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# Mechanistic Study of Erchen Decoction Combined with Taohong Siwu Decoction in Ameliorating Atherosclerosis in ApoE$^{-}$/$^{-}$ Mice by Inhibiting Ferroptosis via the p53/SLC7A11 Pathway

Zhonghao Yuan[1,a], Shiyu Lin[1,a], Binghao Pan[1], Mengqian Liu[1], Ting Guo[1], Hanwen Zheng[1], Yan Chen[1,*]

[1]College of Traditional Chinese Medicine, Changsha Medical University, Changsha, Hunan 410219, China

[a]These authors contributed equally to this work.

[*]Corresponding author

## Abstract

Objective：This study aimed to evaluate whether Erchen Decoction combined with Taohong Siwu Decoction exerts anti-atherosclerotic effects by modulating the p53/SLC7A11 pathway and ferroptosis. Methods：An atherosclerosis model was established by feeding ApoE$^{-}$/$^{-}$ mice a high-fat diet. The mice were randomly divided into five groups: model group, low-, medium-, and high-dose TCM groups, and a simvastatin group, with a normal-diet group serving as control. Following 4 weeks of treatment, aortic plaque formation was examined, serum levels of SOD, MDA, and GSH were assessed, and protein and mRNA expression in aortic tissues were detected via immunohistochemistry and RT-PCR. Results：Compared with the model group, the combined administration of Erchen Decoction and Taohong Siwu Decoction markedly reduced aortic plaque area, elevated serum SOD and GSH activities, and lowered MDA levels ($P < 0.05$ or $P < 0.01$). Additionally, it downregulated mRNA expression of p53, PTGS2, and NOX1 and COX2 protein expression in aortic tissue, while upregulating mRNA expression of SLC7A11, GPX4, and FTH1, as well as FTH1 protein expression. Conclusion: Erchen Decoction combined with Taohong Siwu Decoction may exert anti-atherosclerotic effects by inhibiting p53 expression and activating the SLC7A11/GPX4 axis, thereby alleviating oxidative damage and suppressing ferroptosis.

## Keywords

Erchen Decoction, Taohong Siwu Decoction, Atherosclerosis, Ferroptosis, p53/SLC7A11 Pathway

## 1. Introduction

Atherosclerosis (AS) is a chronic inflammatory disease primarily affecting medium and small arteries, involving multiple processes such as oxidative damage, lipid deposition, and immune regulation, which can lead to vascular structural changes and tissue ischemia [1]. In Traditional Chinese Medicine (TCM), AS is classified under categories such as “chest impediment,” “headache,” and “stroke,” and modern TCM often considers its pathogenesis to involve “phlegm and blood stasis entanglement,” with treatment principles focusing on “strengthening the spleen, eliminating phlegm, and resolving stasis” [2]. Recent studies have identified oxidative damage as a key mechanism in AS development, closely associated with the “phlegm and blood stasis entanglement” pattern [3]. Ferroptosis is a form of programmed cell death characterized by lipid peroxidation, linked to impaired antioxidant systems and

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reactive oxygen species (ROS) accumulation [4]. Our previous research found that Erchen Decoction combined with Taohong Siwu Decoction can improve lipid profiles and plaque formation in AS mice and exhibits antioxidant effects [5]. This study further investigates the anti-AS mechanisms of this formula from the perspectives of oxidative damage and ferroptosis, providing experimental evidence for the TCM theory of "phlegm-stasis-induced pathogenesis."

## 2. Materials and Methods

A total of fifty ApoE$^{-/-}$ mice and ten C57BL/6J wild-type mice were used in this study. All animals were male, 8 weeks old, and of SPF grade, with body weights ranging from 20 to 22 g. They were housed under specific pathogen-free conditions at Liaoning University of Traditional Chinese Medicine, where they had free access to food and water. The animal experiment protocol was approved by the University's Animal Ethics Committee. The two herbal formulas studied were Erchen Decoction and Taohong Siwu Decoction. Erchen Decoction contained Pinellia ternata (15 g), Citrus reticulata (15 g), Poria cocos (12 g), and Glycyrrhiza uralensis (6 g). Taohong Siwu Decoction was composed of Peach kernel (12 g), Safflower (9 g), Angelica sinensis (12 g), Ligusticum chuanxiong (9 g), Red peony root (9 g), and Rehmannia glutinosa (12 g). All herbs were obtained from the university's affiliated hospital and decocted to concentrations of 0.72, 1.44, and 2.89 g/mL. Key reagents and instruments included an HE staining kit (G1120, Solarbio, Beijing), assay kits for GSH (E-BC-K030-M), MDA (E-BC-K025-M), and SOD (E-BC-K020-M) from Elabscience (Wuhan), immunohistochemistry kits (KIT-9710) and DAB chromogen (DAB-0031) from Maxin Biotech (Fuzhou), as well as a reverse transcription kit (B24408) and qPCR Mix (B21202) from Bimake. Major equipment involved a Thermo microplate reader, a Leica optical microscope, a Thermo centrifuge, and an Applied Biosystems 7500 Real-Time PCR system. After 12 weeks on a high-fat diet to induce atherosclerosis, the fifty ApoE$^{-/-}$ mice were randomly divided into five groups: a model group, three TCM dose groups (low, medium, high), and a simvastatin positive control group, with 10 mice per group. The C57BL/6J mice served as the normal control group. From the 9th week, the treatment groups received their corresponding drugs by oral gavage twice daily for 4 weeks. The normal and model groups were given the same volume of saline. After the treatment period, serum and aortic tissue samples were collected.

Aortic plaque morphology was observed using HE is staining on paraffin-embedded sections. Serum levels of SOD, MDA, and GSH were measured with colorimetric kits following the manufacturer's instructions. Protein expression of FTH1 and COX2 in aortic tissue was detected by immunohistochemistry. This process included deparaffinization, antigen retrieval, blocking, incubation with primary and secondary antibodies, DAB development, and hematoxylin counterstaining. Total RNA was extracted from aortic tissues and reverse-transcribed into cDNA. RT-PCR was performed using GAPDH as the reference gene. The relative mRNA expression of p53, SLC7A11, GPX4, FTH1, PTGS2, and NOX1 was calculated by the ΔΔCt method, with primer sequences provided in Table 1.

**Table 1.** Primer sequences for quantitative real-time PCR

| Gene | Primer sequence (5'–3') | Product length (bp) |
|---|---|---|
| p53 | F: GTC AAG AAA GTG GGG CCT GA R: TGA GTG GAA TCT GGG ATT GTG | 125 |
| SLC7A11 | F: GCA TTC CCA GGG GCT AAC AT R: AAT TTC TCC CAT GCG GGT GT | 93 |

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| | | |
|---|---|---|
| GPX4 | F: CCG CTT ATT GAA GCC AGC AC R: TAT CGG GCA TGC AGA TCG AC | 159 |
| PTGS2 | F: ATG CTA CCA TCT GGC TTC GG R: ATG TCC CCA GGT CAC ATT CC | 181 |
| NOX1 | F: GAA TAG CTA CTG CCC ACC CC R: AGC TGA CCC ACA CGT TAG TG | 132 |
| FTH1 | F: CTG AGC CCT TTG CAA CTT CGT C R: TCC TGG TGG TAG TTC TGG CG | 139 |
| GAPDH | F: GGT TGT CTG CGA CA R: TGG TCC AGG GTT TCT TAC TCC | 186 |

Note: F, forward; R, reverse

Experimental data are presented as mean ± standard deviation ($\bar{x} \pm s$) and analyzed using SPSS 22.0 software. For comparisons among multiple groups, homogeneity of variance was first assessed; the LSD method was applied if variances were equal, and the Tamhane method was used if variances were unequal. Comparisons between two groups were performed using an independent-samples t-test. A value of $P<0.05$ was considered statistically significant.

## 3. Results

Figure 1 shows the histological morphology of the aortas in mice from different experimental groups. The aortas in the normal control group exhibited normal structure, whereas the model group developed large plaques. Plaque size was reduced in all treatment groups, with more pronounced improvements observed in the medium- and high-dose groups as well as the positive control group.

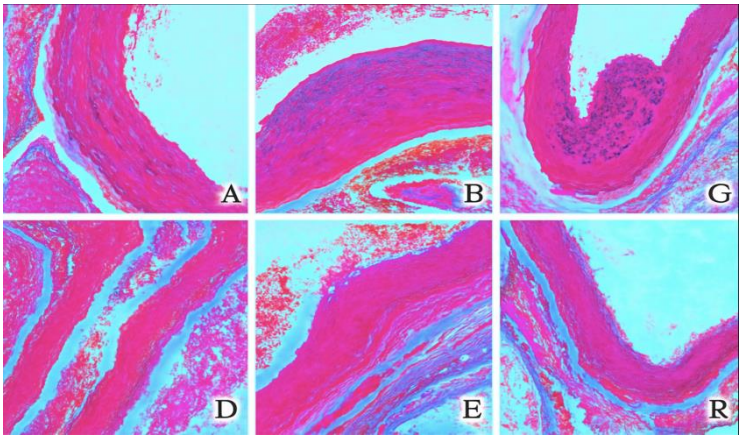


**Fig 1.** Morphological changes of aortic tissues in mice from different experimental groups. *Notes: (A) Normal control group; (B) Model group; (C) Low-dose group; (D) Medium-dose group; (E) High-dose group; (F) Positive control group. The same labeling applies to subsequent figures.*

In the model group, SOD and GSH activities were significantly lower than those in the normal group ($P<0.01$), while MDA levels were significantly higher ($P<0.01$). After treatment, SOD and GSH levels increased and MDA levels decreased, with all differences reaching statistical significance ($P<0.05$ or $P<0.01$).

As shown in Figure 2, COX2 protein expression in the aorta was elevated in the model group, whereas FTH1 protein expression was reduced. In the treatment groups, COX2 expression decreased and FTH1 expression increased, with the medium- and high-dose groups showing particularly notable changes.

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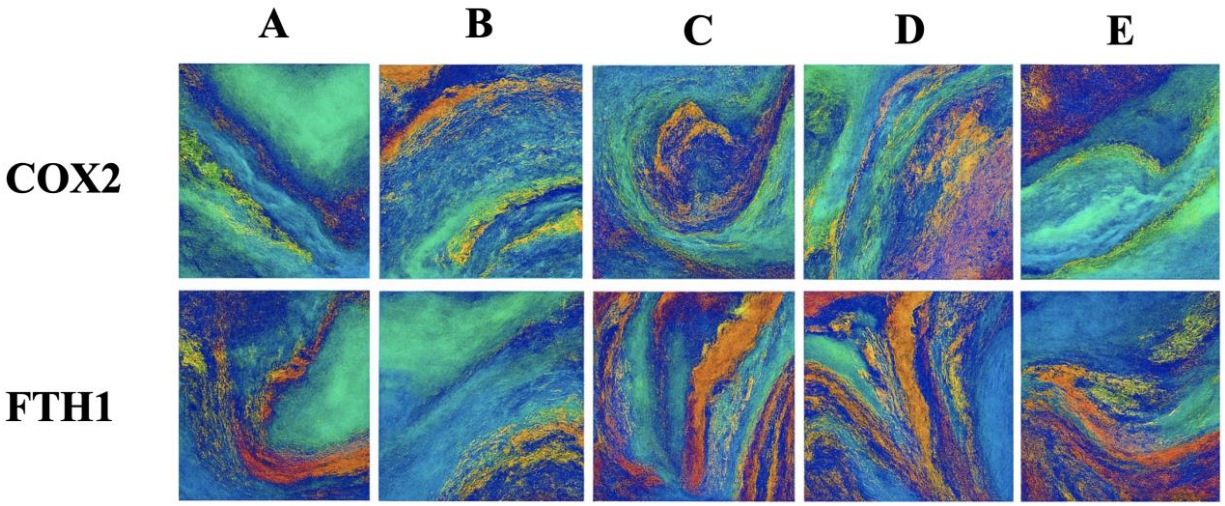


**Fig 2.** Immunohistochemical expression of FTH1 and COX2 in mouse aortic tissues.

Regarding mRNA expression of multiple genes in the aorta, the model group showed significantly higher levels of p53, PTGS2, and NOX1 mRNA compared with the normal group ($P<0.01$), while SLC7A11, GPX4, and FTH1 mRNA levels were significantly lower ($P<0.01$). Drug treatment significantly reversed these gene expression changes ($P<0.05$ or $P<0.01$).

## 4. Discussion

In recent years, the incidence of cardiovascular diseases has continuously increased and is showing a trend toward younger populations, becoming a major threat to human health [6]. Atherosclerosis (AS) is the common pathological basis of most cardiovascular and cerebrovascular diseases, making its prevention and treatment crucial for controlling these conditions. In Traditional Chinese Medicine (TCM), AS is often approached from the perspective of "phlegm-dampness and blood stasis." Erchen Decoction (ECT) from Taiping Huimin Hejiju Fang is effective at drying dampness and resolving phlegm, while Taohong Siwu Decoction (THSWD) from Yizong Jinjian is known for activating blood circulation and removing blood stasis. Modern pharmacological studies indicate that the combination of these two formulas synergistically protects vascular endothelium, exerts anti-inflammatory and lipid-lowering effects, and inhibits thrombosis [7]. Our research focuses on TCM prevention and treatment of AS, emphasizing the theoretical and practical applications of the "phlegm-stasis" approach. Previous work has shown that ECT combined with THSWD can regulate lipid levels, alleviate inflammation, and reduce endothelial oxidative damage in ApoE$^{-/-}$ mice [8].

Ferroptosis, a recently discovered iron-dependent form of cell death closely linked to lipid peroxidation, has attracted increasing attention for its role in cardiovascular diseases, neurodegenerative disorders, and tumors. This study further explores the anti-AS mechanisms of this TCM formula from the perspective of oxidative stress and ferroptosis.

The development of AS is associated with dyslipidemia, oxidative stress, and chronic inflammation. Reactive oxygen species (ROS) play a central role in vascular endothelial injury, leading to endothelial dysfunction, lipoprotein oxidation, and inflammatory activation [9]. Malondialdehyde (MDA), a toxic product of lipid peroxidation, exacerbates intimal damage, whereas superoxide dismutase (SOD) protects tissues by scavenging free radicals [10]. Intracellular glutathione (GSH) and glutathione peroxidase 4 (GPX4) form a critical antioxidant system; GPX4 reduces lipid peroxides with the help of GSH, and its insufficiency triggers ferroptosis. SLC7A11 mediates cystine uptake and is essential for GSH synthesis. p53 can inhibit SLC7A11 transcription, reducing GSH production, while promoting PTGS2 and NOX1 expression, thereby aggravating oxidative damage and inducing ferroptosis.

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This study demonstrates that ECT combined with THSWD increases serum SOD and GSH levels and decreases MDA in AS mice, while downregulating NOX1 and PTGS2 mRNA and COX2 protein expression in the aorta, indicating effective alleviation of oxidative stress. FTH1 protein, involved in iron metabolism homeostasis, is closely linked to ferroptosis, and its decreased expression correlates with ferroptotic activity [10]. The formula also suppresses p53 mRNA expression while upregulating SLC7A11 and GPX4 mRNA levels. In summary, ECT combined with THSWD may mitigate oxidative damage and inhibit ferroptosis by modulating the p53/SLC7A11 pathway, thereby exerting anti-AS effects. Future studies will further explore the underlying mechanisms at the cellular level.

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