

Original Article

ZGC Granules improve HFpEF complicated with atrial fibrillation by inhibiting proinflammatory and pro-fibrotic effects

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Abstract: Objective: This study investigated the efficacy and mechanism of Zhigancao Granules (ZGC) in improving outcomes in patients with heart failure with preserved ejection fraction (HFpEF) complicated by atrial fibrillation (AF), focusing on its anti-inflammatory and anti-fibrotic effects. Method: A total of 220 patients with HFpEF and AF were included and received conventional therapy alone (n = 109) or with ZGC Granules (n = 111) for 3 months, followed by 1-year follow-up. In vitro, HL-1 cardiomyocytes were stimulated with LPS plus palmitic acid and treated with ZGC (0.5, 1.0, 2.0 mg/mL). Cell viability was assessed by CCK-8; mRNA and protein expression of inflammatory and fibrotic markers were measured by qRT-PCR, ELISA, and Western blot. Results: The ZGC group showed significantly greater reductions in B-type natriuretic peptide (BNP), C-reactive protein (CRP), Tumor necrosis factor- α (TNF- α), Interleukin-6 (IL-6), IL-16, and Transforming growth factor-beta 1 (TGF- β 1), along with significantly greater improvements in New York Heart Association (NYHA) functional class, 6-minute walk test (6MWT), Atrial Fibrillation Symptom Scale (AFSS), and Traditional Chinese Medicine (TCM) syndrome efficacy (90.99% vs. 78.90%, $P < 0.05$). At 1 year, the ZGC group had a lower readmission rate (18.02% vs. 28.83%, $P < 0.05$), while mortality and adverse events were similar between groups. In vitro, ZGC treatment restored cell viability and dose-dependently downregulated LPS/palmitic acid-induced inflammatory and fibrotic responses. Conclusion: ZGC Granules improve cardiac function, exercise capacity, and inflammatory/fibrotic markers in HFpEF patients with AF, reduce readmission rates, and exhibit a favorable safety profile. Mechanistically, ZGC suppresses LPS/palmitic acid-induced inflammatory and fibrotic responses in cardiomyocytes, potentially through inhibition of NLRP3/Caspase-1 inflammasome activation.

Keywords: Heart failure with preserved ejection fraction (HFpEF), atrial fibrillation (AF), Zhigancao (ZGC) granules, inflammatory factors, fibrosis

Introduction

Heart failure with preserved ejection fraction (HFpEF) is a subtype of chronic heart failure characterized primarily by a preserved left ventricular ejection fraction (LVEF) of $\geq 50\%$, accompanied by systolic and diastolic dysfunction [1]. The global prevalence of HFpEF continues to rise, accounting for approximately 50% to 60% of all heart failure patients [2]. Meanwhile, the 5-year mortality rate among HFpEF patients reaches 75%, and there has been a significant increase in readmission rates and the burden on the healthcare system [3].

Atrial fibrillation (AF) is the most common arrhythmia complication in HFpEF. Epidemiological studies have shown that the incidence of AF in patients with HFpEF can reach as high as 34% to 41% [4]. There is a close bidirectional pathogenic connection between AF and HFpEF, where AF promotes atrial structural and electrical remodeling, leading to worsening ventricular dysfunction; conversely, the pressure overload and myocardial fibrosis of HFpEF exacerbate the burden of AF, thus creating a vicious cycle that remarkably increases the risk of cardiogenic stroke and all-cause mortality [5].

The activation of inflammation and myocardial fibrosis are the core pathophysiological mechanisms underlying the progression of HFpEF with AF. Numerous clinical and animal experiments have shown that pro-inflammatory factor levels are elevated in the myocardium and serum of HFpEF patients. The overexpression of fibrosis-related factors drives the transdifferentiation of cardiac stromal cells and the deposition of type I and type III collagen, leading to decreased myocardial compliance and abnormal atrial-ventricular electrical conduction in patients [6, 7]. The TGF- β 1/Smad signaling pathway is a key molecular pathway involved in cardiac fibrosis, and is also recognized as a common pathway shared by HFpEF and AF in their mutual progression. TGF- β 1 regulates the transcription of downstream fibrotic genes by promoting Smad2/3 phosphorylation, thereby promoting myofibroblast activation and extracellular matrix proliferation [8, 9]. At present, anti-inflammatory activation and intervention against fibrosis have emerged as key research priorities in HFpEF complicated by AF. However, the mainstream international guidelines currently concentrate on symptom control and comorbidity risk stratification, and there is a lack of specific targeted medications.

Traditional Chinese medicine (TCM) has shown unique theoretical and practical advantages in multi-target and systematic regulation of complex cardiovascular diseases. ZGC Granules originate from *Zhigancao Decoction*, a classic formula created by Zhang Zhongjing, a distinguished physician of the Eastern Han Dynasty. Modern pharmacological studies have confirmed that the main ingredients of ZGC Granules exert multiple effects including immunomodulation, anti-inflammation, anti-fibrosis, antioxidation and cardiomyocyte protection [10]. In vitro studies have demonstrated that glycyrrhizic acid (GA) can markedly suppress the release of inflammatory factors and the activation of the NLRP3 inflammasome [11]. Glycyrrhizin can inhibit TGF- β 1/Smad and p38 MAPK signaling pathways, thereby attenuating myocardial fibrosis [12]. Previous studies have demonstrated that *Zhigancao Decoction* can improve cardiac function and quality of life in patients with heart failure [13], and a randomized controlled trial is currently underway to evaluate its efficacy and safety specifically in HFpEF patients.

However, systematic preclinical and clinical studies are still lacking to verify the regulatory mechanisms of ZGC Granules on inflammatory and fibrotic pathways in HFpEF combined with atrial fibrillation, and the exact targets and molecular mechanisms require further exploration. Based on the above background, the aim of this study is to evaluate the effect of ZGC Granules on improving cardiac function and electrophysiological stability in HFpEF patients with AF, focusing on the TGF- β 1/Smad2/3 signaling pathway and the expression of inflammation-related factors, and to explore its intervention mechanism on myocardial inflammation and fibrosis. This study provides a new theoretical and experimental basis for the prevention and treatment of these complex cardiovascular diseases with traditional Chinese medicine and the development of targeted new drugs.

Materials and methods

Clinical materials and methods

Study subjects: This study was based on a prospective clinical database of patients with HFpEF complicated by atrial fibrillation treated between February 2024 and August 2024. For the purpose of this analysis, patient data were extracted and analyzed retrospectively. Inclusion criteria: (1) Patients aged 18 years or older. (2) Patients who met the diagnostic criteria for HFpEF specified in the *Chinese Guidelines for the Diagnosis and Treatment of Heart Failure*, with LVEF $\geq$ 50% [14]. (3) Patients with chronic heart failure classified as New York Heart Association (NYHA) class II-III [15]. (4) Patients who were diagnosed with permanent atrial fibrillation confirmed by imaging and laboratory examinations. (5) Patients with an EHRA score of II-III according to the 2010 European Heart Rhythm Association atrial fibrillation symptom scale [16]. (6) Patients diagnosed with a pattern of deficiency of both yin and yang according to TCM principles, in accordance with the *Guidelines for Clinical Research on New Traditional Chinese Medicines* [17]. Exclusion criteria: (1) Patients with acute heart failure exacerbation or acute coronary syndrome (e.g., acute myocardial infarction, unstable angina) within the most recent 4 weeks. (2) Patients complicated with primary myocardial diseases (e.g., hypertrophic cardio-

myopathy, dilated cardiomyopathy, restrictive cardiomyopathy). (3) Patients with severe pulmonary hypertension confirmed by echocardiography or right heart catheterization. (4) Patients with severe structural valvular heart disease. (5) Patients with specific cardiac conditions related to tumor radiotherapy or chemotherapy. (6) Patients with severe heart, kidney, liver and other important organ dysfunction. (7) Patients with heterozygous disorders or serious systemic conditions that could affect the assessment of the study endpoint (such as active tumors, severe infections, or immune system disorders). (8) Patients who underwent major cardiac procedures such as surgical or interventional atrial fibrillation ablation and device implantation (e.g., cardiac pacemaker, ICD, CRT) within six months prior to enrollment. (9) Pregnant or lactating patients.

A total of 220 patients were finally enrolled and divided into two groups according to treatment regimens. The conventional treatment group included 109 patients receiving conventional Western medicine, while the ZGC group consisted of 111 patients who were additionally treated with ZGC Granules in addition to the conventional therapy. This study was approved by the Ethics Committee of Zhejiang Hospital. Among the initially considered 240 cases, 20 patients were excluded due to incomplete baseline data ($n = 8$), loss to follow-up before the 3-month endpoint ($n = 7$), or withdrawal of consent for data usage ($n = 5$), resulting in a final analytical cohort of 220 patients.

Treatment methods: The conventional treatment group received standardized conventional Western medical treatment: sacubitril/valsartan tablets 50 mg twice daily (National Drug Approval Number: H20180041, Beijing Novartis Pharma Co., Ltd.); metoprolol tartrate sustained-release tablets 47.5 mg once daily (National Drug Approval Number: H20100040, AstraZeneca Pharmaceuticals Co., Ltd.); spironolactone 20 mg once daily (National Drug Approval Number: H44022137, Baiyunshan Pharmaceutical Factory of Guangzhou Baiyunshan Pharmaceutical Co., Ltd.); digoxin 0.125 mg once daily (National Drug Approval Number: H31020562, Shanghai Xinyi Tianting Pharmaceutical Co., Ltd.); rivaroxaban tablets 15-20 mg once daily (National Drug Approval Number: J20171073, Bayer HealthCare Pharmaceuticals Co., Ltd.).

The ZGC group was additionally administered ZGC Granules on the basis of the medications used in the conventional treatment group. Ingredients: Zhigancao (Radix Glycyrrhizae Preparata) 8 g, Cinnamon Twig 6 g, Fresh Ginger 6 g, Ginseng 4 g, Colla Corii Asini 4 g, Raw Rehmannia Root 20 g, Hemp Seed 10 g, Dwarf Lilyturf Tuber 6 g, Chinese Jujube 5 g. One dose was divided into two sachets. Patients took one sachet in the morning and one in the evening, 30 minutes after meals. Both groups were treated for 3 months.

Key performance indicators: Key indicators: (1) Improvement of cardiac function and exercise tolerance: The NYHA classification system was used to evaluate cardiac function [18] before and after 3 months of treatment by two trained cardiologists. The 6-Minute Walk Test (6MWT) was adopted to assess exercise tolerance [19]. In accordance with the standard operating procedures of the American College of Chest Physicians, the test was conducted along a 30-meter straight corridor. A standard stopwatch (CITIZEN HS20) was used for timing, and the walking distance within 6 minutes was recorded. (2) Symptom of Atrial Fibrillation: The Atrial Fibrillation Symptom Score (AFSS) was used to evaluate symptoms such as palpitations, fatigue and shortness of breath before treatment and 3 months after treatment [20]. The scoring was completed by trained technicians through standardized interviews. All assessments were independently evaluated and reviewed by two physicians; discrepancies were resolved through consensus discussion. (3) Efficacy of TCM syndrome: Referring to the criteria for TCM syndromes of chronic heart failure in the *Guiding Principles for Clinical Research of New Traditional Chinese Medicines (Trial)*, the symptom scoring method was used to quantitatively evaluate primary and secondary TCM symptoms of patients before treatment and 3 months after treatment. Each symptom was scored according to its severity, and the changes in total scores before and after treatment were applied to evaluate the therapeutic effect. The specific criteria were as follows. Markedly effective: The total score for TCM syndromes decreased by $\geq 70\%$ after treatment compared to before treatment; the patient's primary symptoms and signs improved significantly, and subjective symptoms were largely resolved or greatly alleviated. Effective: Patients' TCM

syndrome scores decreased by 30% to 69% after treatment compared to before treatment. Their primary symptoms were apparently alleviated, and quality of life improved. Ineffective: After treatment, the reduction of TCM score was less than 30% or remained unchanged. No obvious improvement was observed in symptoms, and the quality of life showed no improvement or deteriorated.

Secondary indicators: (1) Laboratory indicators: Fasting cubital venous blood (5 mL) was collected from patients in the early morning before treatment and 3 months after treatment, and transferred into EDTA anticoagulant tubes. The samples were centrifuged at 3,500 rpm for 15 min to separate plasma or serum. Brain Natriuretic Peptide (BNP) and C-reactive protein (CRP) were measured using a Roche Cobas e601 automatic electrochemiluminescence analyzer with matching kits (Roche Diagnostics, Germany). Inflammatory markers, including Tumor Necrosis Factor- α (TNF- α), Interleukin-6 (IL-6), and Interleukin-16 (IL-16), as well as Transforming Growth Factor- β 1 (TGF- β 1), were quantified by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Beijing Kemai Biotechnology Co., Ltd.) according to the manufacturers' instructions. All tests were performed in duplicate, and the average values were adopted as the final results. (2) Safety indicators: routine blood test (three-class or five-class blood cell analyzer), serum electrolytes (Na⁺, K⁺, Cl⁻, etc.), liver function (ALT, AST, TBil, etc.), renal function (Scr, BUN, etc.), and 12-lead electrocardiogram examination were performed before and after the treatment. All data of safety indicators were interpreted by senior laboratory physicians and reviewed through quality control procedures, with abnormal results subject to ongoing monitoring. (3) Readmission and mortality: All included patients were followed up from the start of treatment, with a follow-up period of 1 year. Follow-up was conducted through a combination of outpatient re-examination and telephone interview every 3 months. Data regarding heart failure, cardiovascular conditions, all-cause readmission or all-cause mortality were collected during follow-up. For those who failed to attend follow-up on schedule, the research team conducted telephone calls (at least three times on different days) or home visits to ensure complete follow-up data. All follow-up

data were recorded in detail. The follow-up endpoints were the completion of the 1-year follow-up period, loss to follow-up, or patient death. Readmission was defined as one or more unscheduled hospitalizations due to heart failure or recurrence of atrial fibrillation. Mortality was recorded as all-cause mortality and cardiovascular mortality.

Exploration on the mechanism of ZGC Granules in inhibiting pro-inflammatory and pro-fibrotic effects via in vitro experiments

In this study, the HL-1 mouse cardiomyocyte cell line was used as the model for in vitro experiments. After resuscitation, HL-1 cells were cultured in complete medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin mixture and corresponding cell culture additives, in a constant temperature incubator at 37°C with 5% CO₂. The subculture or subsequent experiments were performed when the cells adhered well and reached approximately 80% confluence. HL-1 cells in logarithmic growth phase with good morphology and no contamination were selected for model establishment and drug intervention during the experiment.

Preparation of drug-containing solution of ZGC Granules: Appropriate amount of ZGC Granules was fully dissolved in serum-free medium. The resultant solution was shaken well and centrifuged. The supernatant was collected, sterilized through a 0.22 μ m sterile filter, and prepared as the stock solution for standby use. Prior to experiments, the stock solution was diluted with complete medium to three working concentrations of 0.5, 1.0 and 2.0 mg/mL. All drug-containing culture media were freshly prepared before use to ensure drug stability and experimental reproducibility.

Preparation of palmitic acid working solution: Palmitic acid was heated with an appropriate amount of NaOH solution, then fully mixed with fatty acid-free bovine serum albumin solution to prepare palmitic acid-BSA complex stock solution. The stock solution was diluted with complete medium to 200 μ mol/L prior to experiments. An equal volume of BSA carrier solution was added to the conventional treatment group to eliminate the influence of the carrier on the inflammatory response of the cells and the indicators related to fibrosis.

Detection of cell viability and screening of drug concentrations: The CCK-8 assay was used to assess cell viability to determine the effect of ZGC Granules on HL-1 cells. HL-1 cells were seeded into 96-well plates. After adherence, the cells were treated with different concentrations of ZGC Granules for 24 hours. Subsequently, CCK-8 reagent was added to each well. After further incubation, the absorbance value at 450 nm was measured by a microplate reader to calculate the cell survival rate. According to the cell viability results, concentrations of 0.5, 1.0 and 2.0 mg/mL, which showed no significant cytotoxicity to HL-1 cells, were adopted as the intervention concentrations for subsequent experiments.

Establishment and classification of in vitro micromodels of metabolic inflammation: Given the pathophysiological characteristics of HFpEF, in which metabolic dysfunction, lipotoxicity, and low-grade inflammation collectively contribute to disease progression, this study used lipopolysaccharide (LPS) combined with palmitic acid to stimulate HL-1 cardiomyocytes and establish an in vitro model of the metabolic-inflammatory microenvironment. The experiment was divided into five groups: 1. Control group: HL-1 cells were cultured routinely without treatment with LPS, palmitic acid or drugs. 2. Model group: Cells were stimulated with 1 µg/mL LPS combined with 200 µmol/L palmitic acid. 3. Low-dose group: Cells were treated with 0.5 mg/mL ZGC Granules in addition to the intervention for the model group. 4. Medium-dose group: Cells were treated with 1.0 mg/mL ZGC Granules in addition to the intervention for the model group. 5. High-dose group: Cells were treated with 2.0 mg/mL ZGC Granules in addition to the intervention for the model group.

After 24 hours of intervention, cells and culture supernatants from each group were collected for subsequent detection of proteins, mRNA and inflammatory factors. Each group was set with three replicate wells, and all experiments were independently repeated at least three times.

Detection of inflammatory factors and TGF-β1: The cell culture supernatants of each group were collected and interleukin-1β (IL-1β), TNF-α, IL-6 and TGF-β1 were detected by the enzyme-linked immunosorbent assay. All pro-

cedures were performed in accordance with the instructions provided in the kit manual. The absorbance values were read using a microplate reader, and the concentrations of each index were calculated according to the standard curve. Each sample was tested in duplicate, and the average value was taken as the final result.

Detection of NLRP3, Caspase-1 and TGF-β1 protein expression by Western blot: After collecting HL-1 cells in each group, total protein was extracted with RIPA lysis buffer, and its concentration was measured using the BCA assay. Equal amounts of protein samples were subjected to SDS-PAGE electrophoresis. After protein transfer, the membranes were blocked with 5% skim milk powder. Subsequently, primary antibodies against NOD-like receptor family pyrin domain containing 3 (NLRP3), cysteinyl aspartate-specific proteinase-1 (Caspase-1), TGF-β1 and the reference protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were added, followed by overnight incubation at 4°C. After incubation with secondary antibodies on the following day, chemiluminescence was applied for imaging, and the relative expression of each target protein was calculated by image analysis software. The expression levels of target proteins were normalized to the internal control GAPDH.

Quantitative real-time PCR analysis of genes related to inflammation and fibrosis: Total RNA was isolated from cells of each group using RNA extraction reagent. The concentration and purity of RNA were determined, followed by reverse transcription. Using cDNA as the template, the mRNA expression levels of NLRP3, Caspase-1, IL-1β, TNF-α, IL-6 and TGF-β1 were detected by quantitative real-time PCR (qRT-PCR). With GAPDH as the internal control gene, the relative expression of each target gene was analyzed using the $2^{-\Delta\Delta Ct}$ method. All primer sequences are shown in **Table 1**.

Statistical analysis

All statistical analyses were conducted with SPSS 24.0, GraphPad Prism 9.1 and R 4.1. Measurement data conforming to a normal distribution were reported as mean ± standard deviation (SD). An independent-samples t-test was utilized to compare two groups, while a paired t-test was employed for comparisons

Table 1. Sequences of primers

Sequence	Forward primer	Reverse primer
NLRP3	5'-TCACAATCTCGCCCAAGGAGGAA-3'	5'-AAGAGACCACGGCAGAAGCTAG-3'
Caspase-1	5'-GGCACATTCCAGGACTGACTG-3'	5'-GCAAGACGTGTACGAGTGGTTG-3'
IL-1 β	5'-TGGACCTTCCAGGATGAGGACA-3'	5'-GTTTCATCTCGGAGCCTGTAGTG-3'
TNF- α	5'-GGTGCCTATGTCTCAGCCTCTT-3'	5'-GCCATAGAAGTATGAGAGGGAG-3'
IL-6	5'-TACCACTTCACAAGTCGGAGGC-3'	5'-CTGCAAGTGCATCATCGTTGTTTC-3'
TGF- β 1	5'-TGATACGCCTGAGTGGCTGTCT-3'	5'-CACAAAGAGCAGTGAGCGCTGAA-3'
GAPDH	5'-CATCACTGCCACCCAGAAGACTG-3'	5'-ATGCCAGTGAGCTTCCCGTTTCAG-3'

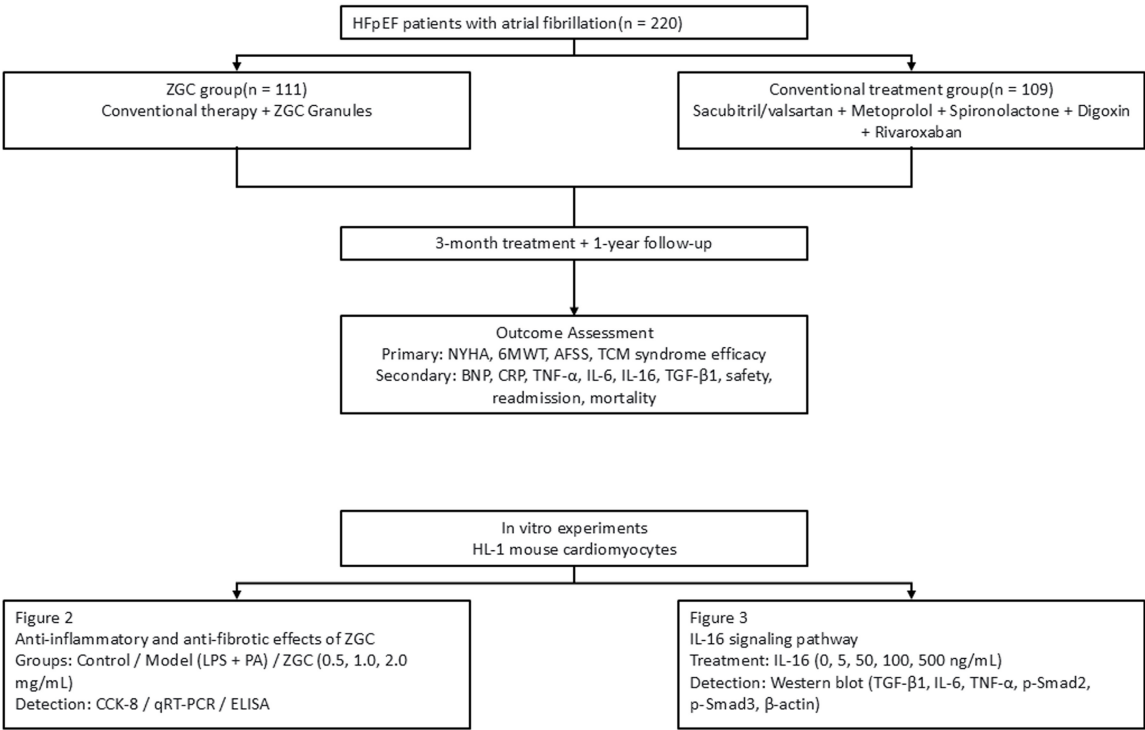


Figure 1. Study workflow. Schematic representation of the overall study design, including patient enrollment, group allocation, treatment protocol, follow-up assessments, and in vitro mechanistic experiments using HL-1 cardiomyocytes.

across different time points in the same group. For multiple-group comparisons, one-way ANOVA was conducted, and Tukey's test was applied for post hoc pairwise comparisons. Non-parametric rank-sum tests were adopted for non-normally distributed data and ranked data. Enumeration data were presented as case numbers and percentages, and between-group comparisons were performed using the χ^2 test or Fisher's exact test. All statistical tests were two-tailed, and $P < 0.05$ indicated statistical significance. For comparisons of changes (Δ values) between groups, analysis of covariance (ANCOVA) was performed with baseline values as covariates. Multivariate logistic regression

analysis was used to identify independent factors associated with TCM syndrome efficacy, with results presented as odds ratios (ORs) with 95% confidence intervals (CIs).

Study flow chart

The general workflow of this study is illustrated in **Figure 1**.

Results

Baseline characteristics of the two groups

There were no statistically significant differences in baseline data such as sex, age, body

Table 2. Comparison of baseline characteristics between the two groups

Indicator	ZGC group (n = 111)	Conventional treatment group (n = 109)	t/ χ^2	P
Male/Female (n)	65 (58.56)/41 (41.44)	68 (62.39)/41 (37.61)	0.337	0.562
Age (years)	69.23 $\pm$ 8.14	68.81 $\pm$ 7.94	0.387	0.699
BMI (kg/m ²)	25.42 $\pm$ 3.22	25.13 $\pm$ 3.32	0.658	0.511
Disease course (years)	3.62 $\pm$ 0.54	3.74 $\pm$ 0.61	1.546	0.123
Hypertension (n, %)	74 (66.67)	70 (64.22)	0.146	0.703
Diabetes mellitus (n, %)	35 (31.53)	37 (33.94)	0.145	0.703
LVEF (%)	58.73 $\pm$ 3.54	58.34 $\pm$ 3.82	0.786	0.433
NYHA class II/III	54 (48.65)/57 (51.35)	47 (43.12)/62 (56.88)	0.677	0.411

Table 3. Comparison of laboratory indicators before and after treatment

Indicator	Group	Pre-treatment	Post-treatment	Intra-group P	Inter-group P (Post-treatment)
BNP (pg/mL)	ZGC group	426.41 $\pm$ 65.22	341.86 $\pm$ 61.23	< 0.001	< 0.001
	Conventional treatment group	432.09 $\pm$ 64.85	377.45 $\pm$ 69.31	< 0.001	
CRP (mg/L)	ZGC group	8.16 $\pm$ 2.22	5.47 $\pm$ 2.05	< 0.001	< 0.001
	Conventional treatment group	8.09 $\pm$ 2.13	6.02 $\pm$ 2.11	< 0.001	
TNF- α (pg/mL)	ZGC group	22.93 $\pm$ 5.63	15.92 $\pm$ 4.18	< 0.001	< 0.001
	Conventional treatment group	23.08 $\pm$ 5.45	17.36 $\pm$ 4.46	< 0.001	
IL-6 (pg/mL)	ZGC group	11.82 $\pm$ 2.64	7.23 $\pm$ 2.11	< 0.001	< 0.001
	Conventional treatment group	12.04 $\pm$ 2.77	8.08 $\pm$ 2.34	< 0.001	
IL-16 (pg/mL)	ZGC group	69.46 $\pm$ 13.28	51.65 $\pm$ 12.53	< 0.001	< 0.001
	Conventional treatment group	68.89 $\pm$ 13.41	56.41 $\pm$ 13.07	< 0.001	
TGF- β 1 (ng/mL)	ZGC group	38.72 $\pm$ 8.43	26.28 $\pm$ 7.14	< 0.001	< 0.001
	Conventional treatment group	39.01 $\pm$ 8.57	29.53 $\pm$ 8.09	< 0.001	

mass index (BMI), disease duration, coexistence of hypertension and diabetes, left ventricular ejection fraction (LVEF), and NYHA classification between the two groups ($P > 0.05$; **Table 2**).

Changes of laboratory indicators before and after treatment in the two groups

There were no significant differences in the levels of BNP, CRP, TNF- α , IL-6, IL-16 and TGF- β 1 between the two groups before treatment ($P > 0.05$). All measured parameters were reduced after 3 months of treatment in both groups. The ZGC group showed significantly greater improvement compared to the conventional treatment group ($P < 0.05$), as shown in **Table 3**.

Comparison of improvement in cardiac functional classification before and after treatment

There was no significant difference in NYHA functional class between the two groups before treatment ($P > 0.05$). Both groups achieved

improvements in cardiac function after treatment. The proportion of patients with NYHA class II or lower was significantly higher in the ZGC group than in the conventional treatment group ($P < 0.05$), as shown in **Table 4**.

Comparison of exercise tolerance and atrial fibrillation symptom scores before and after treatment

There were no significant differences in 6MWT and AFSS scores between the two groups at baseline ($P > 0.05$). Compared with baseline, both groups had higher 6MWT values and lower AFSS scores post-treatment. The improvements were more pronounced in the ZGC group than in the conventional treatment group, with statistically significant differences ($P < 0.05$) (**Table 5**).

Comparison of TCM symptom efficacy before and after treatment

The total effective rate of TCM syndromes at 3 months after treatment was higher in the ZGC

Table 4. Changes in NYHA Functional class before and after treatment

Group	Time point	Class I	Class II	Class III	Intra-group P	Inter-group P (Post-treatment)
ZGC group (n = 111)	Pre-treatment	0 (0.00)	54 (48.65)	57 (51.35)	< 0.001	0.021
	Post-treatment	6 (5.41)	82 (73.87)	23 (20.72)		
Conventional treatment group (n = 109)	Pre-treatment	0 (0.00)	47 (43.12)	62 (56.88)	< 0.001	
	Post-treatment	2 (1.83)	68 (62.39)	39 (35.78)		

Table 5. Changes in 6MWT and AFSS scores pre- and post-treatment

Indicator	Time point	ZGC group (n = 111)	Conventional treatment group (n = 109)	Intra-group P	Inter-group P (Post-treatment)
6MWT (m)	Pre-treatment	342.15 ± 52.13	340.81 ± 50.85	< 0.001	0.002
	Post-treatment	429.58 ± 54.77	406.09 ± 51.35		
AFSS (point)	Pre-treatment	11.46 ± 2.17	11.39 ± 2.31	< 0.001	0.005
	Post-treatment	6.53 ± 2.04	7.38 ± 2.19		

Table 6. TCM syndrome efficacy pre- and post-treatment in two groups [n (%)]

Group	Markedly effective	Effective	Ineffective	Total effective rate
ZGC group (n = 111)	37 (33.33)	64 (57.66)	10 (9.01)	101 (90.99)
Conventional treatment group (n = 109)	23 (21.10)	63 (57.80)	23 (21.10)	86 (78.90)
P	/			0.012

Table 7. Adverse events in the two groups

Group	Gastrointestinal reactions	Mild elevation of ALT/AST	Mildly elevated serum creatinine	Atrial premature beats	Short-term supraventricular tachycardia	Total
ZGC group (n = 111)	2 (1.80)	1 (0.90)	1 (0.90)	1 (0.90)	0 (0.00)	6 (5.41)
Conventional treatment group (n = 109)	2 (1.83)	2 (1.83)	1 (0.92)	1 (0.92)	1 (0.92)	7 (6.42)

group than in the conventional treatment group ($P < 0.05$) (**Table 6**).

Follow-up results of readmission and mortality in the two groups

During the 1-year follow-up period, the ZGC group had a significantly lower readmission rate than the conventional treatment group ($P < 0.05$). No statistically significant difference was observed in all-cause mortality between the two groups ($P > 0.05$).

Treatment safety and management of adverse events in the two groups

During the study, adverse events occurred in both groups, with no statistically significant difference in incidence ($P > 0.05$, **Table 7**). No severe hepatic or renal dysfunction or life-threatening arrhythmias occurred in either group. Adverse events were managed promptly

with symptomatic treatments. Patients with gastrointestinal symptoms received dietary guidance; if symptoms persisted, the study drug was temporarily withheld. In cases of mild renal function abnormalities, suspected medications were paused and renal function was re-evaluated, with most cases returning to baseline within 2 weeks. For allergic reactions, suspected drugs were immediately discontinued and anti-allergic therapy was initiated. For aggravated arrhythmias, electrocardiographic monitoring was intensified and anti-arrhythmic regimens were adjusted. All adverse events resolved with symptomatic management; no patient permanently discontinued the study drug, and all remained eligible for follow-up.

Effect of ZGC Granules on viability of HL-1 cells induced by LPS and palmitic acid

According to the CCK-8 assay results, cell survival rate in the model group was significantly

decreased compared with the control group ($P < 0.05$). Treatment with low, medium and high doses of ZGC groups all restored cell viability at different extents ($P < 0.05$), especially in medium and high ZGC groups (**Figure 2A**). As detected by qRT-PCR, the mRNA expression of NLRP3, Caspase-1, IL-1 β , TNF- α , IL-6 and TGF- β 1 was significantly upregulated in the model group compared with the conventional treatment group ($P < 0.05$). Compared with the model group, the expression levels of the above genes were downregulated at different extent in three ZGC groups ($P < 0.05$), with the most remarkable decrease observed in the high-dose ZGC group (**Figure 2B**). ELISA results revealed that the levels of IL-1 β , TNF- α , IL-6 and TGF- β 1 in cell culture supernatants were significantly higher in the model group than in the conventional treatment group ($P < 0.05$). Compared with the model group, these factors were markedly decreased in all ZGC groups ($P < 0.05$), and the decrease gradually enhanced with the increase of ZGC concentration (**Figure 2C**).

Inhibitory effects of ZGC on NLRP3/Caspase-1 inflammasome and TGF- β 1 protein expression

Compared with the control group, the model group exhibited markedly elevated expression levels of NLRP3, cleaved caspase-1, IL-1 β and TGF- β 1 ($P < 0.05$). In comparison with the model group, the expression of the above proteins was obviously decreased after ZGC intervention ($P < 0.05$). It suggests that ZGC exerts a potent inhibitory effect on LPS and palmitic acid-triggered inflammasome activation and pro-fibrotic protein expression (**Figure 3**).

Changes of indicators between baseline and post-treatment (Δ values)

To further compare the magnitude of improvement between groups, we calculated the changes (Δ) from baseline to 3 months post-treatment. As shown in **Table 8**, the ZGC group exhibited significantly greater improvements than the conventional treatment group in BNP ($F = 16.231$, $P < 0.001$), IL-16 ($F = 4.077$, $P = 0.045$), 6MWT ($F = 5.367$, $P = 0.021$), and AFSS ($F = 172.968$, $P < 0.001$). CRP showed a trend toward greater reduction in the ZGC group ($F = 3.831$, $P = 0.052$), whereas TNF- α , IL-6 and TGF- β 1 did not reach statistical significance (all $P > 0.05$). While **Table 3** compares

post-treatment values between groups, **Table 8** presents the changes from baseline (Δ values), providing complementary information on the magnitude of improvement.

Multivariate logistic regression analysis of factors associated with treatment efficacy

Multivariate logistic regression analysis (**Table 9**) identified ZGC treatment as an independent predictor of TCM syndrome efficacy (OR = 3.865, 95% CI: 1.970-7.583, $P < 0.001$), along with baseline BNP level (OR = 3.865, 95% CI: 2.533-5.898, $P < 0.001$). Other variables including age, sex, hypertension, diabetes, CRP, TNF- α , and IL-6 were not significant predictors in the multivariate model (all $P > 0.05$).

Subgroup analysis of ZGC treatment effect

To assess the consistency of ZGC treatment effects across different patient populations, subgroup analyses were performed stratified by age, sex, hypertension, diabetes, and baseline NYHA class. As shown in **Figure 4**, ZGC treatment consistently favored TCM syndrome efficacy across all predefined subgroups (all ORs > 1.0), indicating that the therapeutic benefits of ZGC are robust and generalizable.

Discussion

Heart failure with preserved ejection fraction (HFpEF) accompanied with atrial fibrillation (AF) is a frequently encountered cardiovascular composite phenotype linked to adverse outcomes. HFpEF is characterized by preserved left ventricular ejection fraction and impaired diastolic function. This condition commonly occurs in elderly patients and is linked to hypertension, diabetes mellitus, obesity, and chronic low-grade inflammation [21, 22]. A bidirectional pathological interaction exists between the two disorders. HFpEF results in increased ventricular filling pressure, elevated left atrial pressure, and myocardial fibrosis, which contribute to the initiation and persistence of atrial fibrillation. Conversely, atrial fibrillation worsens ventricular diastolic filling and cardiac output, thus forming a vicious cycle of heart failure, atrial fibrillation, inflammation and fibrosis [23-25]. Accordingly, for HFpEF patients complicated with atrial fibrillation, the treatment should not focus solely on relieving volume overload, controlling ventricu-

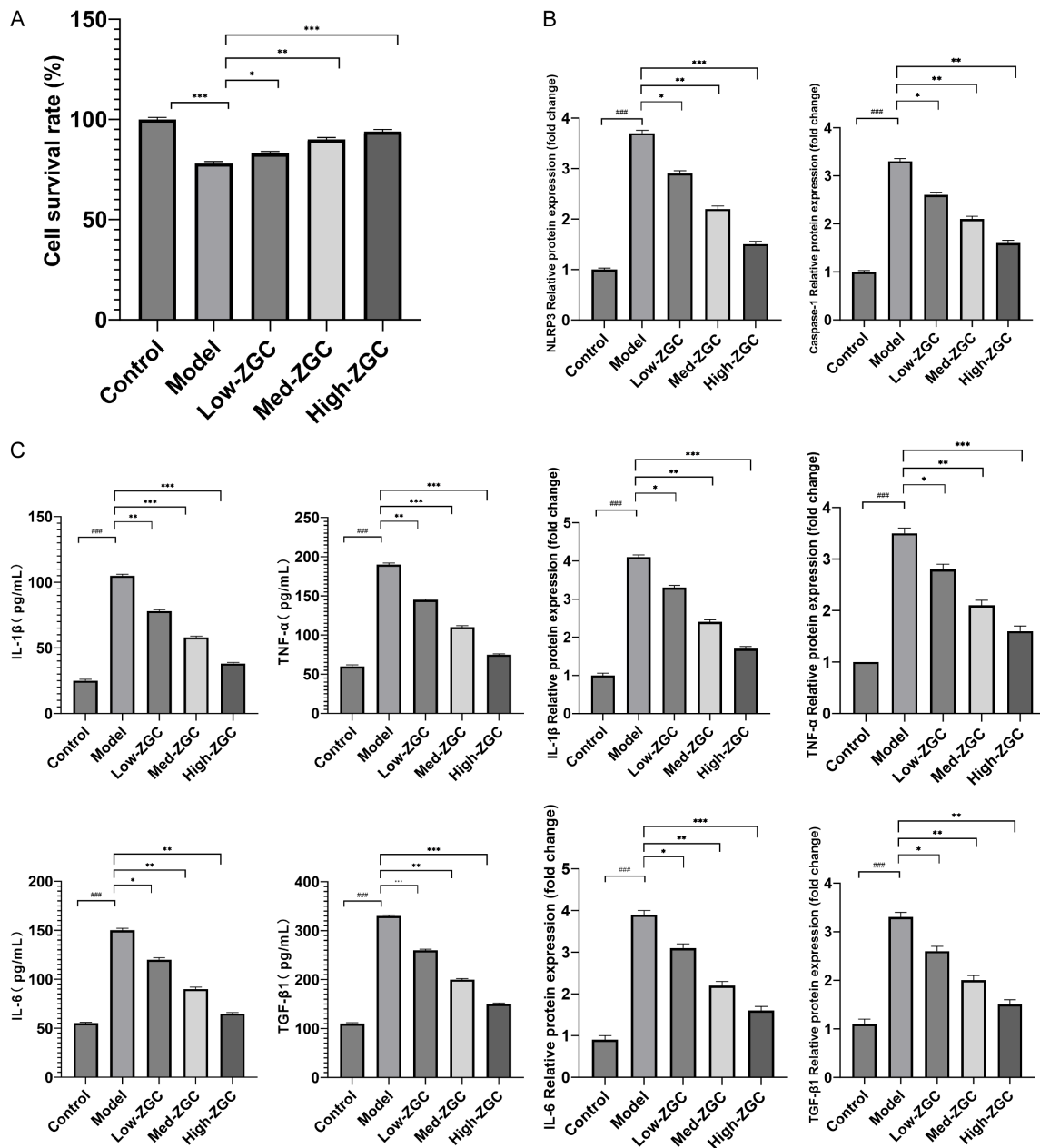


Figure 2. ZGC Granules suppress LPS/palmitic acid-induced inflammatory and fibrotic responses in HL-1 cardiomyocytes. HL-1 cardiomyocytes were pretreated with ZGC Granules (0.5, 1.0, and 2.0 mg/mL) for 24 h followed by stimulation with LPS (1 μ g/mL) plus palmitic acid (200 μ mol/L) for 24 h. A. Cell viability assessed by CCK-8 assay. B. mRNA expression of NLRP3, Caspase-1, IL-1 β , TNF- α , IL-6 and TGF- β 1 determined by qRT-PCR. C. Protein levels of IL-1 β , TNF- α , IL-6 and TGF- β 1 in cell culture supernatants measured by ELISA. Data are presented as mean $\pm$ SD (n = 3). Notes: ###P < 0.001 vs. control group; *P < 0.05, **P < 0.01, ***P < 0.001 vs. model group (one-way ANOVA with Tukey's post-hoc test). ZGC, Zhigancao Granules; LPS, lipopolysaccharide; PA, palmitic acid; CCK-8, Cell Counting Kit-8; qRT-PCR, quantitative real-time polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

lar rate, and initiating anticoagulation. Therapies also need to tackle common pathological pathways: inflammation, myocardial damage and fibrotic remodeling. Given the pivotal roles

of inflammation and fibrosis in this comorbid condition, traditional Chinese medicine formulas with multi-target anti-inflammatory and anti-fibrotic properties may offer therapeutic

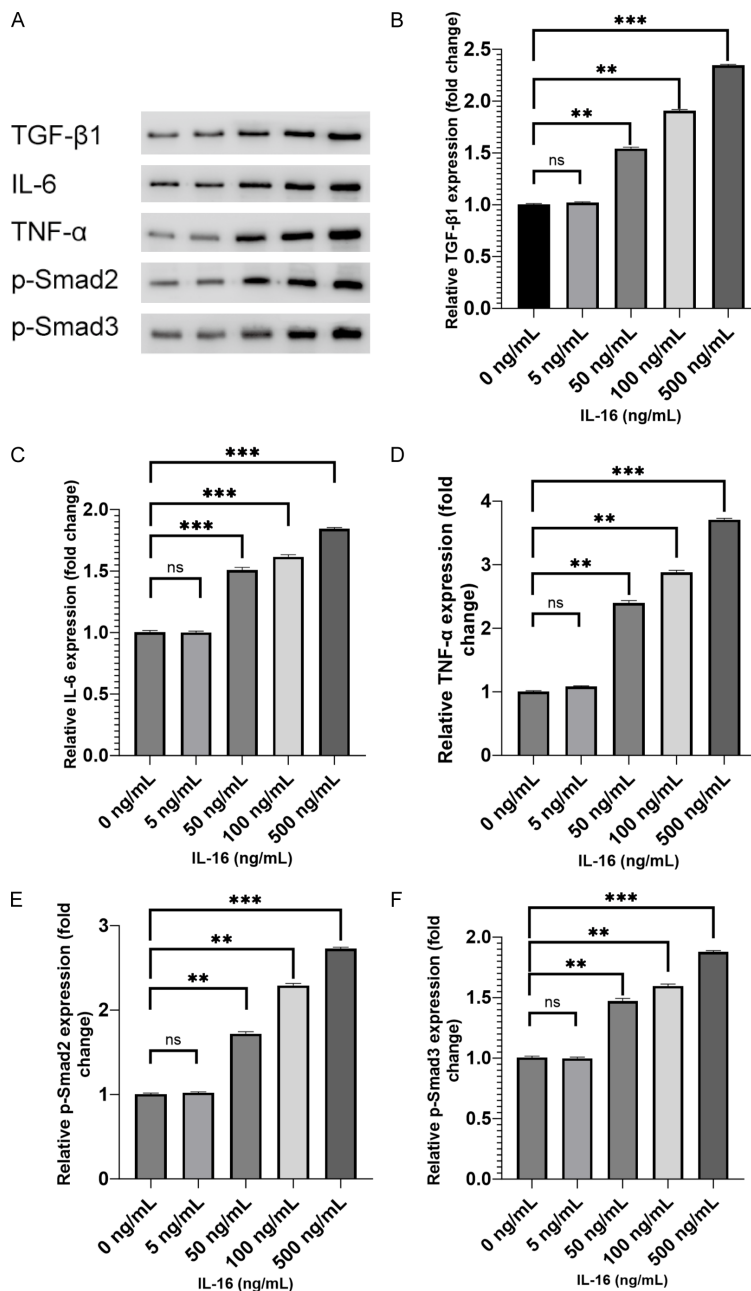


Figure 3. IL-16 promotes inflammatory and fibrotic protein expression in a concentration-dependent manner. HL-1 cardiomyocytes were treated with increasing concentrations of IL-16 (0, 5, 50, 100, 500 ng/mL) for 24 h. A. Representative Western blot bands showing protein expression of TGF-β1, IL-6, TNF-α, P-Smad2, P-Smad3 and β-actin. B. Quantitative analysis of relative protein expression normalized to β-actin. Data are presented as mean ± SD (n = 3). Notes: *P < 0.05, **P < 0.01, ***P < 0.001 vs. 0 ng/mL (one-way ANOVA with Dunnett's post-hoc test). P-Smad2, phosphorylated Smad2; P-Smad3, phosphorylated Smad3.

potential. ZGC Granules have been clinically used for cardiovascular diseases, yet the effects on HFpEF with AF remain unexplored.

the initiation and progression of HFpEF complicated by atrial fibrillation. Patients with HFpEF often manifest metabolic derangements, endo-

This study demonstrates that adjuvant treatment with ZGC Granules on the basis of conventional Western medicine can further alleviate clinical symptoms and enhance cardiac function in patients with HFpEF complicated by atrial fibrillation. Three months post-treatment, serum levels of BNP, CRP, TNF-α, IL-6, IL-16 and TGF-β1 were markedly decreased from baseline in the treatment group, and the reductions were superior to those in the conventional treatment group. Additionally, the treatment group showed better improvements in NYHA classification, 6-minute walk distance and atrial fibrillation symptom scores, along with a higher TCM syndrome response rate. Collectively, these observations reveal that ZGC Granules not only ameliorate clinical manifestations such as palpitations, fatigue, and shortness of breath, but also improve the myocardial micro-environment and cardiac function by mitigating systemic inflammation and downregulating profibrotic factors. The 1-year follow-up further showed that the treatment group had a lower readmission rate than the conventional treatment group. No significant between-group differences were observed in all-cause mortality or adverse event incidence. These findings indicate that ZGC Granules exert promising clinical effects while exhibiting acceptable safety.

Inflammatory activation represents one of the core pathological mechanisms behind

Table 8. Comparison of changes (Δ) in laboratory and functional indicators between the two groups

Indicator	ZGC group (n = 111)	Conventional group (n = 109)	F	P value
Δ BNP (pg/mL)	-84.55 $\pm$ 93.00	-54.65 $\pm$ 90.90	16.231	< 0.001
Δ CRP (mg/L)	-2.70 $\pm$ 3.00	-2.07 $\pm$ 2.94	3.831	0.052
Δ TNF- α (pg/mL)	-7.00 $\pm$ 7.07	-5.72 $\pm$ 6.77	1.880	0.172
Δ IL-6 (pg/mL)	-4.60 $\pm$ 3.39	-3.97 $\pm$ 3.63	1.772	0.185
Δ IL-16 (pg/mL)	-17.81 $\pm$ 19.95	-12.47 $\pm$ 19.26	4.077	0.045
Δ TGF- β 1 (ng/mL)	-12.43 $\pm$ 11.32	-9.48 $\pm$ 12.00	3.519	0.062
Δ 6MWT (m)	-1.34 $\pm$ 67.71	-23.50 $\pm$ 74.09	5.367	0.021
Δ AFSS (points)	-0.08 $\pm$ 3.15	0.85 $\pm$ 3.12	172.968	< 0.001

Δ represents the change from baseline to 3 months post-treatment (post-treatment minus pre-treatment). Data are presented as mean $\pm$ SD. *P* values were calculated by ANCOVA with baseline values as covariates. Negative values indicate improvement for BNP, CRP, inflammatory markers, TGF- β 1 and AFSS; positive values indicate improvement for 6MWT.

Table 9. Multivariate logistic regression analysis of factors associated with TCM syndrome efficacy

Variable	β coefficient	SE	Wald χ^2	OR (95% CI)	P value
ZGC treatment	1.352	0.344	15.459	3.865 (1.970-7.583)	< 0.001
Age (per SD)	-0.139	0.170	0.662	0.871 (0.624-1.215)	0.416
Male (vs. female)	0.457	0.345	1.752	1.579 (0.803-3.105)	0.186
Hypertension (yes vs. no)	0.052	0.354	0.021	1.053 (0.526-2.108)	0.884
Diabetes (yes vs. no)	-0.182	0.366	0.247	0.834 (0.407-1.707)	0.619
Baseline BNP (per SD)	1.352	0.216	39.336	3.865 (2.533-5.898)	< 0.001
Baseline CRP (per SD)	0.062	0.170	0.135	1.064 (0.763-1.486)	0.713
Baseline TNF- α (per SD)	0.179	0.167	1.149	1.196 (0.862-1.660)	0.284
Baseline IL-6 (per SD)	0.227	0.171	1.769	1.255 (0.898-1.754)	0.184

Dependent variable: TCM syndrome efficacy (effective vs. ineffective). OR, odds ratio; CI, confidence interval; SD, standard deviation. Variables with *P* < 0.10 in univariate analysis were entered into the multivariate model. Hosmer-Lemeshow goodness-of-fit test: χ^2 = 8.24, *P* = 0.411.

thelial dysfunction and chronic low-grade inflammation, and persistently elevated pro-inflammatory factors further cause cardiomyocyte injury, coronary microcirculatory dysfunction and myocardial interstitial remodeling [26, 27]. Once atrial fibrillation develops, the rapid, irregular electrical activity within the atria triggers oxidative stress and inflammatory mediator release, further worsening atrial structural and electrical remodeling [28, 29]. The clinical component of this study found that serum levels of CRP, TNF- α , IL-6 and IL-16 were markedly decreased in the treatment group after intervention. This indicates that alongside standard therapy, ZGC Granules can further inhibit inflammatory activity in patients with HFpEF and atrial fibrillation. TNF- α and IL-6 are pivotal cytokines that reflect cardiovascular inflammatory burden, and they contribute to endothelial injury, cardiomyocyte apoptosis and myocardial interstitial remodeling. IL-16 is a pro-inflammatory chemo-

kine involved in chronic inflammatory cell infiltration and myocardial tissue injury [30, 31]. The reduction of these inflammatory factors offers a mechanistic interpretation for the improvements in cardiac function and exercise tolerance following ZGC Granules treatment.

To further explore the mechanism underlying ZGC Granules, we conducted *in vitro* experiments using HL-1 cardiomyocytes. Cells were co-stimulated with LPS and palmitic acid to recapitulate the metabolic and inflammatory microenvironment of HFpEF. LPS triggers classical inflammatory responses, whereas palmitic acid, a saturated fatty acid, induces lipotoxic injury and metabolic stress. Combined stimulation with the two agents closely mimics the concurrent metabolic dysfunction and inflammatory activation characteristic of HFpEF patients [32, 33]. Our results showed that LPS combined with palmitic acid treatment obviously impaired HL-1 cell viability. The mRNA lev-

ZGC Granules in HFpEF with AF

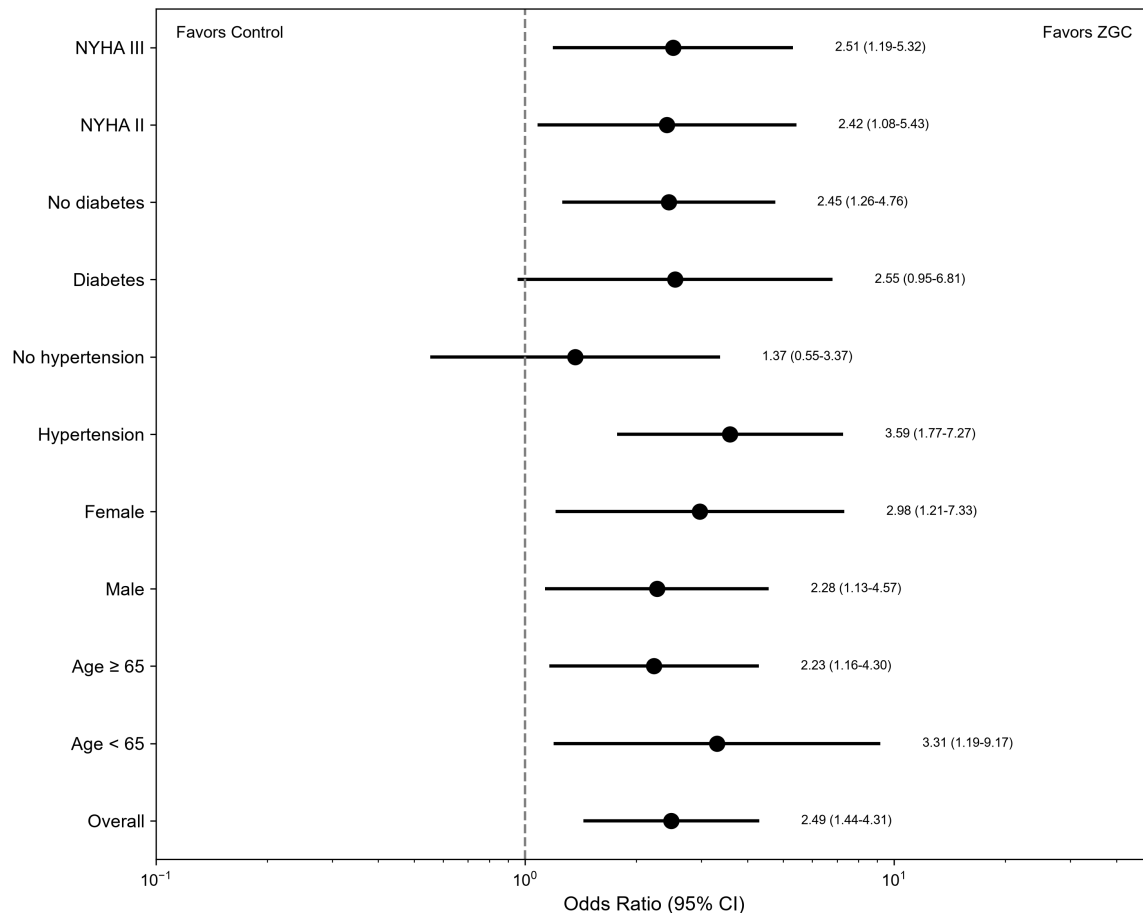


Figure 4. Subgroup analysis of ZGC treatment effect on TCM syndrome efficacy. Forest plot showing odds ratios (ORs) and 95% confidence intervals for the effect of ZGC treatment compared with conventional treatment across different subgroups. The overall effect is shown at the top, followed by subgroup-specific effects stratified by age (< 65 vs. ≥ 65 years), sex, hypertension status, diabetes status, and baseline NYHA class (II vs. III). The vertical dashed line represents the null effect (OR = 1.0). Squares represent point estimates, and horizontal lines indicate 95% CIs. All subgroup-specific ORs exceeded 1.0, indicating consistent therapeutic benefits of ZGC across diverse patient populations.

els of NLRP3, Caspase-1, IL-1 β , TNF- α , IL-6 and TGF- β 1 were significantly upregulated, and the concentrations of these cytokines in cell culture supernatants also increased substantially. These findings confirm that this cell model successfully recapitulates inflammatory reactions and activated fibrogenic signaling pathways in cardiomyocytes under a metabolic-inflammatory microenvironment. After intervention with ZGC Granules, HL-1 cell viability was restored. Both inflammatory and fibrogenic biomarkers were downregulated to different extents, with the high-dose group showing the most remarkable effects. These results demonstrated that ZGC Granules exert protective effects on cardiomyocytes from injury induced by metabolic-inflammatory stress.

The NLRP3/Caspase-1 inflammasome serves as a core molecular platform that bridges metabolic stress and amplified inflammation. Once activated, it facilitates Caspase-1 activation, which further drives the maturation and secretion of IL-1 β and initiates downstream inflammatory cascades [34, 35]. Pro-inflammatory cytokines including IL-1 β , TNF- α and IL-6 not only directly injure cardiomyocytes, but also induce fibroblast activation and collagen accumulation, which elevates myocardial stiffness. These changes ultimately lead to diastolic dysfunction and cardiac structural remodeling in HFpEF [36]. Western blot analysis revealed that the protein levels of NLRP3, cleaved caspase-1, and IL-1 β were significantly elevated in the model group. Treatment with ZGC Granules

substantially downregulated the expression of these proteins. Collectively, these data indicate that the granules suppress NLRP3/caspase-1 inflammasome activation, which in turn limits IL-1 β maturation and secretion and halts the progression of inflammatory cascades. Consistent with these cellular findings, clinical assays also demonstrated decreased levels of inflammatory biomarkers including TNF- α , IL-6 and CRP. Taken together, both clinical observations and in vitro experiments validate the anti-inflammatory properties of ZGC Granules.

Myocardial fibrosis constitutes a key structural substrate for diastolic dysfunction and persistent atrial fibrillation in patients with HFpEF. TGF- β 1 is a critical regulator of fibrotic responses. By activating downstream Smad2/3 signaling pathways, it induces myofibroblast activation and collagen synthesis, and accelerates extracellular matrix deposition. These alterations eventually reduce myocardial compliance, increase atrial conduction heterogeneity, and elevate the risk of atrial fibrillation [37, 38]. The clinical part of this study showed that treatment led to a more substantial decrease in TGF- β 1 levels in the ZGC group compared with the conventional treatment group. In vitro experiments further confirmed that combined LPS and palmitic acid markedly upregulated TGF- β 1 mRNA and protein expression in HL-1 cardiomyocytes, whereas ZGC Granules effectively reversed this trend by suppressing TGF- β 1 expression. These findings indicate that ZGC Granules can not only suppress inflammatory responses but also mitigate the fibrotic tendency of cardiomyocytes and their microenvironment via inhibiting TGF- β 1-driven profibrotic signaling. Within the pathological continuum linking inflammation and fibrosis, activated NLRP3 inflammasome stimulates IL-1 β secretion, which subsequently elevates TGF- β 1 levels and accelerates myocardial fibrosis. ZGC Granules concurrently downregulate key molecules involved in inflammasome signaling and TGF- β 1. It therefore exerts multi-target synergistic effects to modulate and halt the inflammatory-fibrotic cascade in HFpEF complicated by atrial fibrillation.

This study innovatively combined clinical observations with in vitro experiments to explore the mechanisms by which ZGC Granules treat HFpEF complicated by atrial fibrillation, from

both clinical and cellular levels. Clinically, co-administration of the granules and routine Western therapy ameliorated cardiac function, exercise tolerance and atrial fibrillation symptoms, and lowered inflammatory and fibrotic indicators. In vitro assays revealed that under the metabolic-inflammatory microenvironment established by LPS and palmitic acid, ZGC Granules restored HL-1 cell viability, suppressed NLRP3/Caspase-1 inflammasome activation, reduced the secretion of IL-1 β , TNF- α and IL-6, and downregulated TGF- β 1 expression. The complementary evidence from two parts of this study indicate that ZGC Granules alleviate HFpEF combined with atrial fibrillation mainly through a cascade of actions: inhibition of inflammasome activation, reduction of inflammatory cytokine release, and downregulation of TGF- β 1-mediated fibrotic responses.

Nevertheless, several limitations of this study should be noted. Firstly, the clinical component was a retrospective controlled study. While the two groups had comparable baseline data, selection bias and potential confounding factors might still exist. Further verification via prospective, multicenter randomized controlled trials is therefore warranted. Secondly, although the in vitro metabolic inflammation model established in HL-1 cardiomyocytes using LPS and palmitic acid recapitulates cardiomyocyte inflammatory injury, the pathogenesis of HFpEF complicated by AF involves multiple elements, including various cardiac cells (cardiomyocytes, fibroblasts, endothelial cells and immune cells) as well as neurohumoral regulation. A single-cell culture system therefore may fail to faithfully reproduce the sophisticated in vivo pathological conditions. Thirdly, although our in vitro data demonstrated that ZGC treatment significantly reduced Smad2/3 phosphorylation (**Figure 2**) and that IL-1 β stimulation upregulated TGF- β 1, IL-6, TNF- α , P-Smad2 and P-Smad3 in a concentration-dependent manner (**Figure 3**), we did not perform rescue experiments using pathway-specific agonists or inhibitors. Thus, while these findings strongly suggest that ZGC modulates the TGF- β 1/Smad2/3 signaling pathway, the causal relationship remains to be definitively established. Future studies employing pharmacological inhibitors (e.g., SB431542) or gene silencing approaches are warranted to

confirm that the therapeutic effects of ZGC are indeed mediated through this pathway.

In conclusion, ZGC Granules effectively improve clinical outcomes in patients with HFpEF and concomitant atrial fibrillation. It enhances cardiac performance and exercise capacity, relieves atrial fibrillation-associated symptoms, attenuates systemic inflammation and myocardial fibrosis, and lowers the risk of readmission. The underlying therapeutic mechanism may be attributed to the inhibition of NLRP3/Caspase-1 inflammasome activation, reduced secretion of pro-inflammatory cytokines including IL-1 β , TNF- α and IL-6, and suppression of TGF- β 1-mediated fibrogenic responses. Collectively, this study validates the efficacy of ZGC Granules for HFpEF with concomitant atrial fibrillation through complementary clinical and in vitro evidence. More importantly, it offers an innovative strategy for exploring traditional Chinese medicine therapies for complex cardiovascular diseases via the inflammation-fibrosis pathological axis.

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Disclosure of conflict of interest

None.

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