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Research article

Astragaloside IV inhibits angiotensin II-induced atrial fibrosis and atrial fibrillation by SIRT1/PGC-1α/FNDC5 pathway

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ABSTRACT

Aims and objectives: Astragaloside IV (AS-IV) has been found to possess anti-oxidative, anti-inflammatory, and anti-apoptotic properties, but its effect on atrial fibrosis is yet to be determined. This research investigates the protective role of AS-IV in angiotensin II (Ang II)-induced atrial fibrosis and atrial fibrillation (AF).

Methods: C57BL/6 male mice aged 8–10 weeks (n = 40) were subcutaneously administered Ang II (2.0 mg/kg/day) or saline, with AS-IV (80 mg/kg) intraperitoneally administered 2 h before Ang II infusion for 4 weeks. Biochemical, histological, and morphological analyses were carried out. Using transesophageal burst pacing, AF was generated in vivo.

Results: Here, we report that AS-IV treatment inhibited Ang II-induced AF development in mice (58 ± 5.86 vs 15.13 ± 2.16 %, $p < 0.001$). Ang II + AS-IV therapy was effective in reducing the atrial fibrotic area and decreasing the increase in smooth muscle alpha-actin (α-SMA)-positive myofibroblasts brought on by Ang II treatment (fibrotic area: 26.25 ± 3.81 vs 8.62 ± 1.83 %, $p < 0.001$ and α-SMA: 65.62 ± 10.63 vs 17.25 ± 1.78 %, $p < 0.001$). The reactive oxygen species (ROS) production was reduced by pretreatment with Ang II + AS-IV (9.20 ± 0.92 vs 2.63 ± 0.22 %/sec, $p < 0.001$). In addition, Ang II + AS-IV treatment suppressed oxidative stress in Ang II-induced atrial fibrosis (malondialdehyde: 701.78 ± 85.01 vs 504.07 ± 25.62 pmol/mg protein, $p < 0.001$; superoxide dismutase: 13.82 ± 1.25 vs 29.54 ± 2.45 U/mg protein, $p < 0.001$ and catalase: 11.43 ± 1.19 vs 20.83 ± 3.29 U/mg protein, $p < 0.001$, respectively). Moreover, Ang II + AS-IV decreased the expression of α-SMA, collagen III and collagen I (3.32 ± 0.53 vs 1.41 ± 0.20 fold, $p < 0.001$; 3.41 ± 0.55 vs 1.48 ± 0.18 fold, $p < 0.001$; 2.34 ± 0.55 vs 0.99 ± 0.17 fold, $p < 0.001$, respectively) while increasing the protein expression of sirtuin 1 (SIRT1), peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α), and fibronectin type III domain-containing protein 5 (FNDC5) in Ang II-treated mice (0.22 ± 0.02 vs 0.57 ± 0.08 fold, $p < 0.001$; 0.28 ± 0.04 vs 0.72 ± 0.05 fold, $p < 0.001$; 0.38 ± 0.03 vs 0.68 ± 0.06 fold, $p < 0.001$, respectively).

Conclusion: Our data led us to speculate that AS-IV may protect against Ang II-induced atrial fibrosis and AF via upregulation of the SIRT1/PGC-1α/FNDC5 pathway.

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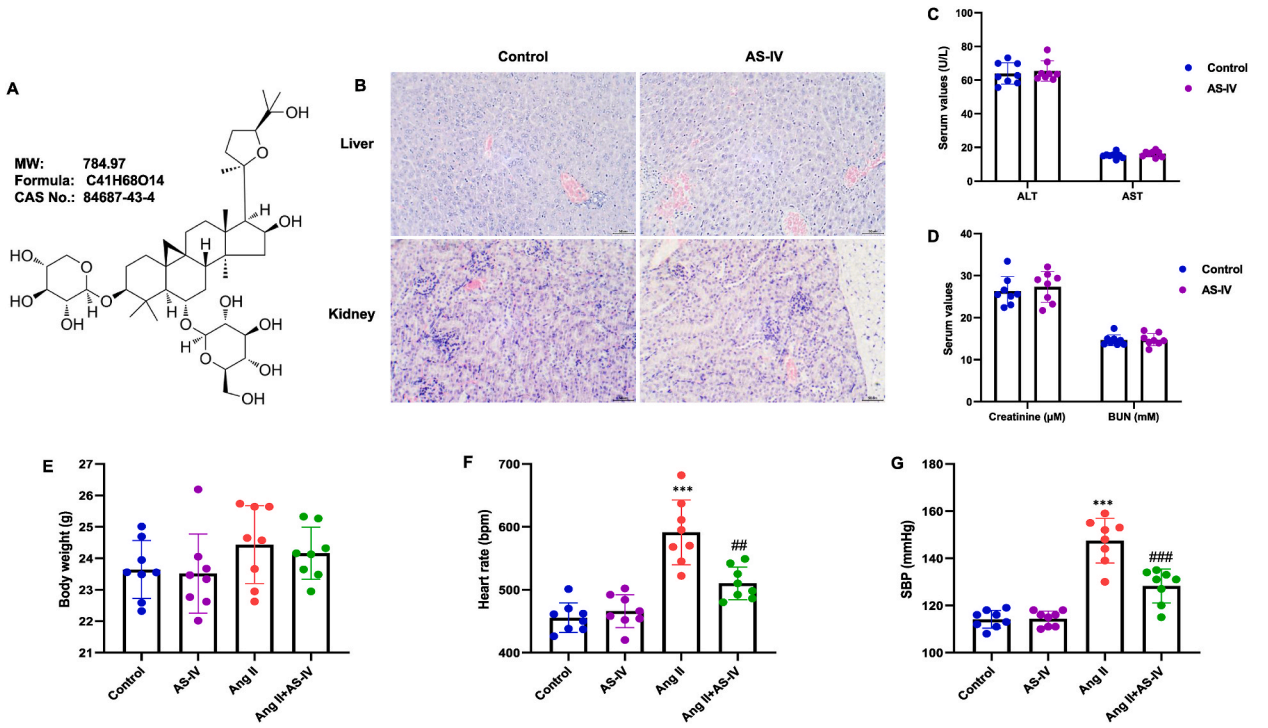

Fig. 1. Preliminary evaluation of safety and physiological parameters in mice. (A) Structural formula, molecular weight, chemical formula and CAS number of AS-IV. (B) The in vivo systematic toxicity of AS-IV was monitored after 28 day administration of AS-IV by intraperitoneal injection (80 mg/kg/day). Hematoxylin and eosin (H&E) stained hepatic and renal slices from the saline and Ang II-infused mice. Scale bar: 50 μm. (C) Hepatic and (D) renal function indicators of mice, including ALT, AST, Creatinine and BUN. Mice were infused with Ang II (2.0 mg/kg/day) and intraperitoneally injected with AS-IV (80 mg/kg/day) for 28 days. Changes in (E) body weight, (F) heart rate and (G) systolic blood pressure (SBP) were monitored in mice. Data are presented as mean ± SD (n = 8 in each group). ***$p < 0.001$ vs. Control group; ##$p < 0.01$, ###$p < 0.001$ vs. Ang II group. **Abbreviation:** CAS, chemical abstracts service; AS-IV, astragaloside IV; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Ang II, angiotensin II.

1. Introduction

Heart failure, stroke, and consequent cardiac morbidity and mortality are among the risks associated with atrial fibrillation (AF), the most common type of cardiac arrhythmia [1,2]. Atrial remodeling significantly influences the pathogenic mechanism of AF, and atrial fibrosis is the common cause of atrial remodeling [3,4]. Moreover, atrial fibrosis can be brought on by angiotensin II (Ang II), which can cause the proliferation of fibroblasts [1]. However, in animals and the biopsies of patients with heart failure, excessive extracellular matrix (ECM) deposition in atrial tissue accelerated atrial fibrosis [5]. This evidence demonstrates a correlation between atrial fibrosis and AF. However, the impact of tissue fibrosis on AF onset and duration remains unclear. In addition, evaluating the underlying mechanisms of atrial fibrosis is essential for generating new therapeutic approaches for AF.

The highly conserved nuclear protein Sirtuin 1 (SIRT1) is extensively expressed in different tissues in the body. After activation, SIRT1 moves from the cytoplasm into the nucleus, which controls the activity of nuclear transcription factors [6]. SIRT1 regulates gene transcription by deacetylating histones, but it also prevents the proliferation, differentiation, energy metabolism, oxidative stress, aging, apoptosis, and other pathological, physiological processes of cells by deacetylating several nonhistones, including tumour suppressor factor (p53), FOXO family of fork protein transcription factors (FOXOs), peroxidase-activated receptor (PPAR-γ), myogenic determinant factor (MyoD), and peroxidase-activated receptor co-stimulating factor-1 alpha (PGC-1α) [7–9]. PGC-1α is an essential protein that is involved in maintaining glycolipid balance, mitochondrial oxidative metabolism, and energy homeostasis [10]. The fibronectin type III domain-containing protein 5 (FNDC5) gene encodes a 203 amino acid protein, which is located on chromosome 1p35.1 [11]. FNDC5 expression is found at the highest levels in the brain and heart [12]. A previous study identified FNDC5 as one of the key genes in the treatment of hypertensive heart disease treated with astragaloside IV [13]. However, the role of AS-IV on Ang ll-induced atrial fibrosis and atrial fibrillation through the SIRT1/PGC-1α/FNDC5 pathway remains unexplored.

A naturally active ingredient called astragaloside IV (AS-IV) was discovered in the Chinese plant *Astragalus membranaceus*. Recent research has demonstrated that AS-IV possesses anti-oxidation, anti-inflammation, and anti-apoptosis activities, as well as the ability to enhance immunity [14–16]. Several pharmacological effects of AS-IV on the liver, neurological system, haematological system, endocrine system, cardiovascular diseases, collagen metabolism, and organ immune system have been demonstrated in recent research [17]. According to its molecular formula, AS-IV is a cycloartane triterpene saponin. The chemical structure of AS-IV is shown in

Fig. 1A. Previous studies have shown that AS-IV possesses antioxidative and anti-inflammatory with cardioprotective effects [18,19]. Moreover, AS-IV has been shown to enhance cardiac function by preventing cardiomyocyte apoptosis and hypertrophy [18,20].

However, how AS-IV protects against Ang II-induced atrial fibrosis and AF remains unclear. Therefore, the current study was performed to investigate the potential protective roles of AS-IV against AF and atrial fibrosis brought on by Ang II and precisely explore the potentially involved molecular mechanism. The ultimate goal is to identify a possible therapeutic target for reducing the incidence of AF.

2. Materials and methods

2.1. Animals and treatment

Shanghai SLRC Laboratory Animal Co., Ltd. (Shanghai, China) provided forty healthy male C57BL/6 mice. All mice were maintained under previously reported animal care conditions [21]. The AF model was established using forty male C57BL/6 mice, aged 8–10 weeks. Mice were subcutaneously infused with either saline or Ang II (2.0 mg/kg/day) via osmotic mini-pumps once daily for 28 days. For four weeks, either Astragaloside IV (AS-IV, 80 mg/kg/day, HY-N0431, MedChemExpress) or an equal volume of saline was administered intraperitoneally once a day. The initial AS-IV injection was given before the commencement of the Ang II infusion for a time interval of 2 h. Utilizing a tail-cuff system (Softron BP98A; Softron Tokyo, Japan), the murine systolic blood pressure (SBP) was assessed on the 28th day before the acquisition of electrocardiogram (ECG) data. The ECG measured the mouse's heart rate. The Animal Care and Use Committee of Shanghai Pudong Zhoupu Hospital approved this study (**ZPYYLL-2018–2**) and confirmed it using the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, updated 1996).

2.2. Arrhythmia induction and persistence

Following a period of 4 weeks involving the administration of AS-IV and Ang II infusions, the mice underwent anesthesia through an intraperitoneal injection containing 1 % pentobarbital sodium. A specialized 8-electrode catheter manufactured by Japan Lifeline in Tokyo, Japan, was carefully inserted through the jugular vein of the mice and subsequently progressed into both the right atrium and ventricle. An automated stimulator was utilized to administer a 2-s burst aimed at inducing AF. The ECG was captured using a computerized data acquisition system (MadLab-4C/501H, ZS Dichuang Co., Ltd., Beijing, China). The specified parameters included a voltage of 20 V, a current of 4 mA, and a wave width of 6 ms. AF was characterized by a swift, irregular atrial rhythm displaying irregular R–R intervals of 1 s. The duration of AF was determined as the timeframe ranging from the conclusion of burst pacing to the initial detection of a P-wave after the rapid, irregular atrial rhythm [22].

2.3. Animal grouping and drug administration

The mice were grouped in a random manner into four distinct categories: (1) the control group (n = 8) received intraperitoneal injections and infusions of saline solution. (2) The AS-IV group (n = 8) received intraperitoneal injections of AS-IV (80 mg/kg/day) along with saline infusions. (3) The Ang II group (n = 8) received saline infusions and Ang II infusions (2.0 mg/kg/day) intraperitoneally. (4) The Ang II + AS-IV group (n = 8) was administered intraperitoneal injections of AS-IV (80 mg/kg/day) and Ang II infusions (2.0 mg/kg/day). The injection of AS-IV (80 mg/kg/day) and infused with Ang II (2.0 mg/kg/day) were given once a day for 28 consecutive days.

2.4. Sample collection

Mice were anesthesized with 1 % pentobarbital sodium by intraperitoneal injection, and the atrial tissue was collected. Some tissue samples were rapidly preserved by snap-freezing in liquid nitrogen to facilitate subsequent RT-qPCR and Western blot analysis. In contrast, a separate set of tissue specimens was immersed in a solution of 4 % paraformaldehyde for fixation purposes, intended for subsequent histological studies.

2.5. Biochemical analysis

Serum was collected to measure biochemical indicators alanine aminotransferase (ALT) (C009-2-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China), aspartate aminotransferase (AST) (C010-2-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. ELISA

Serum creatinine (SCr), blood urea nitrogen (BUN), and irisin (Zell BioGmbH, Germany, ZB-13253S-H9648) concentrations were measured following the manufacturer's instructions (Detect X Serum Creatinine Detection Kit, MI, USA; QuantiChrom Urea Assay Kit, CA, USA). Using the accompanying standard curve, the concentrations were calculated after the 450 nm absorbance was measured in a microplate reader.

Table 1
List of primer nucleotide sequences used in this study.

Genes	Forward primer (5′–3′)	Reverse primer (5′–3′)
α-SMA	TCCTGACGCTGAAGTATCCGATA	GGCCACACGAAGCTCGTTAT
Collagen I	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
Collagen III	TCCCCTGGAATCTGTGAATC	TGAGTCGAATTGGGGAGAAT
NOX2	TGGCGATCTCAGCAAAAGGT	ACCTTGGGGCACTTGACAAA
NOX4	ACCAAATGTTGGGCGATTGTG	GATGAGGCTGCAGTTGAGGT
GAPDH	ACTCCACTCACGGCAAATTC	TCTCCATGGTGGTGAAGACA

Abbreviation: α-SMA, α-smooth muscle actin; NOX2, NADPH Oxidase 2; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

2.7. Histology

Hematoxylin/eosin (HE) staining evaluated the degrees of liver and kidney injury. For paraffin sections, tissue was routinely prepared for embedding in paraffin and then post-fixed overnight in 4 % paraformaldehyde. Sections were cut to a length of 4 μm and immediately placed on organosilane-coated slides. 4 % paraformaldehyde was used overnight to post-fix the tissues. The following 24 h were spent incubating in PBS that had been adjusted with three different buffers to a pH of 7.4 and 10 % sucrose. The slices were then stained with thionin or HE following rehydration and deparaffinization in xylene.

2.8. Immunohistochemistry

Using particular antibodies to stain samples, immunohistochemistry (IHC) is a method that could be used to regulate the distribution and location of target antigens in cells or tissues. Atrial tissue was embedded in paraffin and sectioned into 4 μm serial sections after undergoing treatment with 4 % paraformaldehyde. Slides were either coloured with Masson's trichrome or rabbit polyclonal antibodies against the α-SMA antibody (1:400; ab5694; Abcam, UK) and applied for 20 min at 37 °C. Afterwards, the slides were exposed to a secondary antibody conjugated with biotinylated horseradish peroxidase (HRP). The steps were followed according to the developer's instructions. We used an optical microscope (IX51, Olympus, Japan) to examine the slides.

2.9. Immunofluorescence

Mice's atrial tissue was extracted from frozen sections. Following permeabilization with 0.1 % Triton X-100 (Sigma), the sections were dissolved for 10 min in 1 % bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Subsequently, the slides were blocked for 60 min using 10 % goat serum in PBS. After that, primary anti-tropomyosin (1:250, Sigma T9283) diluted in 0.1 % BSA in PBS was incubated on the slides for an additional night at 4 °C. The secondary antibody was then incubated for an hour at room temperature. The sections were stained with TUNEL (C1086, Beyotime) and DAPI (S0063, Beyotime) after three PBS washes. Finally, an inverted microscope (IX51, Olympus, Japan) was used to examine the samples.

2.10. Oxidative stress

Homogenization of the atrial tissue was done to obtain cell lysate. Subsequently, the cell lysate was refrigerated at −80 °C. The activities of superoxide dismutase (SOD) (S0109, Beyotime), catalase (CAT) (S0051, Beyotime), and malondialdehyde (MDA) (S0131S, Beyotime, Shanghai, China) were measured using a commercial kit, and the amount of oxidative stress was estimated using a previously published protocol [23].

2.11. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Atrial tissue underwent total RNA extraction utilizing TRizol (Invitrogen, USA). Subsequent to the reverse transcription of total RNA, complementary DNA (cDNA) was generated. Real-time quantitative PCR (RT-qPCR) was performed employing an ABI Prism 7700 Real-Time PCR system (Applied Biosystems, USA) with the use of SYBR Green reagent (TaKaRa, Japan) for mRNA amplification. The samples were subjected to denaturation at 95 °C for 3 min, followed by 39 cycles of amplification and quantification (95 °C for 15 s and 58 °C for 15 s, respectively), capped off with a final extension cycle (72 °C for 90 s) in the thermal cycler. The gene expression levels were standardized to the internal control, GADPH, utilizing the 2-ΔΔCt formula [24]. The primers employed in this study are detailed in Table 1.

2.12. Western blotting

Following the extraction of the protein utilizing RIPA buffer and subsequent centrifugation of the lysates to obtain the supernatants, the protein concentrations were measured using a BCA protein assay kit. The 50 μg of protein samples were transferred to a PVDF membrane (Millipore, Bedford, MA) and loaded onto a 10 % SDS-PAGE electrophoresis. The membrane was blocked by a TBS solution containing 5 % skim milk. The following antibodies were incubated on the membrane for one night at 4 °C: FNDC5 (1:400, ab131390,

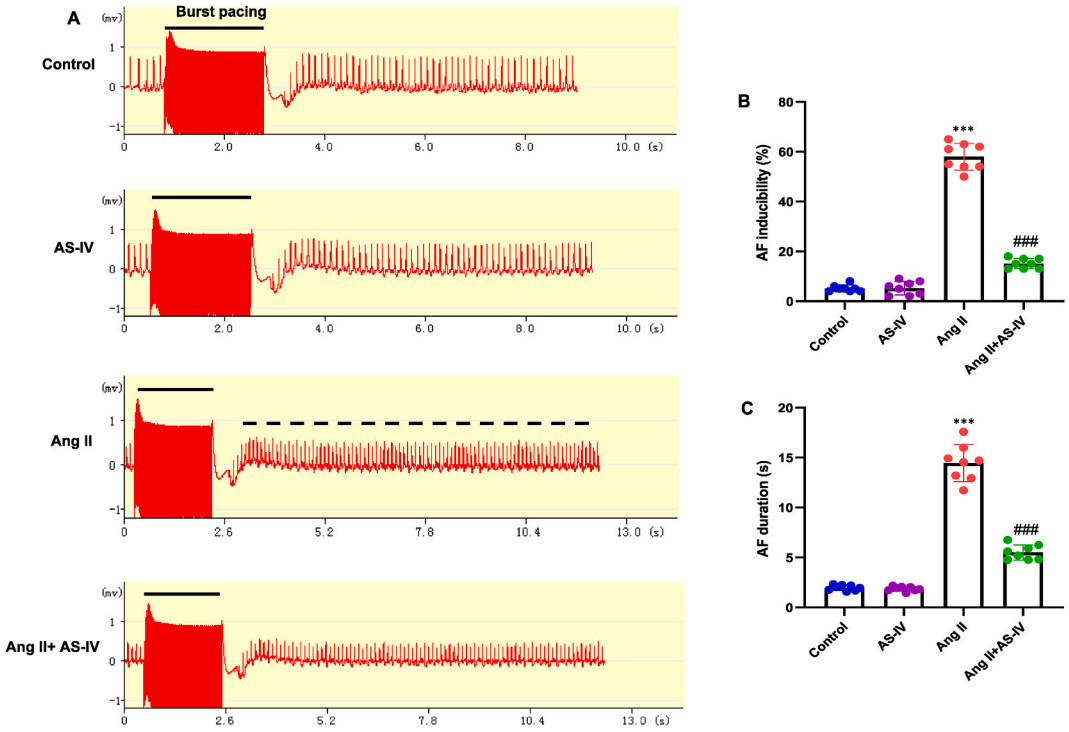

Fig. 2. AS-IV decreases atrial fibrillation (AF) inducibility and AF duration induced by burst pacing. (A) Representative atrial electrogram recordings. Burst pacing is indicated by solid underlines, whereas the induced AF is indicated by dashed underlines. (B) Incidence of successful AF in each group. (C) Average duration of AF induced by burst pacing. Data are presented as mean ± SD (n = 8 in each group). ***p < 0.001 vs. Control group; ###p < 0.001 vs. Ang II group.

rabbit polyclonal, Abcam), PGC-1α (1:500, ab54481, rabbit polyclonal, Abcam), and GAPDH (1:1000, ab9485, rabbit polyclonal, Abcam). These target proteins were found to have immunological reactivity using HRP-conjugated secondary antibodies (1:2000). The ECL (Thermo, Waltham, MA, USA) was used to observe the protein band, and image-processing software (Bio-Rad, Hercules, CA, USA) was used to measure band density.

2.13. Statistical analysis

The mean ± standard deviation (SD) is employed for the purpose of articulating the data. Statistical analyses were carried out utilizing SPSS 20.0 (SPSS, Chicago, IL, USA). Discrepancies among three or more groups were assessed through a one-way ANOVA, with the post hoc comparison of groups conducted using the least significant difference (LSD) method. Statistical one-tailed significance was define as #p < 0.05, ##p < 0.01, ###/***p < 0.001.

3. Results

3.1. Preliminary assessment of the safety and physiological characteristics in mice

In order to examine the potential implications on safety and physiological traits, mice were administered AS-IV intraperitoneally at a dosage of 80 mg/kg/day. After 28 days of intraperitoneal injection of AS-IV (80 mg/kg/day), the in vivo systemic toxicity of AS-IV was evaluated. Hematoxylin and eosin (H&E) stained results showed no negative impact on the hepatic and renal slices from the saline and Ang II-infused mice (Fig. 1B). The same consistent outcome was observed in the case of the hepatic and renal function indicators of mice, including ALT (63.88 ± 6.34 vs 65.39 ± 6.01 U/L, p < 0.633) AST (15.19 ± 1.68 vs 16.18 ± 1.79 U/L, p < 0.273), Creatinine (26.30 ± 3.49 vs 27.32 ± 3.66 μmol/L, p < 0.577) and BUN (14.63 ± 1.27 vs 14.79 ± 1.45 mM/L, p < 0.827) (Fig. 1C and D). To examine physiological characteristics, mice were infused with Ang II (2.0 mg/kg/day) and intraperitoneally injected with AS-IV (80 mg/kg/day) for 28 days. No significant changes observed in body weight (23.65 ± 0.96 vs 23.52 ± 1.42 g, p < 0.818; 23.65 ± 0.96 vs 24.43 ± 1.09 g, p < 0.169; 24.43 ± 1.09 vs 24.16 ± 0.65 g, p < 0.611, respectively) (Fig. 1E), while potentially reduced heart rate (455.63 ± 27.72 vs 466 ± 19.51 bpm, p < 0.419; 455.63 ± 27.72 vs 591.25 ± 33.32 bpm, p < 0.001; 591.25 ± 33.32 vs 510.25 ± 30.12 bpm, p < 0.001, respectively) (Fig. 1F), and systolic blood pressure (SBP) (114.13 ± 4 vs 114.38 ± 3.14 mmHg, p < 0.888; 114.13 ± 4 vs 147.5 ± 8.73 mmHg, p < 0.001; 147.5 ± 8.73 vs 128.25 ± 7.66 mmHg, p < 0.001, respectively) (Fig. 1G) in mice.

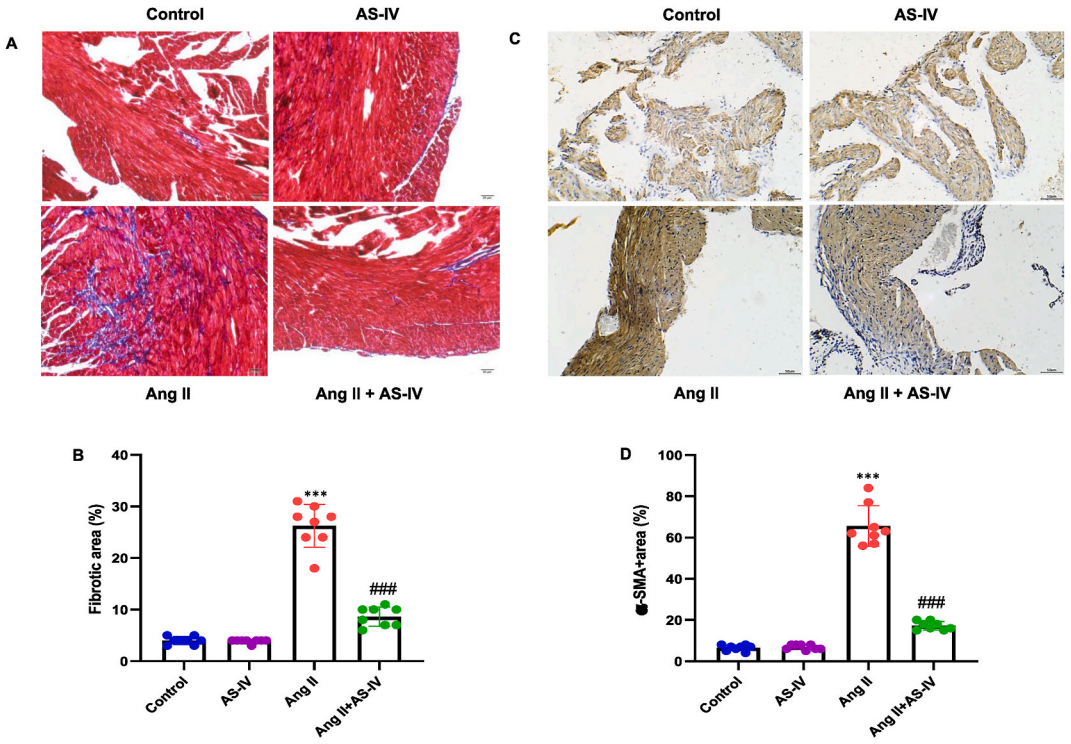

Fig. 3. AS-IV suppresses Ang II-induced atrial fibrosis. (A) Representative images of Masson trichrome staining for atrial tissues. (B) The quantification of atrial fibrosis by calculating the percentage of fibrotic area. (C) Representative images of α-SMA (a marker for myofibroblast differentiation) by immunohistochemistry. (D) The quantification of percentage of α-SMA-positive cells. Data are presented as mean ± SD (n = 8 in each group). ***p < 0.001 vs. control group; ###p < 0.001 vs. Ang II group. **Abbreviation:** α-SMA, α-smooth muscle actin.

3.2. AS-IV attenuated Ang II-induced AF

In order to examine the impact of AS-IV on the regulation of atrial fibrillation (AF) progression, a study was conducted where mice were administered Ang II along with or without AS-IV at a dosage of 80 mg/kg/day for 28 days. The electrocardiogram was recorded subsequent to the implementation of transesophageal fast atrial pacing. The mice in the AF group had typical AF attacks (Fig. 2A). In Ang II-infused mice injected with or without AS-IV, the inducibility and duration of AF were studied. AF inducibility is the heart's tendency to develop atrial fibrillation under specific conditions. However, after giving Ang II, the inducibility of AF was significantly increased (5.13 ± 0.75 vs 58 ± 5.86 %, $p < 0.001$). Ang II + AS-IV treatment significantly attenuated Ang II-induced AF inducibility (58 ± 5.86 vs 15.13 ± 2.16 %, $p < 0.001$), but AS-IV alone did not affect the AF inducibility in saline-treated mice (5.13 ± 0.75 vs 5.25 ± 2.31 %, $p < 0.908$) (Fig. 2B). The overall duration of AF was considerably shortened in the Ang II + AS-IV treated mice compared to the Ang II infusion (14.45 ± 1.99 vs 5.5 ± 0.59 s, $p < 0.001$), with no significant difference observed following the AS-IV therapy alone (1.97 ± 0.26 vs 1.87 ± 0.26 s, $p < 0.415$) (Fig. 2C). The results demonstrated that AS-IV significantly attenuates Ang II-induced AF development in mice.

3.3. AS-IV suppressed Ang II-induced atrial fibrosis

The impact of AS-IV on atrial fibrosis, a defining characteristic of atrial remodeling, was then investigated. Ang II treatment considerably increased the atrial fibrotic area compared to saline therapy (4 ± 0.75 vs 26.25 ± 3.81 %, $p < 0.001$), whereas Ang II + AS-IV treatment significantly reduced this effect (26.25 ± 3.81 vs 8.62 ± 1.83 %, $p < 0.001$) (Fig. 3A and B). In addition, Ang II + AS-IV significantly reduced the increase in α-SMA-positive myofibroblasts that were brought on by Ang II treatment (65.62 ± 10.63 vs 17.25 ± 1.78 %, $p < 0.001$) (Fig. 3C and D).

3.4. AS-IV inhibited oxidative stress in Ang II-induced atrial fibrosis

The levels of significant oxidative stress indicators such as MDA, SOD, and CAT were measured in the lysate of atrial tissue. The formation of ROS in the atrial tissue was significantly enhanced following Ang II (1 ± 0.11 vs 9.20 ± 0.92 %/s, $p < 0.001$), as seen by DHE staining. Additionally, compared to the Ang II group, the DHE-positive cells were considerably reduced after pretreatment with Ang II + AS-IV (9.20 ± 0.92 vs 2.63 ± 0.22 %/s, $p < 0.001$) (Fig. 4A and B). According to Fig. 4C, the MDA content was lower in the

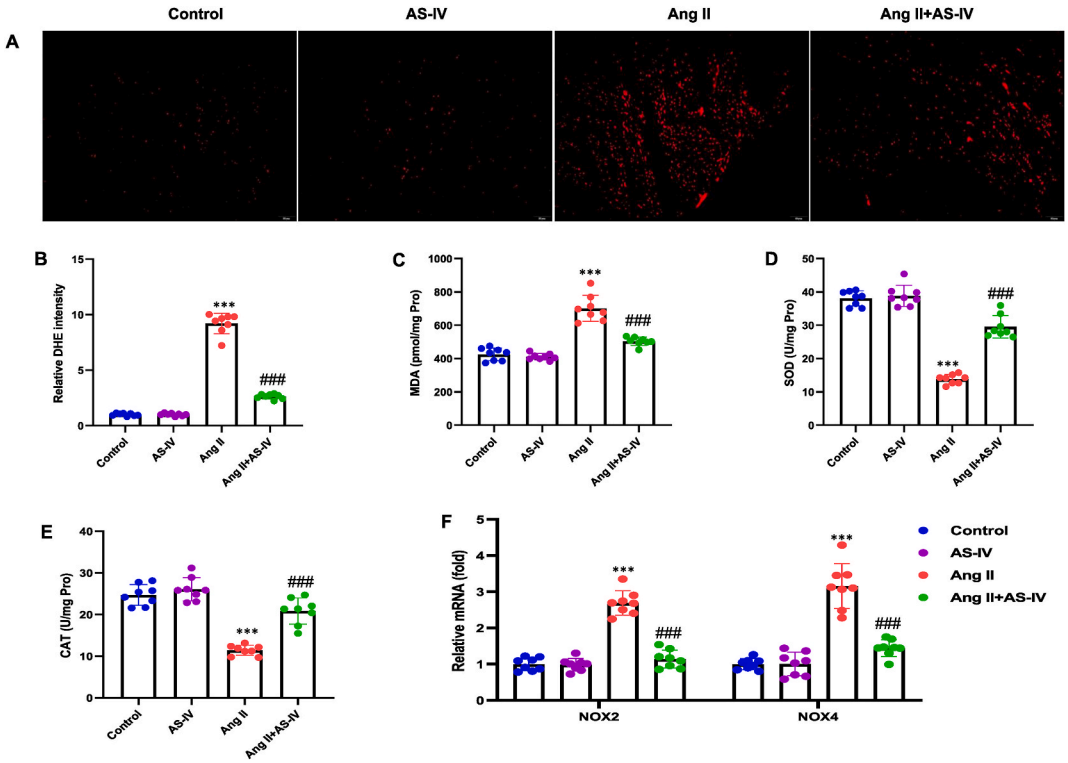

Fig. 4. AS-IV suppresses oxidative stress in atrial tissue of mice after Ang II infusion. (A) Representative images of DHE staining of atrial tissues using DHE staining. (B) Quantification of DHE intensity. The levels of (C) MDA, (D) SOD and (E) CAT in atrial lysate were quantified. (F) RT-qPCR was performed to measure the mRNA expression of NOX2 and NOX4. GAPDH serves as an internal control. Data are presented as mean ± SD (n = 8 in each group). ***p < 0.001 vs. control group; ###p < 0.001 vs. Ang II group. **Abbreviation:** DHE, dihydroethidium; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; NOX2, NADPH oxidase 2; NOX4, NADPH oxidase 4; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Ang II + AS-IV group compared to the Ang II group (701.78 ± 85.01 vs 504.07 ± 25.62 pmol/mg protein, $p < 0.001$). On the other hand, SOD and CAT activities were also noticeably increased in the Ang II + AS-IV group compared to the Ang II group (13.82 ± 1.25 vs 29.54 ± 2.45 U/mg protein, $p < 0.001$ for SOD and 11.43 ± 1.19 vs 20.83 ± 3.29 U/mg protein, $p < 0.001$ for CAT, respectively) (Fig. 4D and E). Moreover, RT-qPCR was performed to measure the mRNA expression of NOX2 and NOX4. The results demonstrated that Ang II + AS-IV treatment significantly reduced the expression of NOX2 and NOX4 compared to the Ang II group (2.69 ± 0.33 vs 1.14 ± 0.24 fold, $p < 0.001$ for NOX2 and 3.15 ± 0.62 vs 1.44 ± 0.23 fold, $p < 0.001$ for NOX4, respectively) (Fig. 4F).

3.5. AS-IV enhanced the expression of SIRT1, PGC-1α and FNDC5 proteins in Ang II-treated mice

We measured the expression of the fibrotic genes such as α-SMA, Collagen I and Collagen III via RT-qPCR. The results showed that Ang II + AS-IV group significantly attenuated the expression of α-SMA, Collagen III and I compared to the Ang II group (3.32 ± 0.53 vs 1.41 ± 0.20 fold, $p < 0.001$; 3.41 ± 0.55 vs 1.48 ± 0.18 fold, $p < 0.001$; 2.34 ± 0.55 vs 0.99 ± 0.17 fold, $p < 0.001$, respectively) (Fig. 5A). In Ang II-induced mice, the protein levels of essential factors of the SIRT1/PGC-1α/FNDC5 pathway were assessed using Western blot (Fig. 5B and Supplementary Fig. S1). Ang II + AS-IV infusion remarkably enhanced the protein expression of SIRT1, PGC-1α, and FNDC5, compared to the Ang II group (0.22 ± 0.02 vs 0.57 ± 0.08 fold, $p < 0.001$; 0.28 ± 0.04 vs 0.72 ± 0.05 fold, $p < 0.001$; 0.38 ± 0.03 vs 0.68 ± 0.06 fold, $p < 0.001$, respectively) (Fig. 5C). We then measured the serum irisin levels in the four mice group by RT-qPCR. The results showed that Ang II treatment significantly reduced the serum irisin levels (53.23 ± 8.28 vs 28.89 ± 3.22 ng/mL, $p < 0.001$), whereas Ang II + AS-IV treatment potentially recovered the irisin level (28.89 ± 3.22 vs 50.95 ± 7.93 ng/mL, $p < 0.001$) (Fig. 5D).

4. Discussion

In the current research, we investigated the potential effects of AS-IV on Ang II-induced atrial fibrosis and AF. The findings demonstrated that AS-IV had no negative effects on the liver or kidney tissues and that the Ang II-induced mice's body mass, heart rate, or systolic blood pressure (SBP) had not changed significantly. In mice treated with Ang II, AS-IV treatment increased the expression of SIRT1, PGC-1α, and FNDC5 and potentially prevented AF, atrial fibrosis, and oxidative stress caused by Ang II. Thus, our results led us

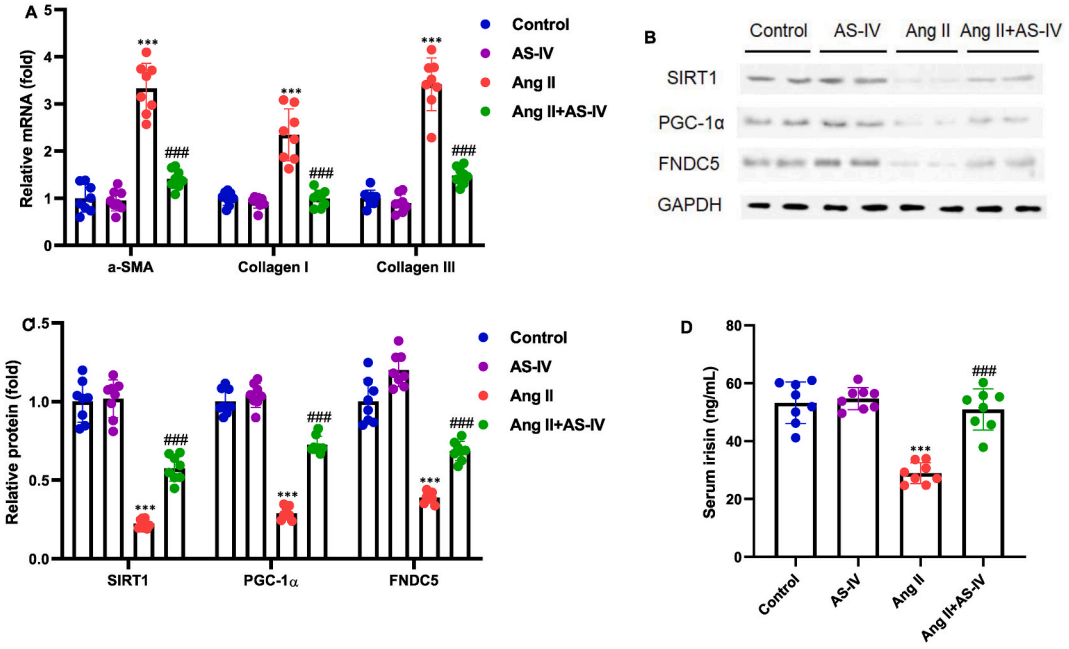

Fig. 5. AS-IV suppressed the expression of fibrotic genes and SIRT1, PGC-1α and FNDC5 proteins in Ang II-treated mice. (A) The mRNA expression of α-SMA, Collagen I and Collagen III. (B) Representative bands of SIRT1, PGC-1α and FNDC5 by Western blot. (C) Quantification of SIRT1, PGC-1α and FNDC5 proteins. Protein expression was normalized to the expression of GAPDH. (D) Serum irisin concentration in the mice of four groups. Data are presented as mean ± SD (n = 8 in each group). ***p < 0.001 vs. control group; ###p < 0.001 vs. Ang II group. **Abbreviation:** SIRT1, sirtuin-1; PGC-1α, peroxisome proliferator-activated receptor gamma co-activator 1-alpha; FNDC5, fibronectin type III domain containing 5.

to speculate that AS-IV could have potential protective effects against Ang II-induced atrial fibrosis and AF.

Mice were intraperitoneally administered with AS-IV (80 mg/kg/day) to examine the possible effects on safety and physiological parameters. The in vivo systemic toxicity of AS-IV was assessed after 28 days of intraperitoneal administration (80 mg/kg/day). The liver and kidney slices from the mice given saline or Ang II revealed no adverse effects (Fig. 1B). The same consistent results were observed in the case of the hepatic and renal function indicators of mice, including ALT, AST, Creatinine and BUN (Fig. 1C and D). Subsequent experiments were carried out to evaluate physiological features. Mice were administered intraperitoneal injections of AS-IV (80 mg/kg/day) and infused with Ang II (2.0 mg/kg/day) for 28 days. Our results showed that mice in the Ang II + AS-IV group did not experience significant changes in body weight but did show a marked reduction in heart rate and SBP (Fig. 1E, F, and 1G).

Several parameters are involved in developing and maintaining AF disorders [25]. The most common cause of structural remodeling and the structural basis for the persistence of AF in patients with AF is atrial fibrosis. AF is associated with structural, electrical, and autonomic remodeling of the left atrium (LA) [26,27]. Atrial fibrosis advancement in the LA aids explicitly in establishing focal activities and re-entries, constructing, and maintaining AF [28]. In both experimental and human AF, myocardial fibrosis is a common feature [29]. However, our data showed that mice in the AF group experienced typical AF attacks (Fig. 2A). Ang II + AS-IV treatment remarkably inhibited Ang II-induced AF inducibility, but AS-IV alone did not affect the AF inducibility in saline-infused mice (Fig. 2B). Overall AF duration was significantly reduced in mice treated with Ang II + AS-IV compared to mice only receiving Ang II infusion, with no significant difference observed following the AS-IV therapy alone ($p > 0.001$) (Fig. 2C). In addition, in comparison to saline therapy, Ang II treatment significantly increased the atrial fibrotic area, whereas Ang II + AS-IV treatment dramatically decreased this effect (Fig. 3A and B). Furthermore, Ang II + AS-IV markedly inhibited the increase in α-SMA-positive myofibroblasts induced by Ang II treatment (Fig. 3C and D). Thus, AS-IV could potentially protect against Ang II-induced atrial fibrosis and AF in mice.

Previous studies reported that AS-IV treatment might reduce oxidative stress by enhancing SOD activity and decreasing nicotinamide adenine dinucleotide phosphate oxidase (NOX) levels [30]. AS-IV, on the other hand, may significantly increase cell viability while decreasing MDA concentration and lactate dehydrogenase (LDH) activity in cardiomyocytes in the hypoxia/reoxygenation (H/R) model [31]. AS-IV may inhibit nicotinamide adenine dinucleotide phosphate (NADPH) oxidase–NF–κB signaling, reducing ROS generation in ISO-treated primary rat cardiac fibroblasts [32,33]. However, our study used atrial tissue lysate to assess several crucial oxidative stress markers, including MDA, SOD, and CAT. Ang II treatment markedly increased the production of ROS in the atrial tissue, as demonstrated by DHE staining. After pretreatment with Ang II + AS-IV, the number of DHE-positive cells decreased significantly compared to the group that only received Ang II ($p < 0.001$) (Fig. 4A and B). Additionally, the MDA content was significantly lower in the group that received Ang II + AS-IV compared to the group that only received Ang II ($p < 0.001$), as shown in Fig. 4C. However, compared to the Ang II group, SOD and CAT activities were potentially higher in the Ang II + AS-IV group (all, $p < 0.001$) (Fig. 4D and E). Moreover, NOX2 and NOX4 expression were considerably decreased by the Ang II + AS-IV treatment compared to the Ang II group (all, $p < 0.001$) (Fig. 4F). Thus, AS-IV suppressed oxidative stress in Ang II-induced atrial fibrosis.

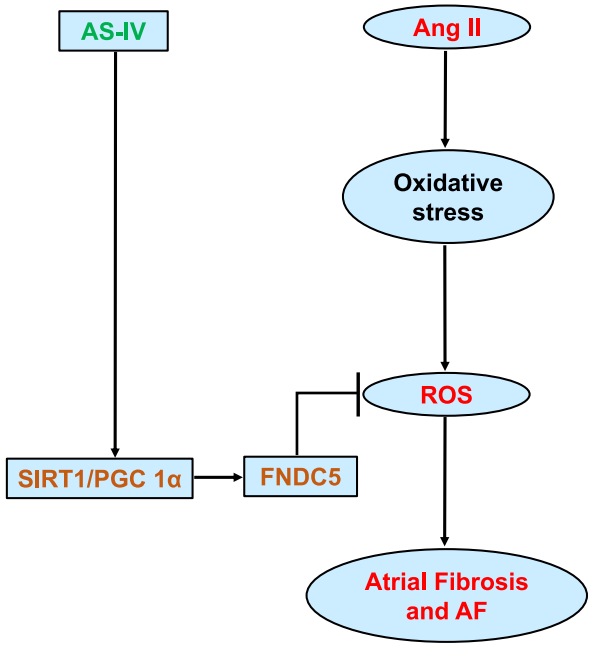

Fig. 6. Schematic illustration of the protective effects of AS-IV against Ang II-induced atrial fibrosis and AF via upregulation of the SIRT1/PGC-1α/FNDC5 pathway.

To further understand the mechanism behind AS-IV's protective effects on Ang II-induced atrial fibrosis and AF, the SIRT1/PGC-1α/FNDC5 signaling pathway was investigated. The nuclear protein Sirtuin 1 (SIRT1), which is highly conserved, is highly expressed in various tissues in the body. SIRT1 plays a role in regulating metabolism and inflammation, while PGC-1α is involved in regulating energy metabolism and mitochondrial biogenesis [34]. FNDC5 protein is involved in regulating energy metabolism and has been shown to be involved in regulating muscle function [29]. However, our study demonstrated that SIRT1, PGC-1α, and FNDC5 protein expression levels were enhanced in the Ang II + AS-IV group, compared to the Ang II group (all, $p < 0.001$) (Fig. 5C). Moreover, Ang II significantly decreased serum irisin levels ($p < 0.001$), but Ang II + AS-IV treatment may have restored irisin levels (Fig. 5D). These findings suggest that AS-IV inhibits Ang II-induced atrial fibrosis and AF in mice by upregulating the SIRT1/PGC-1α/FNDC5 signaling pathway.

5. Limitations

There are still several certain limitations in our study. The efficacy of the AS-IV was not checked in a dose-dependent manner. The study's underlying results have not been validated in preclinical or clinical settings. In addition, more investigation is needed to identify the causative mechanism underlying AS-IV's protective effects against Ang II-induced atrial fibrosis and AF. Furthermore, the use of Ang II in C57BL/6 may not be a suitable model for human AF [35]. However, our study provides significant aspects of AS-IV's protective effects against atrial fibrosis and AF.

6. Conclusion

In the present study, we investigated the potential protective effects of AS-IV on Ang II-induced atrial fibrosis and AF in mice. Our accumulating data suggests that AS-IV treatment significantly inhibited Ang II-induced atrial fibrosis and AF by upregulating the SIRT1/PGC-1α/FNDC5 pathway (Fig. 6). Thus, the study demonstrated that AS-IV would be a potential therapeutic drug against Ang II-induced atrial fibrosis and AF in the future.

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Data availability statement

Due to confidential issues, the datasets generated and/or analyzed during the current work are not publicly available but are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was approved (**ZPYYLL-2018–2**) by Shanghai Pudong New Area Zhoupu Hospital (Zhoupu Hospital affiliated to Shanghai Medical College of Health) Ethics Committee. The authors envisaged all standard protocols in accordance with the 1964 Declaration of Helsinki. All methods carried out in this study were in accordance with ARRIVE guidelines.

CRediT authorship contribution statement

Xinpeng Cong: Writing – original draft, Investigation, Conceptualization. **Xi Zhu:** Investigation, Formal analysis, Data curation. **Xiaogang Zhang:** Investigation, Formal analysis, Data curation. **Zhongping Ning:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, 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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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30984.

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