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Research article

Metabolomics analysis reveals the effects of *Salvia Miltiorrhiza* Bunge extract on ameliorating acute myocardial ischemia in rats induced by isoproterenol

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A R T I C L E I N F O

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A B S T R A C T

Salvia miltiorrhiza Bunge (SM) is a widespread herbal therapy for myocardial ischemia (MI). Nevertheless, the therapeutic signaling networks of SM extract on MI is yet unknown. Emerging evidences suggested that alterations in cardiac metabolite influences host metabolism and accelerates MI progression. Herein, we employed an isoproterenol (ISO)-induced acute myocardial ischemia (AMI) rat model to confirm the pharmacological effects of SM extract (0.8, 0.9, 1.8 g/kg/day) via assessment of the histopathological alterations that occur within the heart tissue and associated cytokines; we also examined the underlying SM extract-mediated signaling networks using untargeted metabolomics. The results indicated that 25 compounds with a relative content higher than 1 % in SM aqueous extract were identified using LC-MS/MS analysis, which included salvianolic acid B, lithospermic acid, salvianolic acid A, and caffeic acid as main components. An *in vivo* experiment showed that pretreatment with SM extract attenuated ISO-induced myocardial injury, shown as decreased myocardial ischemic size, transformed electrocardiographic, histopathological, and serum biochemical aberrations, reduced levels of proinflammatory cytokines, inhibited oxidative stress (OS), and reversed the trepidations of the cardiac tissue metabolic profiles. Metabolomics analysis shows that the levels of 24 differential metabolites (DMs) approached the same value as controls after SM extract therapy, which were primarily involved in histidine; alanine, aspartate, and glutamate; glycerophospholipid; and glycine, serine, and threonine metabolisms through metabolic pathway analysis. Correlation analysis demonstrated that the levels of modulatory effects of SM extract on the inflammation and OS were related to alterations in endogenous metabolites. Overall, SM extract demonstrated significant

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cardioprotective effects in an ISO-induced AMI rat model, alleviating myocardial injury, inflammation and oxidative stress, with metabolomics analysis indicating potential therapeutic pathways for myocardial ischemia.

1. Introduction

Cardiovascular diseases (CVD) remain the principal concern of morbidity and mortality globally, of which about 50 % were caused by ischemic heart disease (IHD) [1–3]. Acute myocardial ischemia (AMI) is among the cardinal pathological features of IHD and is characterized by an acute reduction in blood flow and cardiac oxygen supply, causing an oxygen demand imbalance between the coronary blood and myocardium, which ultimately cause cardiomyocyte apoptosis, cardiac metabolic disorder, myocardial infarction, heart failure, angina pectoris, and even death [4,5]. Both experimental and clinical investigations suggested that, during ischemia myocardial, oxidative stress (OS) and inflammation critically modulate AMI pathogenesis. Over stimulation with isoproterenol (ISO), a synthetic on selective β-adrenergic agonist was demonstrated to promote experimental CVDs, namely, AMI, hypertrophy and infarction, cardiac fibrosis, and heart failure, leading to the oxidative injury of cell membranes, lipid peroxidation, proinflammatory cytokines overproduced, and calcium overload, thereby causing extensive myocardium damage [6,7]. At present, there are multiple proposed AMI treatments, and pharmaceutical intervention is the primary therapy of choice [8]. Medications involving β-blockers, nitrate preparations, antiplatelet drug, statins, and calcium antagonists have been proved to be prevent and alleviate AMI in correlational studies, but they either have some disadvantages or need in-depth study of the mechanisms [9,10]. For this reasons, herbal therapy received increasing attention in IHDs research as safe and effective therapeutic administration [11].

The dried root of *Salvia miltiorrhiza* Bunge (SM), known as Danshen, belongs to genus Salvia of Lamiaceae family and being commonly used for the IHD as a primary therapy in China [12–14]. Studies have demonstrated that SM and its extracts, with widespread pharmacological activities, namely, antioxidant, anti-inflammatory, antiplatelet accumulation, vasorelaxation, free oxygen radicals elimination, antithrombotic, and anti-atherosclerosis, among others [15–18]. The chemical constituents of SM are mainly contained liposoluble tanshinones and water-soluble phenolics, flavonoids, and triterpenoids [19–23]. Recently, it is suggested that salvianolic acid A, and salvianolic acid B possess a strong protective effect against myocardial injury [24–26]. Numerous studies on the beneficial effects of SM mainly focus on lipophilic components, such as tanshinone IIA [27,28]. In contrast, there are limited reports on the cardioprotective influences of water-soluble components against ISO induced AMI from the perspective of metabolomics.

Untargeted metabolomics extensively examines alterations in endogenous metabolites *in vivo* and specific differential bioindicators associated with disease and medicinal therapy, thereby providing a clear understanding of the correlation between drug efficacy, pharmacological activities, and metabolic axes [29,30]. In recent year, an increasing number of studies of metabolomics was used to examine cardioprotective influences of herbal extracts, which contributes to the enhanced elucidation of physiological networks and identification of new bioindicators. Cardiac metabolic aberrations are typically present in most CVDs as the energy supply can have multiple sources, namely, carbohydrates, lipids, lactate, and amino acids [31]. Additionally, dysregulation of cardiac metabolism potentially affects systemic networks, thus, commencing a brutal cycle that encourages CVDs pathogenesis [32]. Therefore, cardiac metabolites from AMI rat SM extract are proposed to offer cardiac protection. Herein, we employed electrocardiography, myocardial ischemic size, histopathological and biochemical evaluations to assess SM extract efficacy in ISO-treated AMI rats. We also employed untargeted metabolomics-based cardiac tissue metabolomics to evaluate the cardioprotective influences of varying SM extract dosages on ISO-treated AMI rats.

2. Materials and methods

2.1. Materials

SM was harvested from Runde Pharmaceutical Co., Ltd. (Anguo, Hebei, China) and received authentication by Professor Genna Ba, School of Mongolian Medicine, Inner Mongolia Minzu University, Tongliao, China. Isoprenaline and 2 % 2,3,5-triphenyl tetrazolium chloride (TTC) solution was acquired from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Circulating alanine transaminase (ALT) and aspartate aminotransferase (AST) were obtained from Shenzhen Icubio Biotechnology Co., Ltd. (Shenzhen, China). ELISA kits were ecquired from Jiangsu Jingmei Biotechnology Co., Ltd. (Jingmei, China) for cytokine content measurement. Hematoxylin and eosin (H&E) stain was obtained from Nanjing Jiancheng Technology Co., Ltd. (Nanjing, China).

2.2. Preparation of SM extract

Primarily, SM was macerated 24h (material-liquid ratio: 1:10) and then it was extracted using distilled water 3 times for 2 h each time in a reflux extractor at 80 °C. Afterwards, the combined extracted solution was filtered with filter paper, concentrated under spray dried and kept at desiccator. Eventually, the yield of extraction was approximately 14 %. In addition, the dried of SM extract was dissolved in 0.5 % CMC-Na for experiment.

2.3. Pharmaceutical Composition analysis

2.3.1. Sample preparation

A stock extract was prepared by resuspending 100 mg SM extract (5:95) in 2 ml of (2:1:1) water, methanol, and acetonitrile (H2O: MeOH: ACN) mixture. Subsequently, the sample underwent 10 min vortex and ultra-sonication at 40 kHz. 20 μl of SM extract was re-diluted with 1000 μl H2O: MeOH: ACN, prior to a 10 min centrifugation at 12,000 rpm, and administration of 2 μl of 1 μg/ml. To increase experimental confidence, 10 μL of 100 μg/mL internal standard (IS) was added and tested through 0.22 μm PTFE filter.

2.3.2. Instrument and acquisition

Phytochemicals were separated with a Zorbax Eclipse C18 column (1.8μm × 2.1 × 100 mm, 5 μm), and LC-MS/MS (Thermo, Ultimate 3000LC, Q Exactive HF) associated with Q-Exactive HF mass spectrometer was employed for analysis. The column and autosampler temperature were maintained at 30 °C and 4 °C, also the flow rate at 0.3 μL/min 2. Mobile phase harbored solvent A (0.1 % formic acid in water) and solvent B (acetonitrile), and employed a gradient elution of 0–5% B at 0–2min, 5–30 % B at 2–6min, 30 % B at 6–7min, 30–78 % B at 7–12min, 78 % B at 12–14min, 78–95 % B at 14–17min, 95 % B at 17–20min, 5 % B at 20–21min, 5 % B at 21–25min. For each mode, the MS conditions were as follows: electrospray voltage, 3.5 kV; capillary temperature, 330 °C; heater temperature, 325 °C; sheath, auxiliary, and purge gas flow rates were 45 arbs, 15 arbs, and 1 arb, respectively; S-Lens RF Leve,55 %; high energy collision dissociation. Mass spectrometry employed a Full Scan (M/Z 100–1500) and data-reliant MS Scan mode (DD-MS2, TopN = 10).

2.3.3. Data processing

Compound Discoverer 3.1 was utilized to correct retention duration, identify and extract peak, and so on. Thermo mzCloud online and Thermo mzValut local databases were employed to recognize these substances, depending on the acquisition mode.

2.4. Effect of SM extract against ISO-induced AMI in rats

2.4.1. Animal protocol

Sixty healthy male Sprague-Dawley rats, weighing 180g–200g were acquired from Changsheng Biotechnology Co., Ltd. (Liaoning, China), and maintained in specific pathogen-free, 12h light/dark cycle, as well as temperature (20–25 °C) and relative humidity (30–40 %) regulated environment with *ad libitum* food and tap water. Our animal protocols received ethical approval from the Inner Mongolian Minzu University (approval No. NM-LL-2021-06-15-1), which followed the guidelines of the care and usage of laboratory Animals.

Following acclimatization for one week, the rats were randomly divided into six groups with ten animals per group. The control group animals received 0.5 % CMC-Na solution orally for 28 days and intraperitoneally (i.p.) injected saline solution for two consecutive days with a gap of 24h on day 27th and 28th; AMI group rats received 0.5 % CMC-Na solution orally for 28 days and i. p. injected ISO at 4 mg/kg concentration on day 27th and 28th with a gap of 24h; AMI + SM treated rats were orally received SM extract at 0.18, 0.9 and 1.8 g/kg doses respectively and i. p. injected ISO at 4 mg/kg concentration for two consecutive days (with a gap of 24h) on day 27th and 28th; and AMI + propranolol group rats were orally received propranolol at 30 mg/kg/d for 28 days and i. p. injected ISO (4 mg/kg/d) for two consecutive days (with a gap of 24h) on day 27th and 28th. Myocardial ischemia was confirmed by elevated levels of serum CK, CK-MB, LDH and AST.

2.4.2. Electrocardiographic assay

For Electrocardiographic evaluation, rats received 50 mg/kg pentobarbital sodium (Sigma-Aldrich, CAS No.: 57-33-0) anesthesia injection. Electrocardiograph (ECG) was recorded 24 h past the last ISO administration. Following anesthesia induction, green, black, and red subcutaneous needle electrodes were attached to the left upper limb, left lower limb, and right lower limb of the rat, correspondingly, and the twelve-lead ECG recordings were consecutively for a minimum of 5min collected with BL-420S (Chengdu Techman Software Co., Ltd) biological signal acquisition and processing system. Respectively record the ECG parameters (heart rate and RR intervals), with the following ECG apparatus calibration: 1 mV/1 cm and 50 mm/s. Furthermore, R–R served as the adjusted heart rate (HR = 60,000/RR).

2.4.3. Sample acquisition and storage

Following ECG recording, all the rats were executed to subsequently collected the blood sample from the abdominal aorta and quickly isolated the heart, liver, spleen, lung, kidney, then blotted dry before weighing. Serum was settled at room temperature (RT) for 30 min, then centrifuged for 10min at 3000 rpm, 4 °C. Whereafter, a small portion of the fresh heart tissues specimens were immediately extracted and cut into small pieces on ice, homogenizing with precooled PBS buffer (ratio at 1:9) at 3500 rpm for 15min to obtain supernatant, which used for the estimation of biological markers. The serum and tissue samples were maintained at −80 °C for additional biological assay.

2.4.4. Biochemical assessment

The circulating level of aspirate transaminase (AST) was determined using an automatic analyzer Applied Biosystem (Ichem-340, Icubio, Shenzhen, China) with commercial kits following kit directions. Cardiac injury was detected via evaluating the serum concentrations of creatine kinase (CK), creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH) via a commercial standard enzyme

Table 1
Characterization of the SM Extract chemical components, as evidenced by LC-MS/MS.

No.	Compound	Molecular Formula	Adduct Ion	RT (min)	Theoretical Value	Test Value	Compound Class	Relative Content (%)	PubChem ID
1	Salvianolic acid B	$C_{36}H_{30}O_{16}$	$[M+H]^-$	7.75	718.1534	718.1539	2-arylbenzofuran flavonoids	12.40 %	13991589
2	4,4'-[Methyleneis(4,1-phenyleneimino)] bis (1-amino −9,10-dioxo-9,10-dihydro-2-anthracenesulfonic acid)	$C_{41}H_{28}N_4O_{10}S_2$	$[M+H]^-$	6.43	800.1247	800.1266	Others	6.01 %	117646
3	Vanillyl mandelic acid	$C_9H_{10}O_5$	$[M+H]^-$	3.06	198.0528	198.0525	Phenols	5.94 %	1245
4	D- (+)-Proline	$C_5H_9NO_2$	$[M+H]^+$	0.82	115.0633	115.0636	Carboxylic acids and derivatives	5.09 %	8988
5	2-[2,7-Bis(2-carboxyethyl)-6-hydroxy-3-oxo-3H-xanthen-9-yl] terephthalic acid	$C_{27}H_{20}O_{11}$	$[M+H]^+$	7.75	520.1006	520.1011	Others	5.03 %	4241719
6	N-({4'-[(3-Butyl-1-{2-chloro-5-[(cyclopropylcarbonyl) amino] phenyl}-5-oxo-1,5-dihydro-4H-1,2,4-triazol-4-yl) methyl]-2-biphenylyl} sulfonyl)-2-chlorobenzamide	$C_{36}H_{33}Cl_2N_5O_5S$	$[M+H]^-$	7.74	717.1580	717.1547	Others	4.15 %	10439848
7	3-(2-{2-[(3-Bromophenyl) sulfanyl] ethoxy}-3-methoxy-5-[(2S,5S)-5-(3,4,5-trimethoxyphenyl) tetrahydro-2-furanyl] phenyl)-1-butyl-1-hydroxyurea	$C_{33}H_{41}BrN_2O_8S$	$[M+H]^+$	0.77	704.1767	704.1781	Others	3.42 %	10508960
8	L-Pyroglutamic acid	$C_5H_7NO_3$	$[M+H]^+$	1.17	129.0426	129.0428	Carboxylic acids and derivatives	3.37 %	7405
9	Lithospermic acid	$C_{27}H_{22}O_{12}$	$[M+H]^+$	7.75	538.1111	538.1116	2-arylbenzofuran flavonoids	2.92 %	6441498
10	Alpha-D-Glucopyranosyl-(1->4)-alpha-D-glucopyranosyl-(1->4)-alpha-D-glucopyranosyl- (1->4)-D-glucopyranose	$C_{24}H_{42}O_{21}$	$[M+H]^-$	0.77	666.2219	666.2223	Organooxygen compounds	2.68 %	446495
11	Aspulvinone E	$C_{17}H_{12}O_5$	$[M+H]^+$	8.31	296.0685	296.0685	Phenols	2.48 %	54675753
12	N-[3,5-Bis(4-morpholinylcarbonyl) phenyl]-2-chloro-4,5-difluorobenzamide	$C_{23}H_{22}ClF_2N_3O_5$	$[M+H]^-$	8.29	493.1216	493.1237	Benzene and substituted derivatives	2.44 %	2974209
13	N-(4-Hydroxy-3-nitrophenyl)-2-[(1-methyl-2,4,6-trioxohexahydro-5-pyrimidinyl) carbonyl] hydrazinecarboxamide	$C_{13}H_{12}N_6O_8$	$[M+H]^+$	0.81	380.0717	380.0722	Others	1.98 %	134141824
14	Caffeic acid	$C_9H_8O_4$	$[M+H]^+$	7.48	180.0423	180.0423	Cinnamic acids and derivatives	1.94 %	689043
15	Salvianolic acid A	$C_{26}H_{22}O_{10}$	$[M+H]^-$	7.49	494.1213	494.1217	Stilbenes	1.76 %	5281793
16	Choline	$C_5H_{13}NO$	$[M+H]^+$	0.81	103.0997	103.1002	Organonitrogen compounds	1.70 %	305
17	Methyl 2-(4-morpholinyl)-5-({2-(trifluoromethyl)-5-[4-(trifluoromethyl) phenyl]-3-furoyl} amino) benzoate	$C_{25}H_{20}F_6N_2O_5$	$[M+H]^+$	0.79	542.1276	542.1254	Others	1.65 %	2810078
18	6-C-xylosylluteolin	$C_{20}H_{18}O_{10}$	$[M+H]^-$	7.06	418.0900	418.0902	Flavonoids	1.59 %	44257908
19	2,3,4,5,6-Pentafluoro-N-{[1-(pentafluorobenzoyl)-4- piperidinyl] methyl} benzamide	$C_{20}H_{12}F_{10}N_2O_2$	$[M+H]^+$	7.75	502.0739	502.0763	Others	1.43 %	2853690
20	(3aS,6R,6aS,9aR,10aS,10bR)-2-[3,5-Bis(trifluoromethyl) phenyl-6-(5-chloro-2-hydroxyphenyl)-8-(3-chlorophenyl) -6a-phenyl-3a,4,6,6a,9a,10,10a,10b-octahydroisoindolo [5,6-e] isoindole-1,3,7,9 (2H,8H)-tetrone	$C_{40}H_{26}Cl_2F_6N_2O_5$	$[M+H]^-$	6.96	798.1123	798.1123	Others	1.42 %	6651724
21	5-(Acetylamino)-2,6-anhydro-3,5-dideoxy-L-glycero-L-altro-non-2-enonic acid	$C_{11}H_{17}NO_8$	$[M+H]^+$	0.87	291.1094	291.0953	Others	1.34 %	5329058
22	N, N'-Bis[(E)-(6-nitro-1,3-benzodioxol-5-yl) methylene] biphenyl-4,4'-diamine	$C_{28}H_{18}N_4O_8$	$[M+H]^-$	7.49	538.1125	538.1110	Others	1.31 %	2245403
23	N-[3-chloro-4-(4-morpholinyl) phenyl]-1,1-dioxo-1,2-benzothiazol-3-amine	$C_{17}H_{16}ClN_3O_3S$	$[M+H]^-$	5.84	377.0601	377.0607	Oxazinanes	1.15 %	2972816
24	4',5-Bisdeoxy-dothistromin	$C_{18}H_{12}O_7$	$[M+H]^+$	7.51	340.0583	340.0580	Anthracenes	1.13 %	137628450
25	2-[2-[(2,3-Difluorophenyl) methylsulfanyl]-4- oxoquinolin-1-yl]-N-(2-imidazole-1-ylethyl)-N- [[4- [4-(trifluoromethyl) phenyl] phenyl] methyl] acetamide	$C_{37}H_{29}F_5N_4O_2S$	$[M+H]^+$	0.75	688.1931	688.1916	Benzene and substituted derivatives	1.07 %	10153533

kit (Jingmei Biotechnology Co., Ltd., Jiangsu, China). To assess OS indices, Catalase (CAT), superoxide glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities in heart tissue were determined via ELISA kits (Jingmei Biotechnology Co., Ltd., Jiangsu, China) following kit directions. In addition, corresponding inflammatory cytokines, the levels of tumor necrosis factor-α (TNF-α), Interleukin (IL)-1β, and interleukin (IL)-6 in heart tissue were determined via a commercial ELISA assay (Jingmei Biotechnology Co., Ltd., Jiangsu, China).

2.4.5. TTC staining

In this study, myocardial ischemia was determined by 2 % 2,3,5-triphenyltetrazolium chloride (TTC) staining. In short, rat hearts were rapidly sliced, four samples per group was subjected to 2 % TTC staining, the fresh heart sections were firstly immersed into 0–4°CPBS buffer, frozen at −20 °C for 30 min. Thereafter, the hearts were transversely sliced (thickness of 2 min) and dyed with 2 % TTC solution for 30min in constant temperature water bath at 37 °C, for fully dyeing to slightly shake the container every 5 min. Lastly, removing the hearts washed with PBS buffer, taking photos quickly and fixed by 4 % paraformaldehyde. The selected slices were quantified with Image-Pro Plus 6.0.

2.4.6. Histological examination

Immediately after sacrifice, the heart was excised out, rinsed with 0.9 % saline solution, prior to a ≥48 h fixation in 10 % formalin at RT, embedding in paraffin blocks, and slicing to form 5 μm sections, with subseuqent hematoxylin-eosin (H&E) staining followed established procedures. The histological changes were captured by a Laser Scanning Confocal Microscope.

2.5. Metabolomics analysis of heart samples

2.5.1. Heart sample preparation

In an EP tube, 25 mg of sample were mixed with 500 μL of extraction solution (methanol: acetonitrile: water = 2:2:1, with isotopically-labelled internal standard mixture), prior to a 4 min homogenization at 35 Hz, and 5 min sonication in ice-water bath, these processes were repeated thrice. Samples then underwent a 1 h incubation at −40 °C, before a 15 min centrifugation at 12000 rpm (RCF = 13800(× g),R = 8.6 cm) and 4 °C. The supernatant was further analyzed via transfer to a new glass vial. Quality control (QC) sample was generated by combining equal volumes of supernatants from all samples.

2.5.2. Metabolomics analysis

MS/MS spectra was obtained using the UHPLC system (Vanquish, Thermo Fisher Scientific) accompanied by a Waters BEH Amide column (2.1 mm × 50 mm, 1.7 μm) and Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo) on the information-dependent acquisition (IDA) mode using the acquisition software (Xcalibur, Thermo). The mobile phase harbored 25 mmol/L ammonium acetate and 25 ammonia hydroxide in water (pH = 9.75) (A) and acetonitrile (B), auto-sampler temperature was set to 4 °C, and 2 μL was administered. The ESI source conditions were as follows: sheath gas flow rate: 50 Arb, Aux gas flow rate: 15 Arb, capillary temperature: 350 °C, full MS resolution: 60000, MS/MS resolution: 30000, collision energy: 20/30/40 in NCE mode, and spray Voltage: 3 kV (positive) or −3 kV (negative), respectively.

2.5.3. Data preprocessing and biomarker identification

Raw information was transformed to the mzXML format with ProteoWizard, and processing was conducted using an XCMS-based in-house program established using R to detect peak, as well as extract, align, and integrate information. Subsequently, the in-house MS2 database (BiotreeDB) was employed to annotate metabolites. Next, orthogonal projections to latent structures-discriminant analysis (OPLS-DA) were conducted for identification of candidate bioindicators using the SIMCA software. The variable projection influence (VIP, in the PLS-DA model) criteria were as follows: >1 and $p < 0.05$ (from Student's t-tests between two groups) for the screening of differential metabolites (DMs). Lastly, metabolic network assessment was conducted via MetaboAnalyst 5.0 software.

2.6. Statistical analysis

Inter-group differences in physiological and ECG parameters are expressed as the mean ± standard deviation (X ± SD). One-way analysis of variance was used to compare multiple groups of data with a post-hoc test. Data analysis was conducted in GraphPad Prism version 8.0.2, and p value < 0.05 was set as the significance threshold.

3. Results

3.1. Identification of major SM extract constituents

In all, we identified 72 and 68 compounds in positive and negative ion modes, respectively, mainly including phenolic acids, flavonoids, prenol lipids, carboxylic acids and derivatives, benzene and substituted derivatives. Our results showed that 25 compounds out of the 140 with content higher than 1 %, of which salvianolic acid B, salvianolic acid A, lithospermic acid, and caffeic acid as the major compounds, representing approximatively 19 %. In addition, the names of 23 candidate compounds, retention time (RT), molecular ion, theoretical value, test value, molecular formula, compound class, relative content, and PubChem ID were listed in Table 1.

3.2. The SM extract-mediated modulation of body and organ weights

Following 28 days of SM exposure, the initial body weight showed no obvious changes between the experimental cohorts ($p > 0.005$), while the final weight in rats that received only ISO exhibited marked reduction, relative to controls ($p < 0.005$), and the body weight was visibly restored to that of SM extract and positive groups. Further, the heart weight of AMI rats was higher than that of control rats, indicating increase in size of the heart. Rats pretreated with SM extract (0.18 g/kg) showed a substantial decrease in the heart weight relative to the ISO cohort ($p < 0.05$), although it did not act in a dose dependent manner. SM extract (0.9, 1.8 g/kg) and propranolol pretreated also changed the heart weight of the animals, when compared with that of AMI group. Additionally, animals that were given SM extract and propranolol demonstrated significant ($p < 0.05$) improvement in their lung weight in comparison with their AMI group (Table 2).

3.3. Effect of SM extract on ECG parameters

Fig. 1A–B presents typical ECG record for all cohorts. At the end of the experiment, a remarkable increase in heart rate (HR) was evident in the AMI cohort ($p < 0.05$) while the RR interval substantially decreased, relative to the controls ($p < 0.05$). Oral administration of SM extract at 0.18, 0.9 and 1.8 g/kg effectively ($p < 0.05$) prevented the aforementioned alterations in ECG parameters. As a positive control, pretreatment with propranolol at 30 mg/kg exhibited the same beneficial outcomes ($p < 0.05$) on ECG parameters (Fig. 1C–D).

3.4. Effect on histopathological parameters

To assess histopathologic alterations, hearts from all groups were evaluated via TTC and H&E staining, respectively. From the results of TTC staining, an obvious myocardial ischemic in AMI group was observed, which were pale white colored. Nevertheless, pretreatment either with SM extract or propranolol marked improved the ischemic injury and SM extract exerted better improvement than propranolol, when compared with AMI group (Fig. 2A). Relative to controls, we observed a marked rise in myocardial ischemic size in the AMI cohort, accounting for an over 30 % ischemic area (Fig. 2B). As illustrated in Fig. 2C, the representative H&E-stained left ventricle tissue sections. The histological assessment of control rat tissues demonstrated a few multinucleated myofibers, but no myocardial necrosis. AMI group revealed myocardial cell necrosis, excess leukocyte invasion as well as cardiac myofibrillar separation with excess collagen accumulation. Nevertheless, SM extract significantly reduced inflammatory cells. Similarly, the propranolol cohort exhibited fewer myonecrosis, and immune cell invasion, relative to the AMI cohort. Pretreatment with SM extract and propranolol effectively attenuated ISO-induced myocardial pathological lesion in AMI rats, especially for the propranolol and SM-1.8 g/kg cohorts.

3.5. The SM extract-mediated modulation of circulating cardiac biochemical indicators

As depicted in Fig. 3, the circulating myocardial enzymes CK, CK-MB, LDH, and AST activities were strongly increased ($p < 0.05$) in the AMI verses control cohort. However, SM extract (0.18, 0.9 and 1.8 g/kg) and propranolol pretreatment showed a significant ($p < 0.05$) reduction in the activities of all serum diagnostic marker enzymes compared to AMI group, which means SM extract can maintain the membrane integrity and permeability, thereby limiting the leakage of these enzymes, indicating that SM extract dramatically improved ISO-induced cardiac injury.

3.6. Effect of SM extract on inflammatory injury and OS in the myocardial tissue

As shown in Fig. 4A–C, the inflammatory response induced by ISO, as indicated by the elevated amounts of TNF-α, IL-1β, and IL-6, together with the reduced activities of the enzymes, when compared to control group ($p < 0.05$). Pretreatment with SM extract (0.9 and 1.8 g/kg) and propranolol recovered myocardial inflammation induced by ISO. The CAT, GSH-Px, and SOD, OS-related enzymatic activities were substantially decreased ($p < 0.05$) in AMI cohort. As evident from Fig. 4C–F, relative to the AMI cohort, the abnormal indexes of myocardial OS restored to different degrees in each administration group, and SM-1.8 g/kg group displayed the best efficacy ($p < 0.05$). Thus, these results suggested that the cardioprotective activities of SM extract were intricately linked to its anti-

Table 2
Body weight and organ weight of the experimental groups.

Parameters	Control	AMI	SM-0.18	SM-0.9	SM-1.8	Propranolol
Initial BW (g)	386.60 ± 10.15	383.60 ± 7.14	369.00 ± 8.15	371.10 ± 14.89	370.40 ± 15.91	359.00 ± 6.35
Final BW (g)	391.50 ± 5.08	349.00 ± 7.64$^{\#\#}$	371.60 ± 6.46	371.80 ± 14.71	350.10 ± 13.21	363.30 ± 4.66
Heart (g)	0.286 ± 0.004	0.340 ± 0.013$^{\#\#}$	0.310 ± 0.005*	0.330 ± 0.008	0.334 ± 0.014	0.316 ± 0.010
Lung (g)	0.436 ± 0.010	0.495 ± 0.018$^{\#\#}$	0.424 ± 0.008**	0.447 ± 0.006*	0.429 ± 0.007**	0.394 ± 0.021**
Liver (g)	2.855 ± 0.069	2.899 ± 0.067	2.957 ± 0.064	2.918 ± 0.052	3.048 ± 0.064	2.992 ± 0.041
Spleen (g)	0.206 ± 0.005	0.175 ± 0.124	0.185 ± 0.002	0.188 ± 0.003	0.171 ± 0.013	0.174 ± 0.012
Kidney (g)	0.667 ± 0.009	0.675 ± 0.015	0.673 ± 0.017	0.694 ± 0.021	0.661 ± 0.022	0.693 ± 0.007

BW: Body weight; Values provided as mean ± SEM (n = 6). $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ versus controls; **$p < 0.01$, *$p < 0.05$ versus ISO cohort.

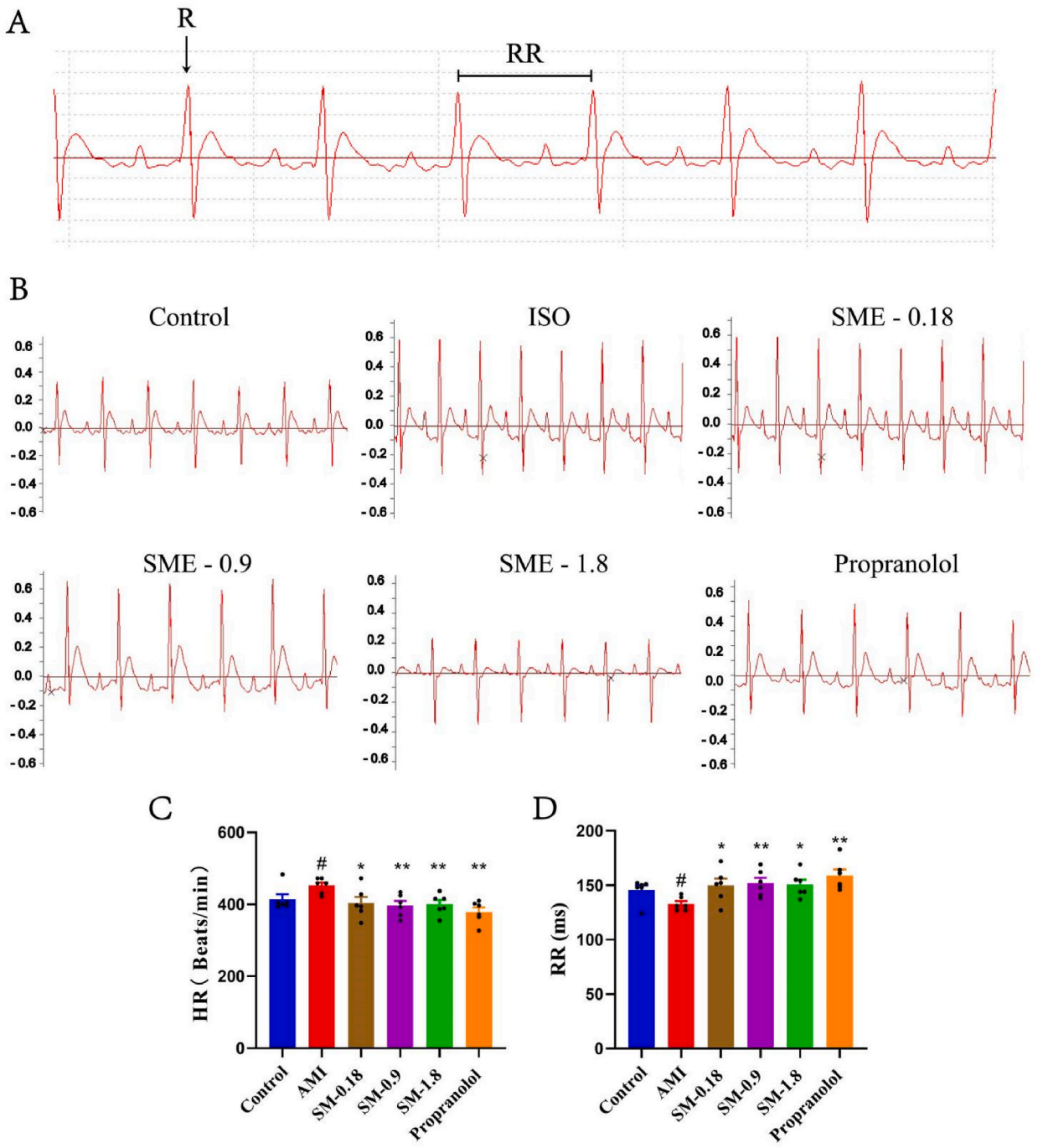

Fig. 1. Effects of SM extract on ISO-induced electrocardiographic changes. (A) Sample plot of RR intervals. (B) Representative lead II ECG records of rats in different groups. heart rate (C) and RR interval (D) changes in different groups. Values provided as mean ± SEM (n = 6). $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. controls; $^{**}p < 0.01$, $^{*}p < 0.05$ vs. AMI cohort.

inflammatory and anti-oxidative activities.

3.7. The SM extract-mediated regulation of myocardial tissue metabolic profiles

To explore the DMs between groups, an OPLS-DA method was employed to filtration and classification of metabolites. The OPLS-DA score plots showed significant metabolites were separated clearly between the control and AMI cohorts, as well as between the AMI and SM-1.8 cohorts (Figure A–B), indicating the endogenous metabolic profile in AMI rats changed significantly. As represented in the

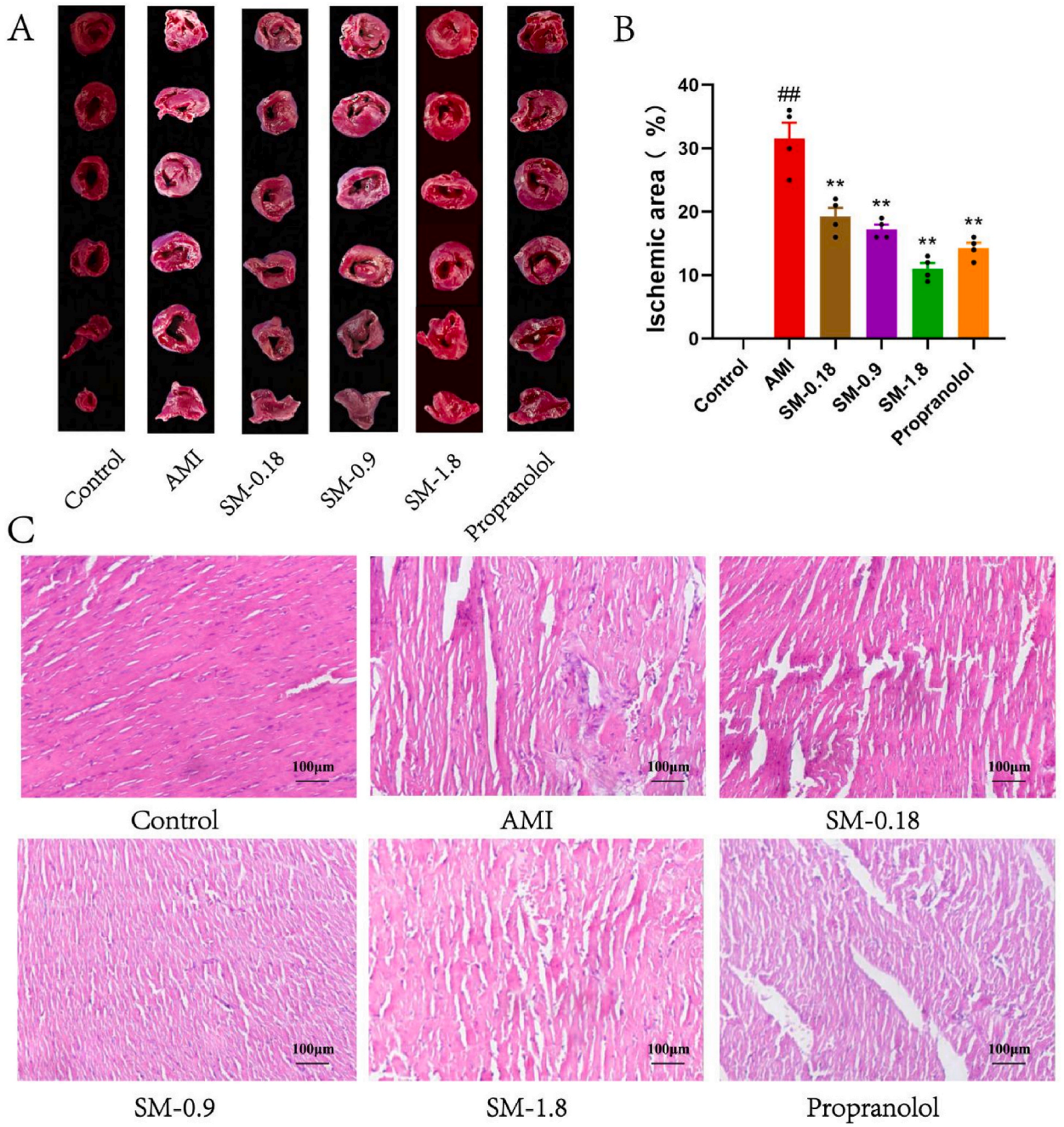

Fig. 2. Typical histopathological images of rat heart. (A) TTC staining of heart tissue. (B) TTC staining of the myocardial ischemic size. (C) H&E staining of heart sections (Enlargement: 10 × , Scale bar: 100 μm). Values presented as the mean ± SEM (n = 4). ##p < 0.01, #p < 0.05 vs. controls; **p < 0.01, *p < 0.05 vs. AMI cohort.

volcano plot (Fig. 5C), 102 DMs were found to be significantly expressed in the control and AMI groups, including 2 upregulated and 100 downregulated metabolites. After SM-1.8 g/kg administration, the levels of 47 metabolites were recovered of which 11 were upregulated and 36 were downregulated (Fig. 5D). According to VIP >1 and $p < 0.05$, we screened 140 DMs, of which 24 common DMs were identified among control, AMI, and SM-1.8 g/kg cohorts (Fig. 5E). Main significant DMs were shown in Fig. 5F–J, including histamine, L-aspartic acid (L-Asp), N-acetyl-L-Asp, 1-methyl-L-Histidine, and glycerylphosphorylethanolamine. As shown in Table 3, the levels of histamine, L-Asp, N-acetyl-L-Asp, 1-methyl-L-Histidine, glycerylphosphorylethanolamine, palmitoleoyl ethanolamide, 15-deoxy-d-12,14-PGJ2, sakacin P, safynol, mukonidine, carnosic acid, norclozapine, lysyl-proline, cinncassiol D3, prolyl-arginine, prolyl-aspartate, L-prolyl-L-proline, 5-L-glutamyl-taurine, PC(P-18:1(9Z)/16:1(9Z)), lysoPE(18:1(9Z)/0:0), 4-(2-aminophenyl)-2,4-

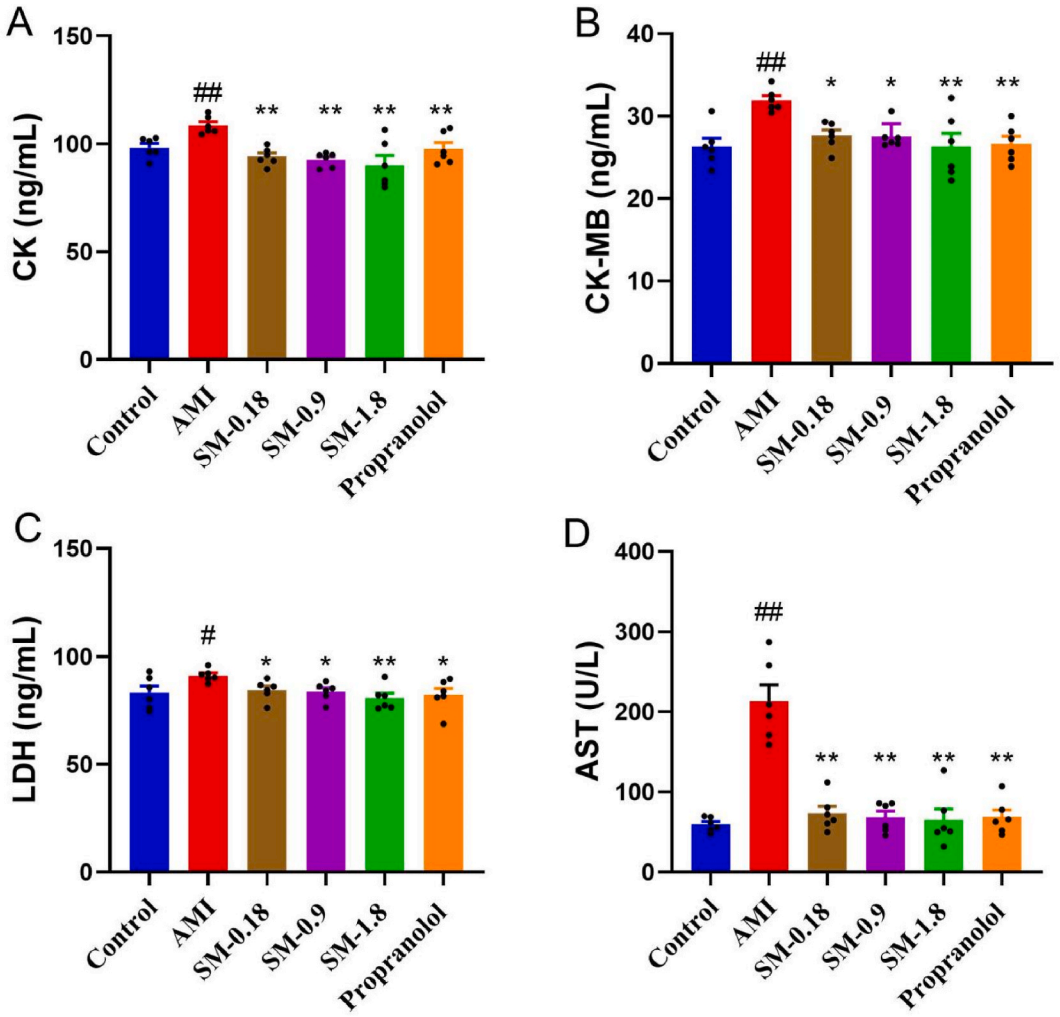

Fig. 3. Effects of SM extract on the serum level of myocardial enzymes. (A) CK, (B) CK-MB, (C) LDH, and (D) AST. Values provided as mean ± SEM (n = 6). $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. controls; $^{**}p < 0.01$, $^{*}p < 0.05$ vs. AMI cohort.

dioxobutanoic acid, 4-acetamido-2-amino-6-nitrotoluene, and 3-hydroxy-2-methylpyridine-4,5-dicarboxylate exhibited a marked rise in the AMI versus control cohort. In contrast, SM-1.8 g/kg pretreatment dramatically reduced the aforementioned metabolites within the heart tissue samples. Moreover, the level of leucyl-hydroxyproline was substantially reduced in the AMI versus control cohort. In contrast, SM-1.8 g/kg pretreatment obviously enhanced the level of this metabolites. Based on these findings, SM extract treatment reversed the contents of most metabolites to that of the control cohort.

3.8. The metabolic pathway analysis in cardiac tissue samples and correlation analysis

According to the identified metabolites, 102 DMs after ISO-induced AMI and 47 DMs after SM extract administration were used for metabolic pathway analysis. As shown in Fig. 6A–B, SM extract modulated numerous metabolic networks, especially those of histidine; alanine, aspartate, and glutamate; glycerophospholipid; and glycine, serine, and threonine metabolisms. Considering the above evidences, we generated an illustration of the altered metabolic networks (Fig. 6C), indicating that SM extract potentially modulates these networks to alleviate AMI. In Spearman's correlation analysis's results, the physiological data, OS, and inflammatory factors revealed strong associations with majority DMs (Fig. 7). For example, ischemic size and cardiac weight were strongly inhibited by the SM intervention and their changes positively correlated with 1-Methyl-L-Histidine. The secretion of OS cytokines was improved by SM and negatively correlated with L-Asp and N-Acetyl-L-Asp. In addition, serum CK-MB was significantly inhibited by the SM intervention and its changes were positively correlated with histamine and glycerylphosphorylethanolamine.

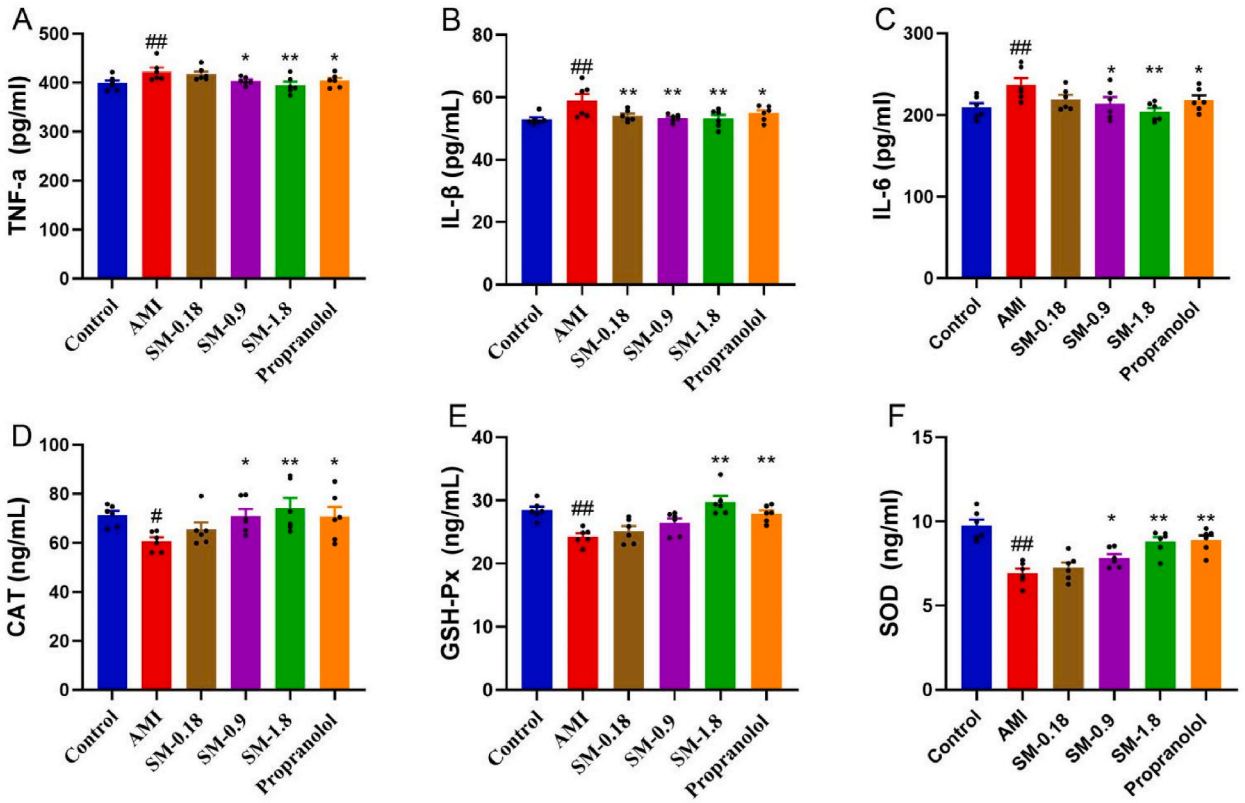

Fig. 4. Influences of SM extract on myocardial tissue biochemical indicators in AMI rats. (A) TNF-α, (B) IL-1β, (C) IL-6, (D) CAT, (E) GSH-Px, and (F) SOD. Values provided as mean ± SEM (n = 6). $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. controls; $^{**}p < 0.01$, $^{*}p < 0.05$ vs. AMI cohort.

4. Discussion

In the present study, we proved that differential SM extract dosages display excellent therapeutic effect on ISO-triggered AMI rat through the investigation of the ECG, histopathology, TTC staining, and clinical biochemical indicators. Specifically, we found 140 compounds, of which phenolic acids were recognized as main bioactive ingredients via LC-MS/MS analysis. Notably, SM extract pretreatment strongly diminished RR intervals prolongation, and elevation of heart rate in ECG. Our results also illustrated that SM could reduce the serum CK, CK-MB, LDH, and AST contents, and improved the histopathological injuries in AMI rats. Moreover, SM extract ameliorated ISO-induced AMI by enhancing antioxidant enzymes CAT, GSH-Px, and SOD activities, while reducing contents of proinflammatory cytokines TNF-α, IL-1β, and IL-6. Additionally, metabolomic analysis suggested that the SM extract-mediated cardioprotective activity was primarily modulated via suppression of inflammatory responses via regulating the glycerophospholipid metabolism in ischemic heart tissue. Additionally, SM extract further modulated the histidine; alanine, aspartate, and glutamate; and glycine, serine, and threonine metabolisms via oxidation of amino acids during MI.

Salvia Miltiorrhiza Bunge, is a widely used medicinal herb in Asia for various cardiovascular diseases. Recent studies have suggested that water-soluble phenolic compounds such as salvianolic acid B and A, lithospermic acid, caffeic acid, extracted from SM exerts distinct benefits in treating ischemic heart diseases according to the multiple components and multiple targets [33]. OS is widely recognized as a critical factor in the mechanisms of ISO-induced myocardial damage [34]. Phenolic acids' actions are mainly attributed to their antioxidative capacity [35,36]. Similar results were also reported for phenolic acids pretreatment in ISO-induced acute ischemia [24]. We can speculate that the anti-inflammatory property of SM extract in ISO-induced AMI is a result of its primary constituent phenolic acids. Several studies reported that some phenolic acids from herbal medicines have cardioprotective effects on IHD by preventing arteriosclerosis and myocardial hypertrophy [37]. Taken together, our results in line with previous reports, the phenolic compounds were recognized as main bioactive ingredients of SM water extract via LC-MS/MS analysis. Indeed, existing literature supports the notion that both water and alcohol extracts of SM exhibit robust cardioprotective effects. The water extract, rich in water-soluble compounds such as salvianolic acids and protocatechualdehyde, has been associated with antioxidant and anti-inflammatory properties, contributing to cardiovascular protection [23–26]. On the other hand, the alcohol extract, containing predominantly fat-soluble compounds like tanshinones, has demonstrated efficacy in improving cardiovascular health through mechanisms such as vasodilation, anti-thrombotic effects, and anti-arrhythmic activities [27–29]. Future research endeavors could focus on elucidating the signaling pathways and molecular interactions associated with each extract, aiming to identify any unique or synergistic actions that contribute to their observed cardiovascular benefits.

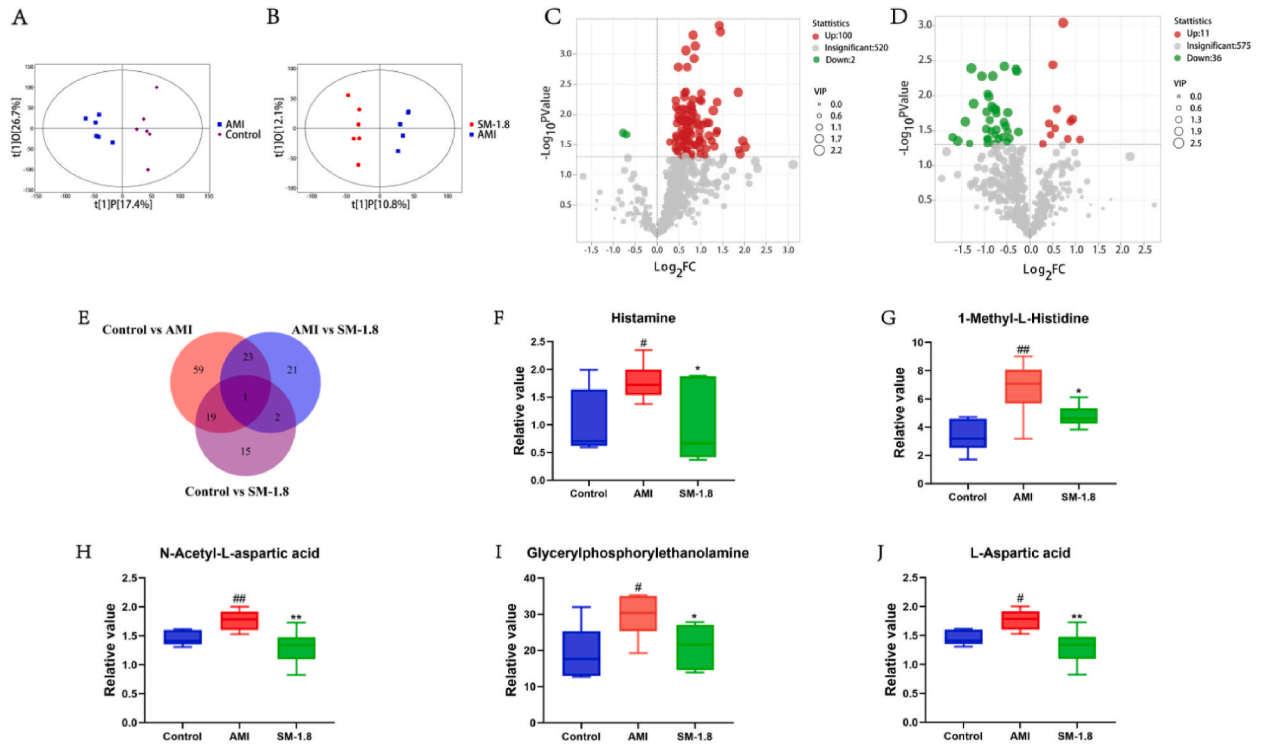

Fig. 5. Influences of SM extract on metabolic profiles of heart tissue samples after ISO induced AMI. (A) Orthogonal partial least squares discriminant analysis (OPLS–DA) of the control vs. AMI cohort. (B) OPLS-DA of AMI vs. SM-1.8 g/kg cohort. (C) A volcano plot of control vs. AMI cohort. (D) A volcano plot of AMI vs. SM-1.8 g/kg cohort. (E) Venn illustration of differential metabolites (DMs). (F)–(J) Five typical DMs. Values provided as mean ± SEM (n = 6). $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. controls; $^{**}p < 0.01$, $^{*}p < 0.05$ vs. AMI cohort.

Table 3
Differential metabolites (DMs) identified via cross-comparison of rat heart samples between various cohorts (control and AMI cohorts, and AMI and SM-1.8 g/kg cohorts).

No.	Metabolites	AMI vs. Control		Trend	*p*-Value	SM – 1.8 vs. AMI		Trend	*p*-Value
		VIP	FC			VIP	FC		
1	Histamine	1.51	1.74	↑	0.0214	1.79	0.55	↓	0.0321
2	L-Aspartic acid	1.79	1.36	↑	0.0075	2.29	0.82	↓	0.0045
3	N-Acetyl-L-aspartic acid	1.78	1.54	↑	0.0051	2.34	0.68	↓	0.0038
4	1-methyl-L-histidine	1.68	2.02	↑	0.0044	1.65	0.71	↓	0.0458
5	Glycerylphosphorylethanolamine	1.63	1.53	↑	0.0234	1.77	0.71	↓	0.0325
6	Palmitoleoyl ethanolamide	2.00	2.28	↑	0.0224	2.50	0.42	↓	0.0131
7	15-Deoxy-d-12,14-PGJ2	2.07	2.44	↑	0.0086	1.68	0.52	↓	0.0418
8	L-prolyl-L-proline	1.51	1.60	↑	0.0390	2.22	0.56	↓	0.0142
9	Mukonidine	1.52	1.38	↑	0.0410	1.90	0.54	↓	0.0225
10	Carnosic acid	1.62	1.56	↑	0.0377	1.94	0.53	↓	0.0094
11	Norclozapine	1.98	2.73	↑	0.0004	2.34	0.48	↓	0.0053
12	Lysyl-proline	1.71	1.86	↑	0.0111	2.40	0.57	↓	0.0053
13	Cinncassiol D3	1.74	1.97	↑	0.0058	1.65	0.59	↓	0.0363
14	Prolyl-arginine	1.94	2.02	↑	0.0149	2.53	0.41	↓	0.0041
15	Sakacin P	2.02	1.83	↑	0.0007	2.01	0.69	↓	0.0274
16	Safynol	1.47	3.63	↑	0.0385	1.96	0.31	↓	0.0395
17	Prolyl-aspartate	1.75	1.40	↑	0.0085	2.25	0.81	↓	0.0042
18	5-L-glutamyl-taurine	1.72	1.96	↑	0.0239	2.22	0.54	↓	0.0104
19	PC(P-18:1(9Z)/16:1(9Z))	1.61	1.72	↑	0.0316	2.15	0.60	↓	0.0307
20	LysoPE (18:1(9Z)/0:0)	1.50	1.32	↑	0.0286	2.02	0.84	↓	0.0332
21	4-(2-Aminophenyl)-2,4-dioxobutanoic acid	1.56	1.61	↑	0.0211	2.05	0.60	↓	0.0179
22	4-Acetamido-2-amino-6-nitrotoluene	1.59	2.19	↑	0.0367	2.35	0.37	↓	0.0236
23	3-Hydroxy-2-methylpyridine-4,5-dicarboxylate	1.61	1.48	↑	0.0132	1.86	0.74	↓	0.0408
24	Leucyl-hydroxyproline	1.65	0.58	↓	0.0202	1.75	1.78	↑	0.0417

Table 3 summarizes the marked modulation of candidate ISO-induced rat heart metabolites (n = 6). "↑/↓" represents elevated or reduced metabolites. FC represents "fold change."

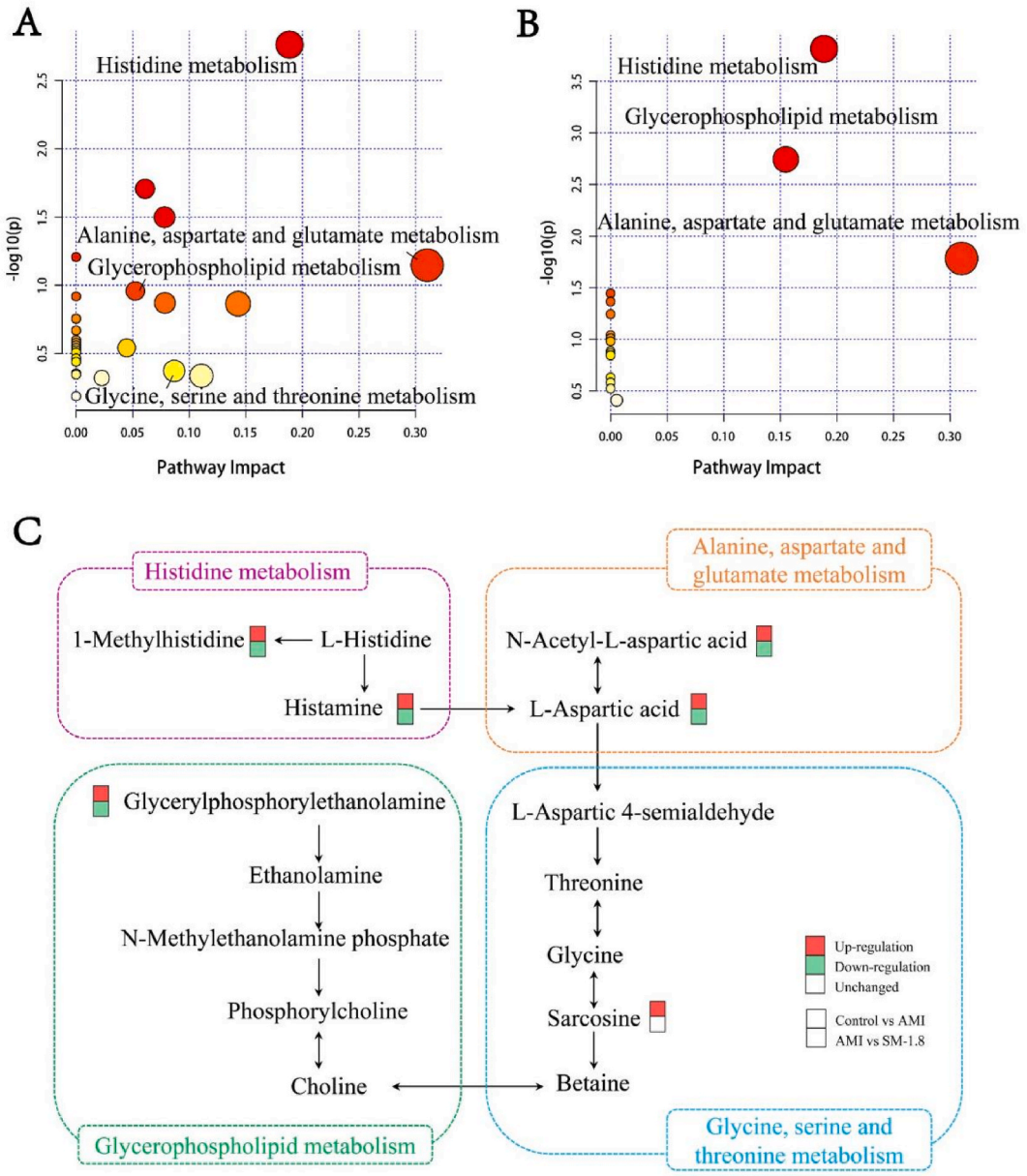

Fig. 6. Candidate SM extracted-related metabolic networks in AMI rats. (A) Impaired metabolic networks in control vs. AMI cohort. (B) Impaired metabolic networks in AMI vs. SM-1.8 g/kg cohort. The size and color of individual circles represent significance of network stratified by p-value (red: enhanced p-values and yellow: diminished p-values) and network impact score (larger circle = higher impact score), respectively. (C) An illustration of the estimated metabolic networks.

Isoproterenol (ISO) is a well standardized model for myocardial injury induction, and it displays comparable morphological changes and ECG aberrations to clinically pertinent in the features of human AMI [38]. Injections of ISO can cause myocardial hyperfunction might be due to increased chronotropism and inotropism, which contributes to imbalance between oxygen demand and supply to cardiomyocytes [39,40]. Multiple investigations revealed that the low-dose ISO administered to rats generated excessive OS and inflammation in the myocardium and aggravate myocardial ischemia, developing into atherosclerosis-like symptoms [41]. According to literature reports, the ECG abnormalities are the prominent criteria generally used for definitive diagnosis of AMI. Myocardial ischemia disturbs the cells' membrane potential, which engenders electrical potential difference. Based on our measurements of cardiac electrical activity using ECG modalities, SM extract enhanced myocardial contractility and pumping ability in ISO-triggered rat in the form of prolongation of RR intervals, and increase in heart rate. RR interval and heart rate variation represents the potential boundary difference between ischemic and normal myocardium [42]. The HR impairment is considered as a marker of poor prognosis in IHD, and the risk of increased HR is closely related to heart failure [43,44]. H&E and TTC staining being commonly

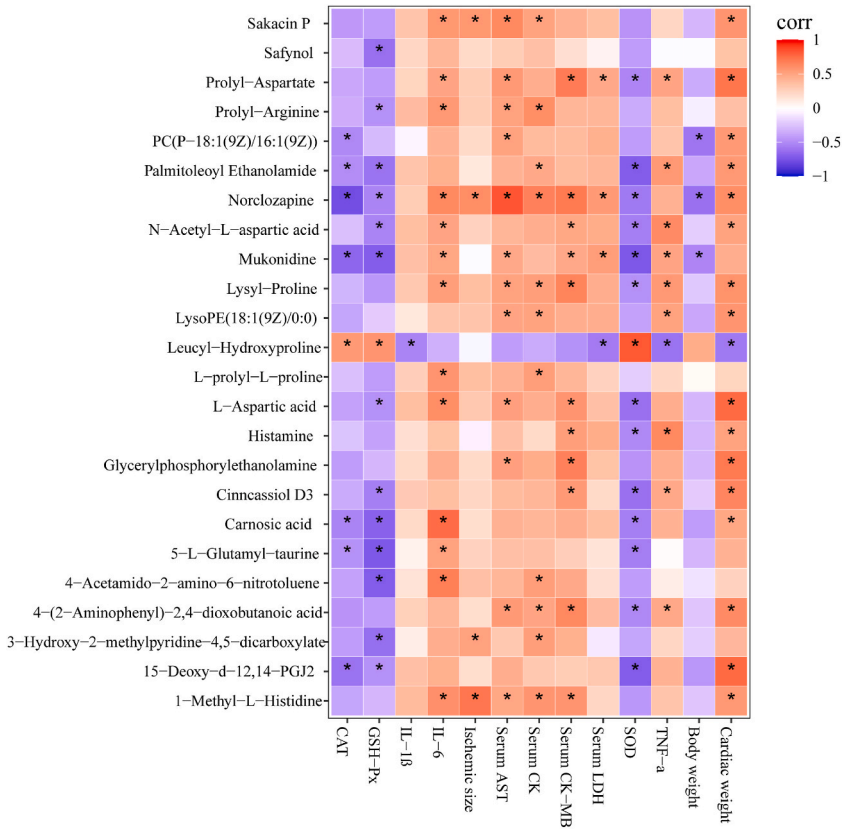

Fig. 7. The Spearman's correlation heatmap of obviously altered metabolites and metabolic syndrome-associated indexes. Positive association in red and negative association in blue. *$p < 0.05$.

used to assessment of myocardial infiltrating leukocytes and ischemic in correlation study [45]. Morphological changes in myocyte ultrastructure, inflammatory cell infiltration, and the deposition of collagen fibers in myocardium due to administration of ISO are substantial [46]. In this study, our findings corroborated with prior investigations, indicating the successful development of the rat model and anti-AMI property of SM extract.

In this study, a significant increased level of circulating CK, CK-MB, LDH, and AST were found in AMI rats, which was consistent with the previous experimental results and demonstrated that the ISO-induced rat model of AMI was successful established. Cardiomyocyte possess excess marker enzymes, ISO administration will cause cardiomyocyte necrosis, massive cell lysis, and these enzymes are secreted into the extracellular fluid, resulting in prolonged ischemia and glucose deprivation [47,48]. Additionally, contents of LDH, CK, and CK-MB, biomarkers of myocardial injury and clinically employed AMI diagnostic biomarkers. CK-MB isoenzyme activity is useful as an index for the early diagnosis of AMI because it is present mainly in cardiac tissues [49]. Moreover, circulating CK-MB is substantially more specific for myocardial damage than circulating total CK. Hence, LDH leakage and CK secretion into the culture supernatant are regarded as myocardial membranal damage biomarkers [50].

Following the administration of ISO, the activity of the endogenous antioxidant system of the heart decreased significantly, generating an oxidation and antioxidant system imbalance that accumulates in cardiomyocytes and manifest as oxidative damage. Enhanced endogenous myocardial antioxidant is recognized as the initial cellular defense mechanism against OS that counters several reactive oxygen species (ROS) formation, such as, superoxide, hydrogen peroxide and hydroxyl radical. SOD, as an intracellular oxygen radical scavenger, facilitates the formation O_2 and H_2O_2 from O_2^-, and protects organisms from superoxide anions. A robust fall in CAT, GSH-Px, and SOD activities leads to the impaired function and integrity of the myocardial membrane, which is commonly observed during MI. In addition, numerous studies demonstrated that MI is an inflammatory vascular disease, which is strongly modulated by invasion of inflammatory cells. As prominent and early mediators of, proinflammatory cytokines critically regulate the response to cardiac injury [51]. Proinflammatory cytokines are pleiotropic polypeptides that are intricately linked to inflammation and cytokines release, which exacerbates myocardial injury via multiple mechanisms [52,53]. Collectively, our results revealed that SM extract possesses antioxidant properties which are exerted via suppression of myocardial tissue OS and simultaneous augmentation of the antioxidant defense system in a rat model of AMI. Also, the outcome of this study showed a significant reduction of inflammatory markers TNF-α, IL-1β, and IL-6 content in the heart, in agreement with previous studies.

Amino acids, an integral enzyme substrates and regulators, are critical for numerous metabolic networks, namely, protein synthesis, reflection the metabolic and functional state of the body modulation of the nervous system, maintenance normal operation of

immune system, etc [54,55]. Numerous investigations revealed that elevated amino acids contents are strongly associated with abnormal energy homeostasis as ischemic myocardium will cause accumulation of amino acids [56]. Histamine, precursor of antioxidant dipeptide, is an amino acid containing imidazole ring and widely exists in organisms, with strong vasodilation function [57]. Three major reactions contribute to histidine metabolism and degradation *in vivo*, including deamination, amination, and decarboxylation, in which histamine is synthesized in the cytosol via catalytic decarboxylation of histidine via histidine decarboxylase (HDC) [58,59]. Prior investigations revealed that histidine metabolism disorder is linked to the OS reactions, resulting in an oxidation and antioxidant system imbalance [60]. The increased histamine and 1-methyl-L-histidine content in AMI group confirmed that AMI resulted in abnormal histidine metabolism in the body, while the SM extract group recovered the differential metabolities content to the normal level, indicating that inhibited abnormal histidine metabolism. This result indicated that SM extract played a role in the regulation of histidine metabolism. Several studies suggested that alanine, aspartate, and glutamate metabolism was found to be the usually disrupted pathway in the heart [61]. In our study, we found that L-Asp and N-acetyl-L-Asp were related to alanine, aspartate, and glutamate metabolism. Relative to the control cohort, the metabolites L-Asp and N-acetyl-L-Asp contents in the AMI group were elevated, indicating that AMI leads to abnormal alanine, aspartate, and glutamate metabolism in the body. All three metabolites in the SM extract group showed a callback trend, indicating that SM extract could inhibit abnormal alanine, aspartate, and glutamate metabolism and attenuate the damage of AMI to the body.

Studies have shown that glycerophospholipids are major lipid bilayer products and essential signaling molecules, involving in many pathophysiological events, namely, inflammation and apoptosis [62]. Additionally, structurally different phospholipids were identified as bioindicators for diverse CVDs types [63–66]. According to substituents, the glycerophospholipid mainly classified as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine, phosphatidic acid, and phosphatidylinositol [67]. Prior investigations involving ISO-induced MI animal model research also confirmed a robust permutation in phospholipids [68,69]. It has been recently discovered that the PCs mediated anti-oxidation, anti-inflammatory, and anti-fibrotic properties [70]. In general, the increased LysoPEs may induce arrhythmogenic [71]. In our findings, the levels of glycerylphosphorylethanolamine, PC(P-18:1(9Z)/16:1(9Z)), and LysoPE (18:1(9Z)/0:0) were strongly upregulated in the AMI versus normal rats. Nevertheless, the SM extract-1.8 g/kg pretreatment maintained normal circulating PC and LysoPE levels, suggesting that SM extract potentially sustains glycerophospholipid stability against glycerophospholipid metabolic disorders during AMI.

In summary, the abovementioned studies provide supporting evidence for the importance of histidine; alanine, aspartate, and glutamate; glycerophospholipid; and glycine, serine, and threonine metabolisms in the ISO-induced cardiac injury. This research has certain limitations. Among them, the underlying mechanism behind SM extract action requires additional and extensive research. Hence, we warranted more studies on this subject to elucidate the mechanism behind the SM extract-mediated protection in AMI using the aforementioned potential metabolites.

5. Conclusions

In summary, SM water extract mainly comprising salvianolic acid B, lithospermic acid, salvianolic acid A, and caffeic acid, demonstrated robust cardioprotective effects in an ISO-induced AMI rat model. The extract not only effectively reduced myocardial ischemic size, normalized electrocardiographic patterns, and improved histopathological features but also exhibited specific metabolic influences, with several amino acids and lipids metabolites identified through LC-MS/MS analysis, primarily associated with histidine, alanine, aspartate, and glutamate metabolism, glycerophospholipid metabolism, and glycine, serine, and threonine metabolism, underscoring its potential as a protective agent for myocardial ischemia.

Data availability statement

Data will be made available on request.

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Ethics statement

The animal study protocol was approved by the Animal Ethical Committee of Inner Mongolia Minzu University (Ethics approval number: NM-LL-2021-06-15-1).

CRediT authorship contribution statement

Xiyele Mu: Writing – original draft, Investigation, Funding acquisition, Formal analysis. **Hongzhen Yu:** Writing – original draft, Investigation, Formal analysis. **Huifang Li:** Investigation, Formal analysis. **Lan Feng:** Software, Investigation, Formal analysis. **Na Ta:**

Software, Formal analysis. **Ling Ling:** Investigation, Formal analysis. **Li Bai:** Investigation, Formal analysis. **Rure A:** Investigation, Formal analysis. **Almaz Borjigidai:** Writing – review & editing, Funding acquisition. **Yipeng Pan:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Minghai Fu:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30488.

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