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Britanin alleviates myocardial damage in rats with acute myocardial infarction by inhibiting the JAK2/STAT3 signaling pathway

Yiqing Zhang [1, 2#], Xian Li [1#], Hezi Jiang [1], Guoxing Zhang [3*], Yafeng Zhou [1*]

[1] Department of Cardiology, The Fourth Affiliated Hospital of Soochow University, Medical Center of Soochow University, Suzhou Dushu Lake Hospital, Suzhou City, Jiangsu Province, P. R. China
[2] Department of Cardiology, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou City, Jiangsu Province, P. R. China
[3] Department of Physiology and Neurosciences, Medical College of Soochow University, Suzhou City, Jiangsu Province, P. R. China

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ABSTRACT

Objective(s): Acute myocardial infarction (AMI) is a life-threatening condition featuring coronary occlusion-induced myocardial ischemia-hypoxia, followed by tissue damage driven by calcium overload, inflammation, and pyroptosis. Current therapies offer limited myocardial protection. Britanin, a sesquiterpene lactone from Inula linearifolia, has anti-inflammatory and antioxidant properties, while its role in AMI remains unclear. This study explored Britanin's therapeutic effects on rat AMI and underlying mechanisms, focusing on the JAK2/STAT3 signaling pathway.

Materials and Methods: Network pharmacology, molecular docking, and ProTox3.0 toxicity prediction were used for target screening and safety evaluation. Rat AMI models were established by left anterior descending ligation and treated with Britanin (5/10 mg/kg). Echocardiography, serum biochemistry, histopathological staining, Western blotting, and qPCR were used to assess cardiac function, myocardial injury, fibrosis, and molecular changes.

Results: Pharmacology identified 30 overlapping targets, including JAK2, and highlighted JAK2/STAT3 as a key pathway. Britanin bound JAK2 strongly (binding energy <-8.2 kcal/mol); its LD_{50} was 150 mg/kg, indicating safety. Britanin improved cardiac function indices, alleviated myocardial fibrosis, reduced serum injury markers, and down-regulated components of the JAK2/STAT3 pathway, the NLRP3 (NOD-like receptor pyrin domain-containing 3) inflammasome, and pyroptosis markers (all $P<0.05$), without liver or kidney toxicity.

Conclusion: Britanin alleviates AMI-induced myocardial damage in rats by improving cardiac function, reducing injury and fibrosis, and inhibiting inflammation and pyroptosis through suppression of the JAK2/STAT3 pathway, positioning it as a potential AMI therapeutic agent.

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Introduction

Acute myocardial infarction (AMI) remains a leading cause of mortality and morbidity worldwide, with rising incidence in middle-aged and older adults and a worrying upward trend in younger individuals (1, 2). Risk factors include chronic diseases (obesity, diabetes, hypertension) and unhealthy lifestyles (smoking, irregular sleep)(3). Despite advances in reperfusion therapy and standard pharmacologic management, 5-year mortality from post-AMI myocardial remodeling remains as high as 30% (4, 5), largely attributable to unresolved inflammation and pyroptosis-mediated myocardial injury (6). Existing therapeutic agents fail to effectively target the inflammatory-pyroptotic cascade, highlighting an urgent unmet clinical need for novel interventions that can mitigate these pathological processes.

Inflammation plays a pivotal role in AMI progression, from the initial ischemic phase to post-infarction remodeling. The NLRP3 (NOD-like receptor pyrin domain-containing 3) inflammasome, a key mediator of inflammatory responses, activates Caspase-1, inducing pyroptosis and releasing pro-inflammatory cytokines (IL-1β, IL-18)(7, 8). The JAK2/STAT3 pathway, a critical signaling cascade in inflammation, regulates NLRP3 inflammasome activation by mediating mitochondrial translocation of NLRP3 (6, 9). Inhibiting these pathways has emerged as a promising strategy for AMI treatment (10, 11).

Britanin, a natural sesquiterpene lactone isolated from *Inula linearifolia*, has demonstrated robust anti-inflammatory and cytoprotective properties in preclinical

**Corresponding authors:* Guoxing Zhang. Department of Physiology and Neurosciences, Medical College of Soochow University, Suzhou City, Suzhou 215123, China. Tel: +86-15895587582, Fax: +86-51265880127, Email: zhangguoxing@suda.edu.cn; Yafeng Zhou. Department of Cardiology, The Fourth Affiliated Hospital of Soochow University, Medical Center of Soochow University, Suzhou Dushu Lake Hospital, Institute for Hypertension of Soochow University, Suzhou 215000, China. Tel: +86-13862593866, Fax: +86-512-67505200, Email: dryafengzhou@163.com
# These authors contributed equally to this work

studies (12, 13). It alleviates cerebral ischemia-reperfusion injury by activating the Nrf2 antioxidant pathway (14) and suppresses NLRP3 inflammasome activation in macrophages, thereby mitigating NLRP3-related diseases (13). Given its established efficacy in targeting inflammation and cell death pathways, Britanin holds potential for AMI treatment. However, its therapeutic effects on AMI and, critically, whether these effects are dependent on the JAK2/STAT3 signaling pathway (a key regulator of NLRP3 pyroptosis in AMI) remain unexplored.

This study integrated network pharmacology, molecular docking, and animal experiments to investigate the therapeutic effects of Britanin on AMI and elucidate the underlying mechanisms, with a focus on the JAK2/STAT3 pathway. The results provide new insights into the development of natural product-based therapies for AMI.

Materials and Methods

Materials

Britanin (purity >98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies against JAK2 (#ab108596), p-JAK2(#YP0155), STAT3 (#ab68153), p-STAT3(#YP0250), and GAPDH (#ab8245) were obtained from Abcam (Cambridge, UK). Antibodies against NLRP3 (#YM8024), IL-18 (#YM8085), IL-1β (#YM8498), Caspase1 (#YM8437), Cleaved-Caspase1 (Cle-Caspase-1, #YC0002), and GSDMD (#YM8489) were purchased from Immunoway (Plano, TX, USA). HRP-conjugated secondary antibody (#FDR007) was supplied by Hangzhou Fude Biotechnology (Hangzhou, China). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA, USA). The CCK-8 kit was purchased from Beyotime (Shanghai, China). The SYBR GREEN PCR kit and reverse transcription kit were from Vazyme (Nanjing, China).

Animals

Male Sprague-Dawley rats (200-220 g) were purchased from Sibeifu (Beijing) Biotechnology Co., Ltd. Rats were housed in a specific pathogen-free (SPF) facility at 17-25 ˚C with a 12 hr light/dark cycle and 50-60% humidity, with free access to food and water. All experimental procedures were approved by the Ethics Committee of Soochow University (Ethics No.: 202503A0154) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Network pharmacology analysis

Target prediction

The molecular structure of Britanin (PubChem CID: 5281713) was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Potential targets of Britanin were predicted using Swiss Target Prediction (http://www.swisstargetprediction.ch/) with a probability score >0.1.

AMI-related targets were collected from Genecards (https://www.genecards.org/), OMIM (https://omim.org/), DisGeNet (https://www.disgenet.org/), and PharmGKB (https://www.pharmgkb.org/) databases using "acute myocardial infarction" as the keyword. Duplicate targets were removed using Excel.

PPI network construction and enrichment analysis

Overlapping targets between Britanin and AMI were identified using the Bioinformatics online platform (https://www.bioinformatics.com.cn/) to generate Venn diagrams. The protein-protein interaction (PPI) network was constructed using STRING (https://string-db.org/) with a confidence score > 0.7 and visualized using Cytoscape 3.9.0. Hub targets were screened using Degree and Betweenness algorithms.

Gene Ontology (GO) terms (biological process, cellular component, molecular function) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using Metascape (https://metascape.org/) with $P<0.05$ and false discovery rate (FDR) <0.1 as cut-off criteria.

Molecular docking

The 3D structures of hub targets (EGFR: 5CNO, ABCB1: 8Y6I, PTGS2: 5F19, CREBBP: 4NYX, JAK2: 8BA3) were downloaded from the RCSB Protein Data Bank (RCSB PDB, https://www.rcsb.org/). The structure of Britanin was converted to PDBQT format using AutoDockTools 1.5.7. Water molecules and endogenous ligands were removed from target proteins, and hydrogen atoms were added. Grid boxes were placed around the targets' active sites. Docking simulations were performed using AutoDock Vina, and binding energies were calculated. PyMOL 2.5 was used to visualize the binding modes.

Toxicity prediction

The potential toxicity of Britanin was predicted using ProTox3.0 (https://tox.charite.de/). The median lethal dose (LD_{50}) was calculated to assess acute toxicity.

AMI model establishment and drug treatment

Rats were randomly divided into four groups (n=10 for the control group, n=6 for the other groups): (1) Control group: Sham operation (no coronary ligation)+intraperitoneal injection of normal saline+co-solvents (polyethylene glycol+Tween 80); (2) Surgical group: AMI induction+injection of normal saline+co-solvents; (3) Low-dose Britanin group: AMI induction+injection of Britanin (5 mg/kg); (4) High-dose Britanin group: AMI induction+injection of Britanin (10 mg/kg). The sample size (n=6 per group) was determined using G*Power 3.1 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) with the following parameters: α (type I error)=0.05, 1-β (statistical power)=0.8, and effect size f=0.5, which is consistent with the effect size of similar cardiovascular pharmacology studies. This sample size was sufficient to detect meaningful differences in key outcome measures (e.g., cardiac function indices, protein expression levels) between groups.

AMI was induced by ligating the left anterior descending coronary artery with a 6/0 suture under 2-3% isoflurane anesthesia. On post-operative day 2, serum myocardial enzymes (LDH, CK-MB, TNI) were detected to confirm successful modeling. Drug administration was performed daily for 7 days starting from post-operative day 1.

Echocardiography

On post-operative day 8, rats were anesthetized with 1-2% isoflurane. Echocardiography was performed using a Vevo 2100 ultrasound system (VisualSonics, Toronto, Canada) equipped with a 30 MHz probe. Parameters including left ventricular internal dimension at end-diastole (LVIDd), left ventricular internal dimension at end-systole (LVIDs), left ventricular end-diastolic volume (LVEDV), left ventricular

end-systolic volume (LVESV), ejection fraction (EF), and left ventricular fractional shortening (LVFS) were measured.

Serum biochemical analysis

Blood samples were collected from the jugular vein and centrifuged at 3500 rpm for 15 min to obtain serum. An automatic biochemical analyzer (Hitachi 7600, Tokyo, Japan) was used to detect serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea (UREA), creatinine (CREA), total cholesterol (TC), low-density lipoprotein (LDL), LDH, CK-MB, and TNI.

Histopathological analysis

Rats were sacrificed, and hearts were perfused with normal saline and fixed in 4% paraformaldehyde. Tissues were embedded in paraffin, sectioned at 5 μm, and stained with Masson-Brückner trichrome and Sirius red. Stained sections were examined under a light microscope (Leica DM4000B, Wetzlar, Germany). The collagen volume fraction (CVF) was calculated using Image-Pro Plus 6.0.

Western blotting

Myocardial tissues were homogenized in RIPA lysis buffer containing protease inhibitors. Protein concentration was determined using a BCA kit. Equal amounts of protein (50 μg) were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked with 5% non-fat milk for 1 hr, then incubated with primary antibodies (1:1000 dilution) at 4 ˚C overnight. After washing, membranes were incubated with HRP-conjugated secondary antibodies (1:5000 dilution) for 2 hr at room temperature. Bands were detected using an ECL kit (Millipore, Billerica, MA, USA) and visualized with a ChemiScope 6000 imaging system (Shanghai Qinxiang, China). GAPDH was used as an internal control.

qPCR analysis

Total RNA was extracted from myocardial tissues using TRIzol reagent. RNA concentration and purity were determined using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was synthesized using a reverse transcription kit. qPCR was performed using a SYBR GREEN PCR kit on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). The reaction conditions were as follows: 95 ˚C for 30 sec, followed by 40 cycles of 95 ˚C for 10 sec and 60 ˚C for 30 sec. Relative mRNA expression was calculated using the $2^{-}\Delta\Delta Ct$ method, with GAPDH as an internal control. Primer sequences are listed in Table 1.

Statistical analysis

Data are presented as the mean±standard deviation (SD). Statistical analyses were performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Prior to multiple group comparisons, Levene's test was conducted to verify the homogeneity of variances. If variances were homogeneous, one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was used to compare differences among groups. If variances were heterogeneous, Welch's ANOVA with Dunnett's T3 correction was applied to avoid Type I error. Comparisons between two independent groups were analyzed using the independent samples t-test. A two-tailed *P*-value<0.05 was considered statistically significant.

Results

Identification of overlapping targets and PPI network

A total of 97 potential targets of Britanin and 1473 AMI-related targets were identified. Thirty overlapping targets were obtained, including JAK2, PTGS2, and MAPK1. The PPI network of overlapping targets contained 30 nodes and 74 edges (Figure 1B and 1C). Hub targets screened

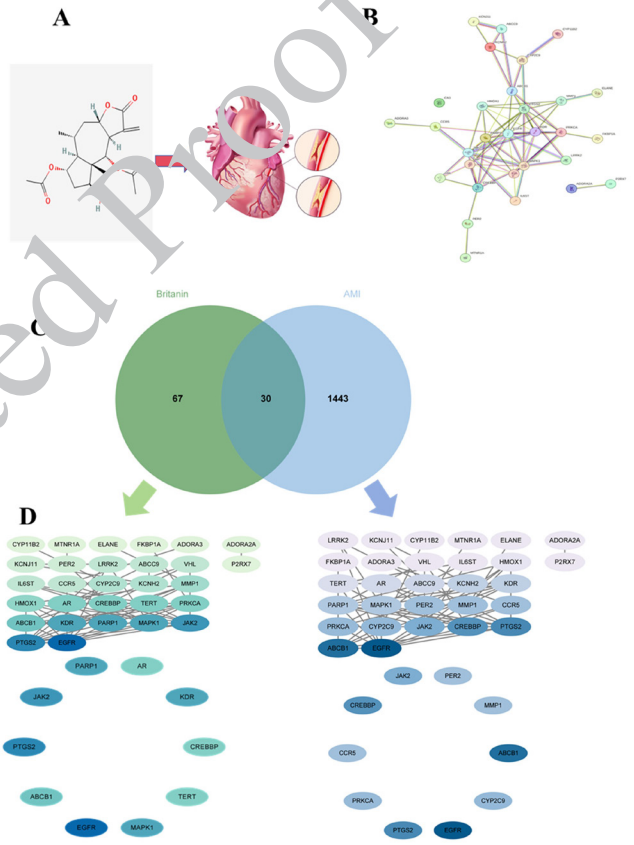

Figure 1. A) Molecular structure of Britanin. B) Protein-protein interaction (PPI) network diagram. C) Venn diagram of predicted targets of Britanin and targets of acute myocardial infarction (AMI). D) Top 10 targets obtained by the degree algorithm using Cytoscape software

Table 1. Primer list of target genes used for RT-qPCR

Gene	Forward primer	Reverse primer
Interleukin-18 (IL-18)	5-TCAGCTCTTCTACCAGCAAACA-3	5-CACTTCCAACTGAGAGGCTGT-3
Interleukin-1β (IL-1β)	5-GACTTCACCATGGAACCCGT-3	5-GGAGACTGCCCATTCTCGAC-3
Janus Kinase 2 (JAK2)	5-AGCCTTGTCATTTGTGTCGTTAAT-3	5-ACGGCAAAGGTCAGGAAGTATTT-3
Signal Transducer and Activator of Transcription 3 (STAT3)	5-TGTCAGATCACATGGGCTAAGTT-3	5-TGAAACCCATGATGTACCCTTCA-3
Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)	5-CTCAGTTGCTGAGGAGTCCC-3	5-ATTCGAGAGAAGGGAGGGCT-3

by Degree and Betweenness algorithms included EGFR, ABCB1, PTGS2, CREBBP, and JAK2 (Figure 1D).

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis

GO analysis showed that overlapping targets were enriched in biological processes (BP) such as response to inorganic substances and metal ions, and regulation of systemic processes; cellular components (CC) including membrane rafts and plasma membrane protein complexes; and molecular functions (MF) such as heme binding and tetrapyrrole binding (Figure 2A).

KEGG analysis indicated that overlapping targets were mainly enriched in the JAK2/STAT3, PI3K/AKT, HIF-1α, and lipid metabolism pathways (Figure 2B). These results suggested that the JAK2/STAT3 pathway might be a key pathway involved in the therapeutic effects of Britanin in AMI.

Molecular docking results

Molecular docking results showed that Britanin had strong binding affinity with EGFR (-8.5 kcal/mol), PTGS2 (-7.9 kcal/mol), CREBBP (-7.6 kcal/mol), and JAK2 (-8.2 kcal/mol), with binding energies <-1.2 kcal/mol. However, the binding energy between Britanin and ABCB1 was -0.8 kcal/mol, indicating weak affinity (Figure 3). These results confirmed that Britanin could form stable complexes with key targets except ABCB1.

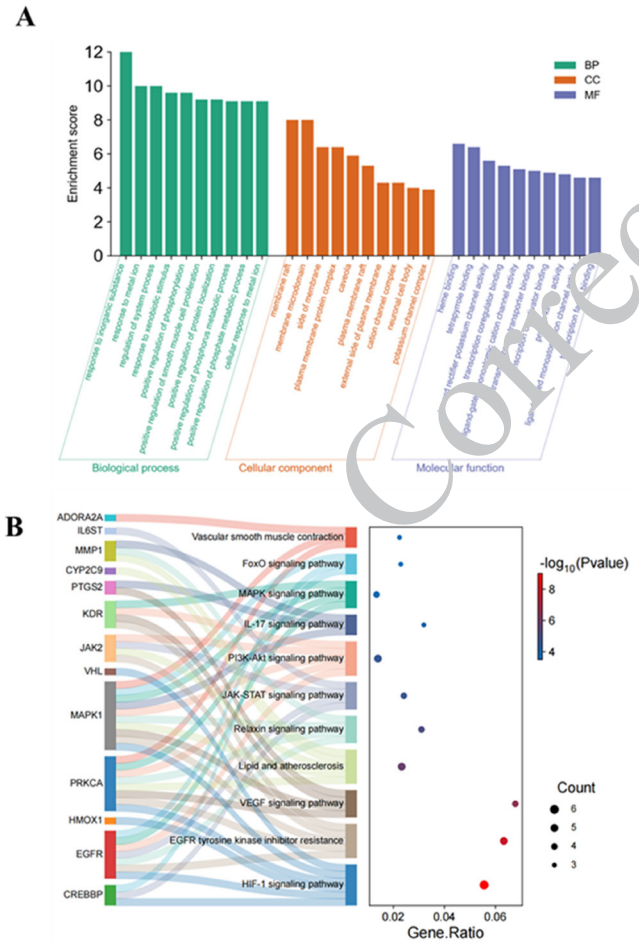

Figure 2. Enrichment analysis results and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway association visualization
A) Bar plot showing the results of enrichment analysis, where the length of each bar represents the enrichment degree and the color indicates the statistical significance. B) Sankey diagram of KEGG pathway analysis, illustrating the hierarchical association between enriched items and corresponding KEGG pathways, with the width of the flow lines reflecting the number of related genes or molecules

Toxicity prediction of Britanin

ProTox3.0 prediction showed that the LD_{50} of Britanin was 150 mg/kg, which is classified as "moderately toxic" (LD_{50}: 50-300 mg/kg) according to the toxicity classification standard (Figure 4). This indicated that Britanin was safe at the doses used in this study (5 mg/kg and 10 mg/kg).

Britanin improves cardiac function in AMI rats

Echocardiography results showed there were no marked differences in EF, LVFS, LVIDd, LVIDs, LVEDV, and LVESV

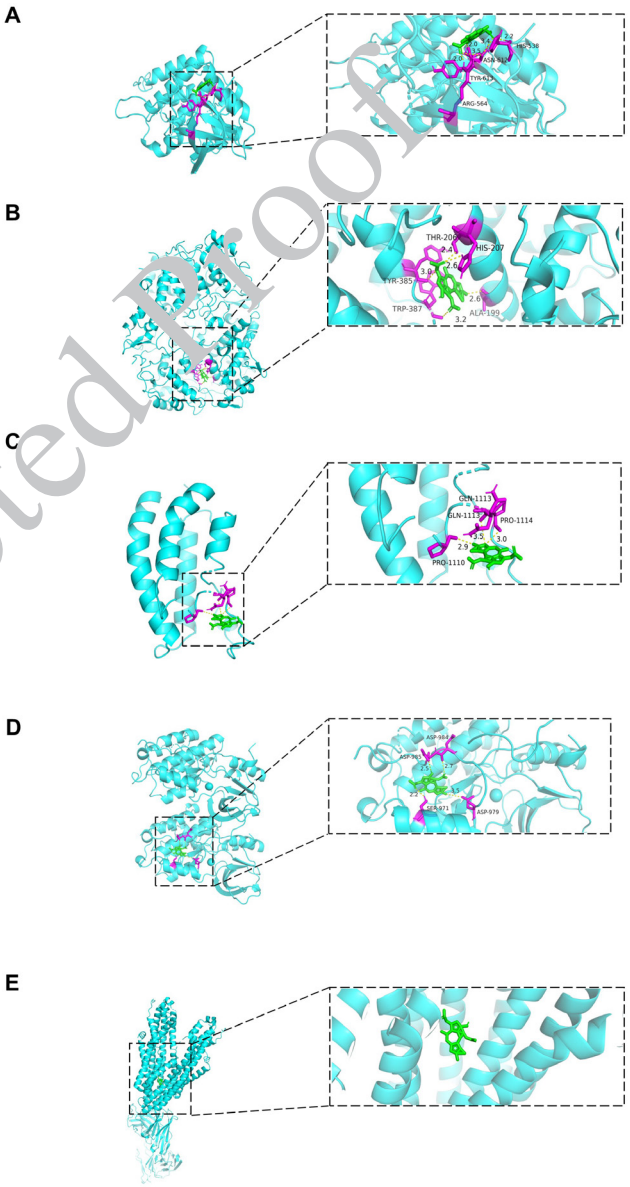

Figure 3. A) Macroscopic and microscopic three-dimensional molecular docking models of Britanin and JAK2 (8BA3). B) Macroscopic and microscopic three-dimensional molecular docking models of Britanin and PTGS2 (5F19). C) Macroscopic and microscopic three-dimensional molecular docking models of Britanin and CREBBP (4NYX). D) Macroscopic and microscopic three-dimensional molecular docking models of Britanin and EGFR (5CNO). E) Macroscopic and microscopic three-dimensional molecular docking display of Britanin and ABCB1 (8Y6I) without hydrogen bond formation

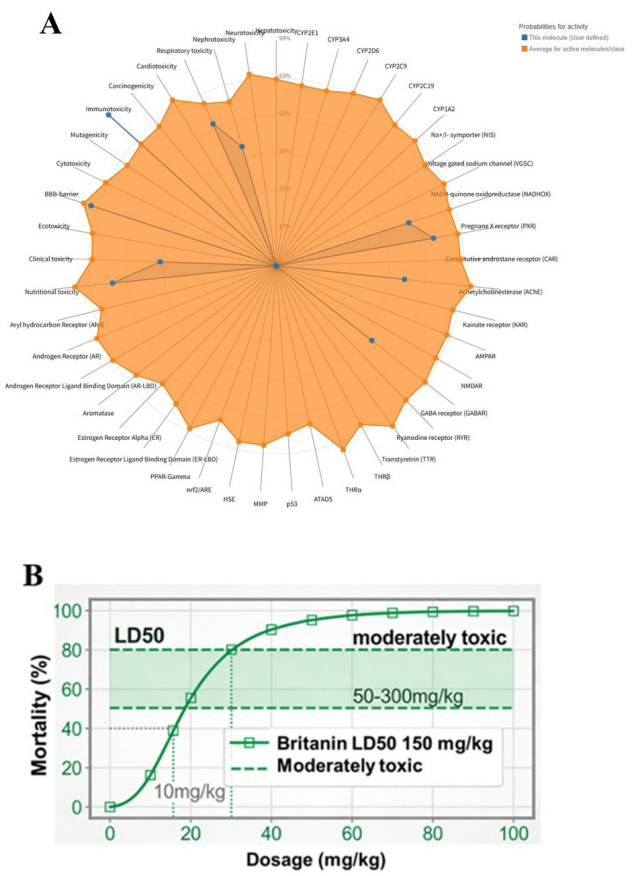

Figure 4. A). Britanin toxicity radar chart is designed to quickly illustrate the confidence of positive toxicity results compared to the class average. B) ProTox3.0-predicted median lethal dose (LD_{50}) curve of Britanin and its toxicity classification. The LD_{50} curve illustrates the relationship between Britanin dosage and the corresponding mortality rate in the prediction model. The predicted median lethal dose (LD_{50}) of Britanin wa determined to be 150 mg/kg, which lies within the range of 50–300 mg kg and is therefore classified as moderately toxic. The vertical dashed li s mark the experimental doses (5 mg/kg and 10 mg/kg) used in this study, which are significantly lower than the LD_{50} value, indicating good safe y of Britanin at the applied doses

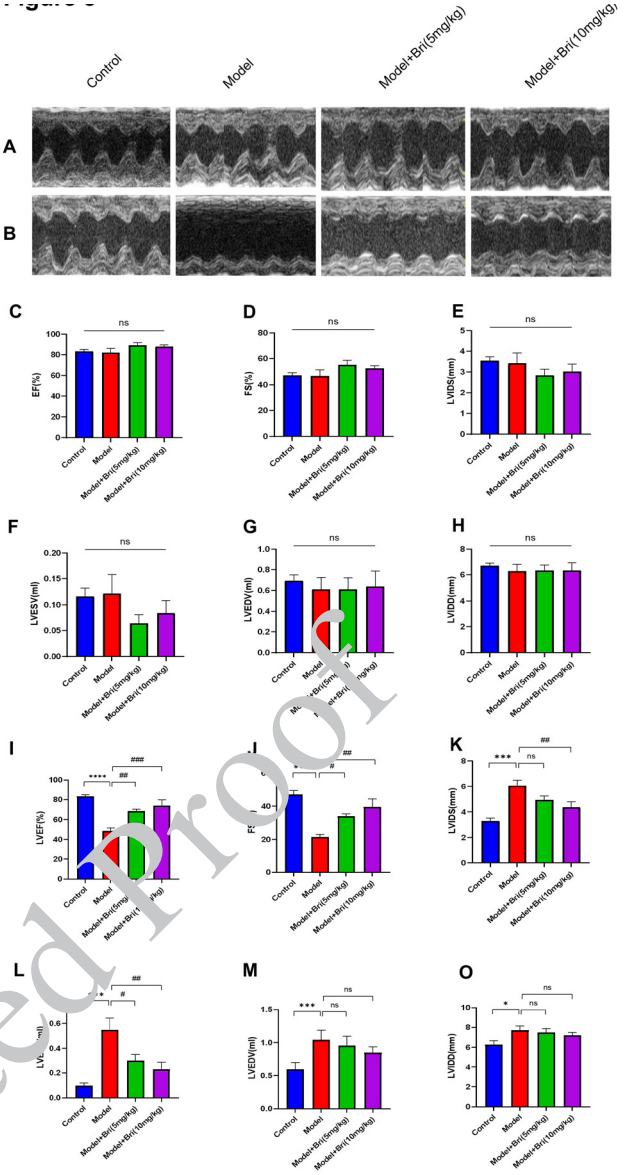

Figure 5. A) Echocardiographic images of rats in each group before surgery. B) Echocardiographic images of rats in each group on the 8th day after surgery. C-H) Statistical analysis of echocardiographic parameters of ejection fraction (EF), left ventricular fractional shortening (LVFS), left ventricular internal dimension at end-systole (LVIDS), left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV), left ventricular internal dimension at end-diastole (LVIDD) in each group before surgery. I-O) Statistical analysis of echocardiographic parameters of EF, LVFS, LVIDS, LVESV, LVEDV, LVIDD in each group on the 14th day after surgery

N=6 in each group, $^{*}P<0.05$ vs Control, $^{***}P<0.001$ vs Control, $^{****}P<0.0001$ vs Control, $^{\#}P<0.05$ vs Model, $^{\#\#}P<0.01$ vs Model, $^{\#\#\#}P<0.001$ vs Model

before surgery (Figure 5C-H). On the 8th day after surgery (7 days after continuous drug administration), the NLRP3 surgical group had significantly decreased EF and LVFS, and increased LVIDd, LVIDs, LVEDV, and LVESV compared with the control group (Figure 5I-O). After treatment with Britanin, EF and LVFS were significantly increased, and LVESV and LVIDs were significantly decreased compared with the surgical group. There was no significant difference in LVIDd and LVEDV between the surgical group and Britanin-treated groups. These results indicated that Britanin improved cardiac function in AMI rats.

Britanin reduces myocardial infarct size and fibrosis

TTC staining showed that the surgical group had a large white infarcted area, while Britanin treatment significantly reduced the infarct size (Figure 6A-C). Masson staining revealed disorganized myocardial fibers, necrotic cells, and increased collagen deposition in the surgical group. Britanin treatment reduced collagen deposition and improved myocardial tissue integrity ($P<0.05$)(Figure 6D-E). Sirius red staining showed that the surgical group had increased red-stained collagen fibers, while Britanin treatment significantly reduced the collagen volume fraction (Figure 6F). These results indicated that Britanin reduced myocardial infarct size and fibrosis in AMI rats.

Britanin regulates serum biochemical indicators

Compared with the control group, the surgical group had significantly increased serum levels of LDH, CK-MB, and TNI (Figure 7A-C). Britanin treatment significantly reduced myocardial enzyme levels, with a greater effect in the high-dose group.

There was no significant difference in serum AST, ALT, UREA, and CREA levels between the surgical group and Britanin-treated groups (Figure 7D-I). For blood lipids,

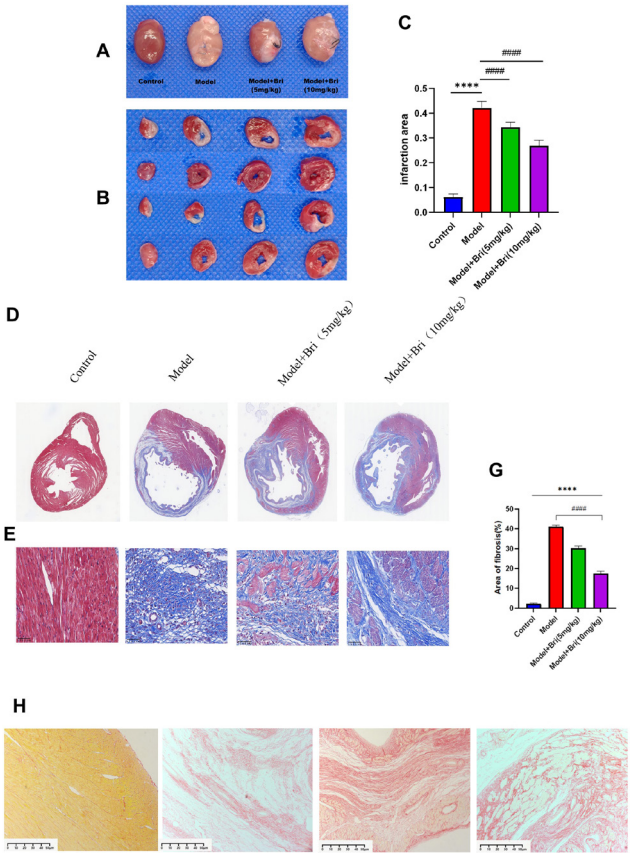

Figure 6. A) Representative cardiac images of rats in each group. B) Triphenyltetrazolium Chloride (TTC) staining of myocardial infarction among groups. C) Statistical analysis of TTC staining; D) Masson staining results of cardiac tissues of rats in each group. E) Proportion of fibrotic area in Masson staining of cardiac tissues of rats in each group. F) Statistical analysis of Masson staining. G) Sirius Red staining of cardiac tissues of rats in each group
N=6 in each group, $^{****}P<0.0001$ vs Control, $^{####}P<0.0001$ vs Model

TC levels in Britanin-treated groups differed from those in the control group, but there was no significant difference between Britanin-treated groups and the surgical group (Figure 7J, M). TG levels showed no significant difference in serum between the surgical group and Britanin-treated groups (Figure 7K, O). LDL levels in the surgical group and Britanin-treated groups were significantly lower than the control group. Still, there was no significant difference between Britanin-treated groups and the surgical group (Figure 7L, P). These results indicated that Britanin had no adverse effects on liver and kidney function and reduced myocardial injury.

Britanin inhibits the JAK2/STAT3 signaling pathway

Western blotting results showed that compared with the control group, the surgical group had significantly increased protein expression of JAK2, STAT3, NLRP3, IL-1β, IL-18, Caspase-1, and Cle-Caspase-1 (Figure 8A-G). Britanin treatment significantly down-regulated these protein levels, with a more significant effect in the high-dose group.

qPCR results showed that, compared with the control group, the surgical group had significantly higher mRNA expression of JAK2, STAT3, IL-1β, and IL-18 (Figure 8H-K). Britanin treatment significantly down-regulated these mRNA levels. These results indicated that Britanin inhibited the JAK2/STAT3 signaling pathway and reduced inflammation in AMI rats.

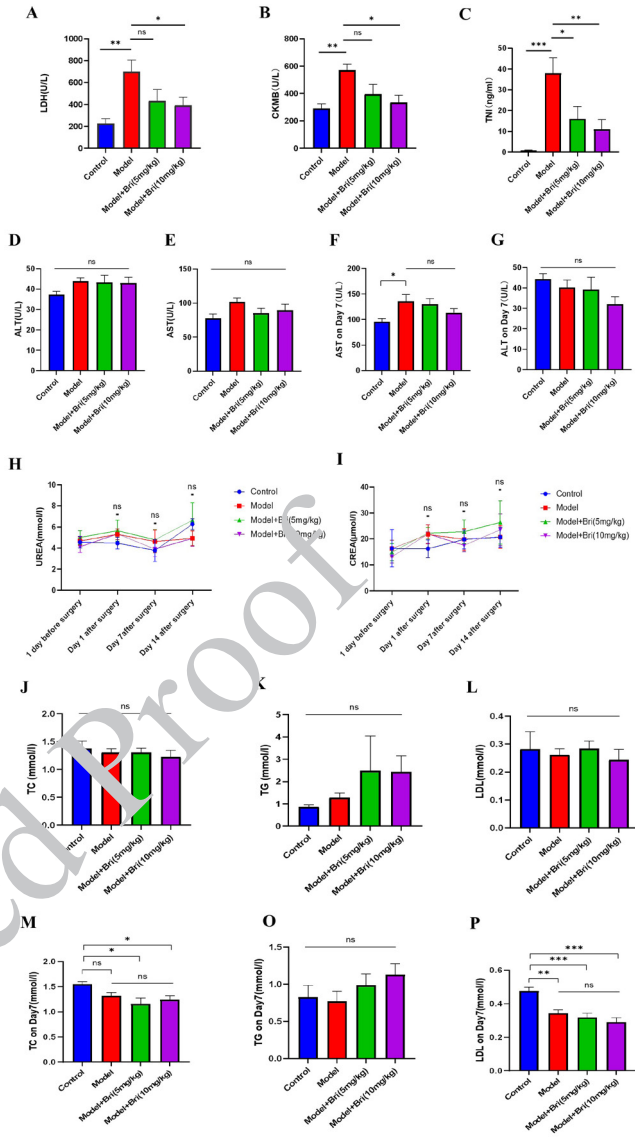

Figure 7. Effects of Britanin on serum myocardial enzyme levels, liver and kidney function indices, and blood lipid profiles in rat experimental groups (A-C) Comparison of serum myocardial enzyme levels among groups: Compared with the control group, the surgical group showed a significant increase in serum levels of lactate dehydrogenase (LDH) (A), creatine kinase - myocardial band (CK - MB) (B), and cardiac troponin I (TNI) (C). After Britanin treatment, the levels of these three myocardial enzymes decreased significantly, and the high-dose Britanin group exhibited a more pronounced inhibitory effect on the elevation of myocardial enzyme levels. (D-I) Comparison of serum liver and kidney function indices among groups: There were no significant differences in serum levels of aspartate transaminase (AST) (D, E), alanine transaminase (ALT) (F, G), urea (UREA) (H), and creatinine (CREA) (I) between the surgical group and the Britanin - treated groups. (J-P) Comparison of serum blood lipid levels among groups: Regarding total cholesterol (TC) (J, M), the levels in the Britanin-treated groups differed from those in the control group, but there was no significant difference between the Britanin-treated groups and the surgical group. For triglycerides (TG) (K, O), no significant difference was observed in serum levels between the surgical group and the Britanin-treated groups. In terms of low-density lipoprotein (LDL) (L, P), serum levels in the surgical and Britanin-treated groups were significantly lower than in the control group. At the same time, there was no significant difference between the Britanin-treated groups and the surgical group.
N=6 in each group, $^{*}P<0.05$ vs Control, $^{**}P<0.01$ vs Control, $^{***}P<0.001$ vs Control

Britanin suppresses myocardial NLRP3

Western blotting results showed that, compared with the control group, the surgical group had significantly increased expression of Caspase-1, Cle-Caspase1, and GSDMD (Figure 9A-E). Britanin treatment significantly down-regulated these protein levels, with a more significant effect in the high-dose group. These results indicated that Britanin suppressed myocardial pyroptosis in AMI rats.

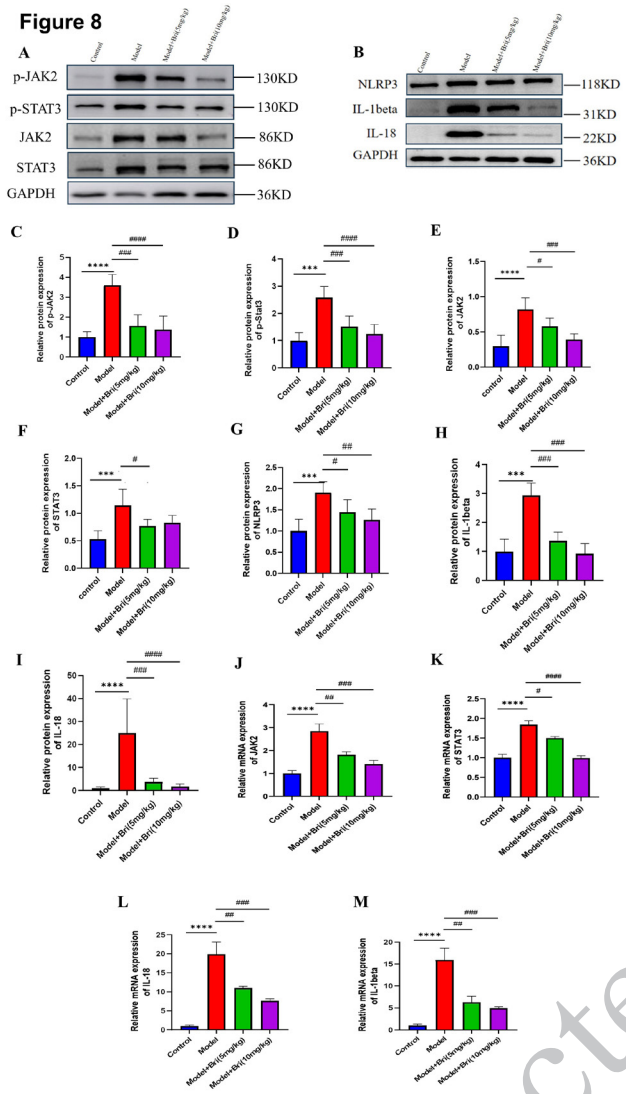

Figure 8. Effects of Britanin on rat protein expression levels of Janus kinase 2/ signal transducer and activator of transcription 3 (JAK2/STAT3) signaling pathway-related proteins and NOD-Like receptor pyrin domain-containing 3 (NLRP3) inflammasome-associated cytokines detected by Western blotting

(A) Representative Western blotting images of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3). B) Quantitative analysis of JAK2. C) Quantitative analysis of STAT3. (D) Representative Western blotting images of NLRP3, IL-1β, and IL-18. E) Quantitative analysis of NLRP3. F) Quantitative analysis of IL-1β. G) Quantitative analysis of IL-18. An internal reference protein, GAPDH, was used to normalize protein loading. (H-K) Quantitative real-time polymerase chain reaction (qPCR) detection results of target gene mRNA expression: Compared with the control group, the surgical group (AMI model group) showed a significant increase in the mRNA expression levels of Janus kinase 2 (JAK2, H), signal transducer and activator of transcription 3 (STAT3, I), interleukin-18 (IL-18, J), and interleukin-1β (IL-1β, K). N=6 in each group, $^{***}P$<0.001 vs Control, $^{****}P$<0.0001 vs Control, $^{\#}P$<0.05 vs Model, $^{\#\#}P$<0.01 vs Model, $^{\#\#\#}P$<0.001 vs Model. $^{\#\#\#\#}P$<0.0001 vs Model

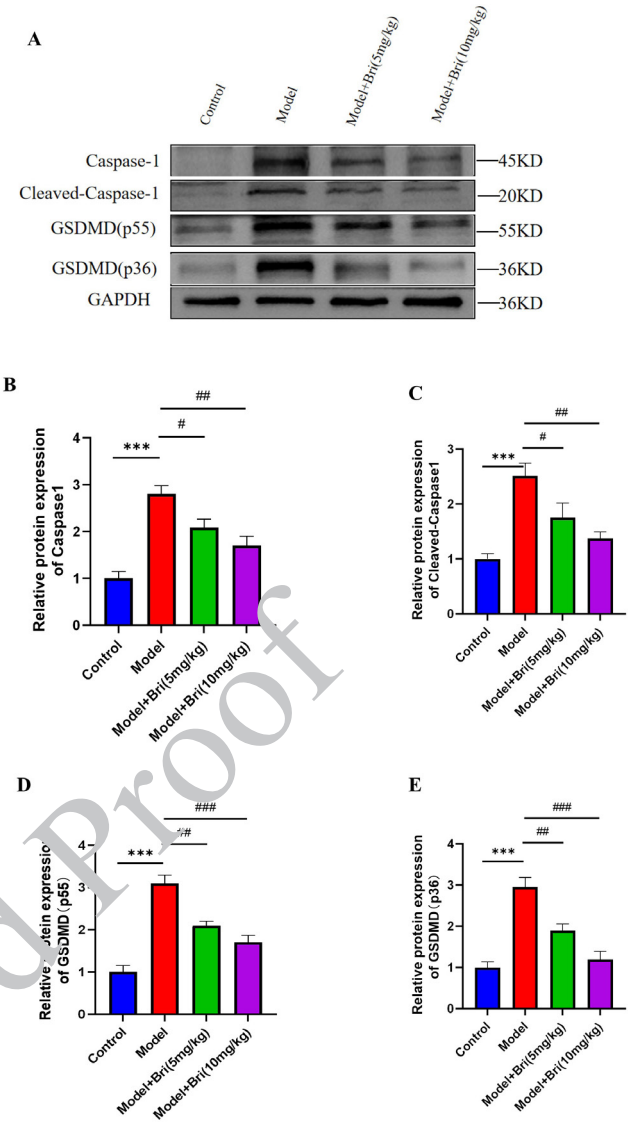

Figure 9. Effects of Britanin on the protein expression levels of myocardial pyroptosis-related molecules in acute myocardial infarction (AMI) rats

(A) Representative Western blotting images of Caspase-1, Cle-Caspase1, Gasdermin D (GSDMD) (p55, p36). B) Quantitative analysis of Caspase-1. C) Quantitative analysis of Cleaved-Caspase1. (D) Quantitative analysis of GSDMD (p55). E) Quantitative analysis of GSDMD (p36). An internal reference protein, GAPDH, was used to normalize protein loading. N=6 in each group, $^{***}P$<0.001 vs Control, $^{\#}P$<0.05 vs Model, $^{\#\#}P$<0.01 vs Model, $^{\#\#\#}P$<0.001 vs Model

Discussion

This study systematically investigated the therapeutic effects of Britanin on AMI in rats and its underlying mechanisms using network pharmacology, molecular docking, and *in vivo* experiments. The results demonstrated that Britanin improved cardiac function, reduced myocardial infarct size and fibrosis, inhibited inflammation, and suppressed pyroptosis, all of which were associated with inhibition of the JAK2/STAT3 signaling pathway.

AMI is characterized by myocardial ischemia-hypoxia and subsequent inflammatory responses, which contribute to myocardial damage and adverse remodeling (15-17). The JAK2/STAT3 pathway, a key regulator of inflammation, is activated in AMI and promotes the expression of pro-inflammatory cytokines and the assembly of the NLRP3 inflammasome (6, 9). In this study, network pharmacology and KEGG analysis identified the JAK2/STAT3 pathway as a key candidate for Britanin's therapeutic effects. Molecular docking confirmed that Britanin had strong binding affinity with JAK2, further supporting the JAK2/STAT3 pathway as a target of Britanin.

Echocardiography results showed that Britanin improved EF and LVFS and reduced LVESV and LVIDs, indicating improved systolic cardiac function. However, there was no significant change in LVIDd and LVEDV, possibly due to the short treatment duration (7 days). Myocardial cells are terminally differentiated and cannot proliferate, so myocardial repair relies on fibroblast proliferation and collagen deposition, which are slower processes (18-21).

Histopathological analysis showed that Britanin reduced myocardial infarct size and fibrosis. Excessive fibrosis after AMI impairs cardiac function, while moderate fibrosis maintains cardiac structure (20-22). Britanin reduced collagen deposition and improved myocardial tissue integrity, suggesting a protective role in myocardial remodeling.

Serum biochemical analysis showed that Britanin reduced the levels of LDH, CK-MB, and TNI, which are specific markers of myocardial injury (10, 23-25). This indicated that Britanin mitigated myocardial damage. Additionally, Britanin had no adverse effects on liver and kidney function, confirming its good safety profile, consistent with the LD_{50} prediction (150 mg/kg).

Inflammation and pyroptosis are key drivers of AMI progression (26, 27). The NLRP3 inflammasome activates Caspase-1, which cleaves GSDMD, leading to pyroptosis and the release of IL-1β and IL-18 (7, 28). The JAK2/STAT3 pathway regulates NLRP3 inflammasome activation by mediating mitochondrial translocation of NLRP3 (6). In this study, Britanin down-regulated the expression of JAK2, STAT3, NLRP3, IL-1β, and IL-18, and reduced the levels of Caspase1, Cleaved-Caspase1, and GSDMD. These results indicated that Britanin inhibited the JAK2/STAT3 pathway, thereby suppressing NLRP3 inflammasome activation and pyroptosis, and alleviating inflammation.

Previous studies have reported that natural products such as artemisinin and baicalein alleviate AMI by inhibiting the NLRP3 inflammasome (29, 30). This study extends these findings by demonstrating that Britanin exerts its therapeutic effects through the JAK2/STAT3-NLRP3-pyroptosis axis, thereby providing a new mechanism for natural product-based AMI treatment.

Limitations of this study include the lack of *in vitro* experiments to confirm the direct effect of Britanin on the JAK2/STAT3 pathway and the short follow-up period for evaluating long-term effects. Future studies will focus on *in vitro* validation and long-term outcome assessment.

Conclusion

Britanin alleviates AMI-induced myocardial damage in rats by improving cardiac function, reducing infarct size and fibrosis, inhibiting inflammation, and suppressing pyroptosis. The underlying mechanism involves the inhibition of the JAK2/STAT3 signaling pathway. These findings support Britanin as a potential therapeutic agent for AMI.

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Authors' Contributions

Y Z and X L: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original Draft, Visualization. These authors contributed equally to this work and share first authorship. H J: Investigation, Resources, Data Curation. G Zh: Conceptualization, Supervision, Funding Acquisition, Writing – Review & Editing, Project Administration. Y Zh: Conceptualization, Methodology, Supervision, Writing–Review & Editing, Project Administration, Funding Acquisition, Corresponding author. All authors have read and agreed to the published version of the manuscript, and confirm that the order of authorship accurately reflects the relative contributions of each individual to the work.

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration

We have not used any AI tools or technologies to prepare this manuscript.

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