Forward primer: CAGAGAGGAGAAGTGGTGCGA</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: GCAGTAGAGTCATGGGAG</td>
</tr>
<tr>
<td>SMAD4</td>
<td>Forward primer: AAGCAATGACGCCGTCTGTA</td>
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<tr>
<td></td>
<td>Reverse primer: CTGGGTTGAGCTGCTTGTG</td>
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<tr>
<td>SMAD7</td>
<td>Forward primer: GTCAGGGACCACAGCATCGC</td>
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<tr>
<td></td>
<td>Reverse primer: GGGAGTGGTGTGACCTTTTG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward primer: CAGAGAGGATGCTTGCTGCTOC</td>
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<tr>
<td></td>
<td>Reverse primer: GATGGGCTTCGCCTGTGATGA</td>
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</table>

Table 1 Primer sequences used in RT-qPCR experiment

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Figure 3 Comparative analysis of echocardiographic function among rat groups. (A) Echocardiograms of the left ventricle of rats in each group. (B) Histograms compare the LVEF, LVFS, LVEDV, and LVESV values of rats in each group. **P < 0.01 vs. Sham group, ^P < 0.01 vs. Model group, n = 8. LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVFS, left ventricular fractional shortening.

Figure 4 Comparison of serum NT-proBNP levels of rats in each group. **P < 0.01 vs. Sham group, ^P < 0.01 vs. Model group, n = 8. NT-proBNP, N-terminal brain natriuretic peptide precursor.

Figure 5 Comparison of serum IL-1β and cTn levels in rats of each group. (A) Comparison of serum IL-1β. (B) Comparison of serum cTn. **P < 0.01 vs. Sham group, ^P < 0.01, *P < 0.05 vs. Model group, n = 8. IL-1β, interleukin-1β; cTn, cardiac troponin.
Effect of electroacupuncture on myocardial fibrosis in HF rats

Comparison of myocardial tissue morphology: HE staining. In the Sham group, there were no significant abnormalities in myocardial fibers and interstitium, with uniform staining and absence of necrotic cells. In the Model group, myocardial fibers were corrugated and altered, forming a fibrous scar, and myocardial cells had vacuoles of varying sizes, and the nuclei of the cells were consolidated or dispersed. Compared to the Model group, the myocardial tissue necrosis in the Model + EA group was significantly reduced, and the myocardial fibers were relatively orderly arranged (Figure 6).

Comparison of myocardial tissue fibrosis: Masson staining. Compared to the Sham group, the myocardial CVF was increased in the Model group ($P < 0.01$). Compared to the Model group, CVF was decreased in the Model + EA group ($P < 0.01$) (Figure 7).

Myocardial tissue collagen deposition typing results: PSR, immunohistochemical staining, RT-qPCR. Compared to the Sham group, there was a marked increase in the deposition of type I and type III collagen in the Model group ($P < 0.01$). Compared to the Model group, EA significantly reduced the deposition of type I collagen and type III collagen ($P < 0.01$). These findings were further supported by immunohistochemical staining results. RT-qPCR analysis revealed that the mRNA expression levels of type I and type III collagen were elevated in the Model group in comparison to the Sham group ($P < 0.01$), whereas electroacupuncture reduced collagen production at the RNA level ($P < 0.05$) (Figure 6).

Electroacupuncture attenuates myocardial fibrosis in HF rats through the TGF-β1/Smad3 pathway

Comparative analysis of the relative expression levels of TGF-β1 and SMAD2/3/4/7 mRNA in rat myocardial tissues across different groups. Compared to the Sham group, the relative expression of TGF-β1 and SMAD2/3/4 mRNA in the myocardial tissue of rats in the Model group was higher ($P < 0.01$), and the relative expression of SMAD7 mRNA was lower ($P < 0.01$). Compared to the Model group, the Model + EA group decreased the relative expression of TGF-β1 and SMAD2/3/4 mRNA ($P < 0.01$) and increased the relative expression of SMAD7 mRNA ($P < 0.01$) (Figure 9).

Figure 6 HE staining of rat myocardial tissue in each group

Figure 7 CVF in rat myocardial tissue across different group. (A) Masson’s trichrome staining highlights collagen fibers in blue and cardiomyocytes in red. (B) Histograms quantifying the CVF within the myocardial tissue of each group. **$P < 0.01$ vs. Sham group, ***$P < 0.01$ vs. Model group, n = 3. CVF, collagen volume fraction.
Figure 8 Myocardial tissue collagen deposition typing results in rats of all groups. (A) PSR of rat myocardial tissues across group. Type I collagen is highlighted in yellow and red, while type III collagen appears green when viewed under a polarized light microscope. (B) Histogram of the volume fraction of type I and III collagen in rat myocardial tissues across group. (C) Immunohistochemical staining of rat myocardial tissues across group. (D) The histograms represent a semi-quantitative analysis of type I and III collagen in rat myocardial tissues, as measured by IOD. (E) Comparative analysis of relative mRNA expression levels of type I and III collagen in rat myocardial tissues across different groups. **P < 0.01 vs. Sham group, *P < 0.01, *P < 0.05 vs. model group, n = 3. PSR, picro sirius red staining; IOD, integrated optical density.

Figure 9 Comparative analysis of TGF-β1 and SMAD2/3/4/7 mRNA expression levels in rat myocardial tissues across various groups. **P < 0.01 vs. Sham group, *P < 0.01 vs. Model group, n = 3.
TGF-β1, SMAD3/7, p-SMAD3 protein expression levels in rat myocardial tissue of each group. Compared to the Sham group, the Model group demonstrated increased protein expression levels of TGF-β1, SMAD3, and p-SMAD3 in rat myocardial tissues (P < 0.01), while the expression level of SMAD7 was reduced (P < 0.01). In contrast, the Model + EA group showed a significant decrease in the protein expression of TGF-β1, SMAD3, and p-SMAD3 (P < 0.01), along with an upregulation of SMAD7 expression (P < 0.01) (Figure 10).

Discussion

In this study, LAD ligation was used to establish the model. Following 4 weeks of modeling, the Model group exhibited a decrease in LVEF and LVFS, along with an increase in LVEDV and LVESV, indicating a decline in left heart function and the onset of left ventricular ejection dysfunction. Histological examination using HE staining revealed disorganized and rippled myocardial fibers, disrupted interstitial arrangement, and vacuolization in cardiac myocytes. Conversely, in the Model + EA group, left heart function was notably improved, cardiac insufficiency was reduced, and myocardial fibers exhibited a more organized pattern, suggesting that electroacupuncture effectively ameliorated cardiac function post-HF. Serum NT-proBNP, a crucial HF biomarker, significantly increased in the Model group, indicating elevated anterior and posterior loads on ventricular cells and the progression of HF. Elevated levels of serum IL-1β and TNFα indicated severe myocardial injury and necrosis, all of which were mitigated following electroacupuncture treatment, suggesting an improvement in HF.

Myocardial fibrosis is marked by an overabundance of ECM proteins in myocardial tissue, leading to scar formation that impairs cardiac function and contributes to HF development [22]. The ECM comprises predominantly type I collagen fibers (70%) and type III collagen fibers (12%). Type I collagen provides structural support to cells, while type III collagen determines myocardial tissue elasticity, offers a framework for myocardial cells, imparts myocardial wall stiffness, and aids in signal transmission [23, 24]. Following myocardial injury, the progressive deposition of type I and III collagen in the infarcted area exacerbates, disrupting the balance between ECM accumulation and breakdown, resulting in cardiac dysfunction and hastening HF progression [25]. Therefore, the regulation of ECM collagen is crucial in mitigating HF. Our observations revealed a substantial increase in collagen deposition, particularly type I and III collagen, in the infarcted region of the model rats post-HF onset, indicating a disrupted structural equilibrium in myocardial tissue, heightened fibrous collagen degradation, and severe cardiac fibrosis. After 7 days of electroacupuncture, compared to the Model group, the deposition of type I and III collagen in the Model + EA group significantly decreased. Suggesting that electroacupuncture can attenuate the expression and accumulation of type I and III collagen in the myocardium of HF rats, thereby ameliorating myocardial fibrosis. These findings align closely with those reported by Wu et al. [26, 27].

Members of the TGF-β superfamily primarily regulate cell proliferation, growth, and differentiation. TGF-β1 is closely associated with tissue fibrosis, serving as a pivotal factor in the initiation of fibrosis through its promotion of collagen accumulation within the ECM. [28]. Activation of the TGF-β1/Smad pathway leads to TGF-β1 binding to its receptor on the plasma membrane, triggering the phosphorylation of transcription factors SMAD2 and SMAD3, thereby mediating signaling. Phosphorylated SMAD2 and SMAD3 form complexes with SMAD4 in the cytoplasm, translocating to the nucleus [29, 30]. This process induces the expression of type I and III collagen to promote fibrosis, while low levels of SMAD7 can inhibit the TGF-β1 pathway by blocking SMAD3 phosphorylation [31–34]. Previous research has shown that electroacupuncture can suppress this pathway [35]. To investigate this, we assessed the expression of this pathway using RT-qPCR and western blot techniques. Compared to the Sham group, revealed a significant upregulation of TGF-β1 and SMAD2/3/4 mRNA in the Model group. Additionally, protein levels of TGF-β1 and p-SMAD3 were notably elevated. Electroacupuncture treatment upregulates SMAD7 expression, suggesting its potential to inhibit pro-fibrotic factors in this pathway, enhance anti-fibrotic factor expression, reduce collagen deposition, alleviate myocardial fibrosis, and enhance cardiac function.

Electroacupuncture, a traditional Chinese medicine therapy, exerts

Figure 10 Comparative analysis of TGF-β1, SMAD3, SMAD7, and p-SMAD3 expression in rat myocardial tissues across various groups. (A) The western blot chart. (B) Comparison of TGF-β1, SMAD3/7, p-SMAD3 protein expression in each group. **P < 0.01 vs. Sham group, ***P < 0.01, *P < 0.05 vs. Model group, n = 3.
its effects on various physiological systems, including the nervous, endocrine, and immune systems. The results of the experiments indicate that electroacupuncture significantly reduces myocardial fibrosis and effectively treats HF by modulating the TGF-β/SMADs pathway. Furthermore, this ELISA indicated that electroacupuncture exhibited a certain anti-inflammatory effect, which was similar to the results of some scholars [19, 36]. Therefore, electroacupuncture may be an important therapeutic method to exert the advantages of TGF-β/SMADs pathway in anti-fibrosis. Thus, the beneficial anti-inflammatory properties of electroacupuncture could mitigate the negative consequences of inhibiting the TGF-β/SMADs pathway, potentially playing a pivotal role in harnessing the pathway’s advantages to counteract fibrosis. Importantly, electroacupuncture is non-invasive, drug-free, and surgery-free, with no associated risks or side effects. Consequently, electroacupuncture offers significant advantages in the treatment of HF.

The TGF-β1/SMADs pathway involves SMAD2/4, alongside the key pro/anti-fibrotic factors SMAD3/7, in the context of myocardial fibrosis. Despite reports of higher SMAD3 expression in the heart compared to SMAD2, some scholars question the conclusiveness of this finding [37, 38]. Our findings demonstrated that the SMAD2 mRNA expression levels in the Model group remained elevated in comparison to the Sham group, paralleling the results reported by Hadi Khalil [39]. Furthermore, a notable reduction in SMAD2 mRNA expression was observed following electroacupuncture. This observation raises the possibility that electroacupuncture may mitigate fibrosis and preserve cardiac function through modulation of the TGF-β1/SMADs pathway, warranting further investigation in this direction.

Conclusion

In conclusion, electroacupuncture demonstrates the potential to ameliorate cardiac function decrease serum cardiac enzymes and NT-proBNP levels, and mitigate HF in rats with HF. These effects may be attributed to the downregulation of TGF-β1 and SMAD2/3/4 expression, upregulation of SMAD7 expression, reduction in collagen deposition, and enhancement of the collagen type I to collagen type III ratio, thereby achieving an anti-myocardial fibrosis outcome. Our research offers novel insights into the mechanism by which traditional acupuncture alleviates HF and provides an experimental foundation for its clinical application. Future research may employ TGF-β1/SMADs-specific inhibitors to delve deeper into the role of the TGF-β1/SMADs signaling pathway in the therapeutic effects of electroacupuncture on HF.

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