Original Paper

Aloe-Emodin Relieves High-Fat Diet Induced QT Prolongation via MiR-1 Inhibition and I_{K1} Up-Regulation in Rats

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Key Words

High-fat • QT interval • Aloe-emodin • I_{K1} • MiR-1

Abstract

Background/Aims: High-fat diet (HFD) causes cardiac electrical remodeling and increases the risk of ventricular arrhythmias. Aloe-emodin (AE) is an anthraquinone component isolated from rhubarb and has a similar chemical structure with emodin. The protective effect of emodin against cardiac diseases has been reported in the literature. However, the cardioprotective property of AE is still unknown. The present study investigated the effect of AE on HFD-induced QT prolongation in rats. **Methods:** Adult male Wistar rats were randomly divided into three groups: control, HFD, and AE-treatment groups. Normal diet was given to rats in the control group, high-fat diet was given to rats in HFD and AE-treatment groups for a total of 10 weeks. First, HFD rats and AE-treatment rats were fed with high-fat diet for 4 weeks to establish the HFD model. Serum total cholesterol and triglyceride levels were measured to validate the HFD model. Afterward, AE-treatment rats were intragastrically administered with 100 mg/kg AE each day for 6 weeks. Electrocardiogram monitoring and whole-cell patch-clamp technique were applied to examine cardiac electrical activity, action potential and inward rectifier K^+ current (I_{K1}), respectively. Neonatal rat ventricular myocytes (NRVMs) were subjected to cholesterol and/or AE. Protein expression of Kir2.1 was detected by Western blot and miR-1 level was examined by real-time PCR *in vivo* and *in vitro*, respectively. **Results:** *In vivo*, AE significantly shortened the QT interval, action potential duration at 90% repolarization (APD_{90}) and resting membrane potential (RMP), which were markedly elongated by HFD. AE increased I_{K1} current and Kir2.1 protein expression which were reduced in HFD rats. Furthermore, AE significantly inhibited pro-arrhythmic miR-1 in the hearts of HFD rats. *In vitro*, AE decreased miR-1 expression levels resulting in an increase of Kir2.1 protein levels in cholesterol-enriched NRVMs. **Conclusions:** AE prevents HFD-induced QT prolongation by repressing miR-1 and upregulating its target Kir2.1. These findings suggest a novel pharmacological role of AE in HFD-induced cardiac electrical remodeling.

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Introduction

Hyperlipidemia and other life-style related diseases, are characterized by the metabolic disorder of blood lipids and cholesterol [1]. Numerous studies have found that metabolic disorder is an important risk factor for cardiovascular diseases, such as atherosclerosis, coronary artery disease, and even sudden cardiac death [2]. Epidemiological studies have suggested that high-fat diet (HFD) contributes to hyperlipidemia and obesity [3]. Hypercholesterolemia induced pro-arrhythmic neural and electrophysiological remodeling was associated with prolonged action potential duration, longer QTc intervals, increased repolarization dispersion, and increased ventricular vulnerability to fibrillation [4]. Some experimental and clinical studies have shown that HFD is related to QT prolongation, atrial fibrillation [5, 6]. However, underlying mechanisms are not fully understood, and better therapeutic measurements are urgently needed.

Recently, the use of traditional Chinese medicine has been gaining increasing attention. For instance, the pervasive effects of Tanshinone and Berberin in the prevention and cure of common cardiovascular diseases has been clarified [7, 8]. In the current study, Aloe-emodin (Fig. 1A) is a monomer of phenanthrenequinones extracted from rhubarb and has a similar chemical structure with emodin [9]. Several studies have reported the protective effect of emodin against cardiac diseases. For example, Feng et al. demonstrated the protective effect of emodin against metabolic disorders by selectively inhibiting 11 β -hydroxysteroid dehydrogenase type 1 in diet-induced obese mice [10]. In addition, another study also found that emodin treatment can ameliorate hypercholesterolemia by binding with sodium deoxycholate [11]. Until now, various pharmacological effects of AE including anti-neoplasticity [12], anti-inflammation [13], and strengthening of immunity [14], have been elucidated. However, the potential role of AE in the cardiovascular system is limited. Thus, the present study focused on the relationship between AE and HFD-induced cardiac dysfunction.

MicroRNAs (miRNA, miR) are a group of endogenous non-coding RNA, approximately 22 nucleotides in length, which play important roles in post-transcriptional gene regulation [15]. Numerous studies suggest that miRNAs are implicated in the development of cardiovascular diseases [16, 17]. Among them, many studies have demonstrated that miR-1 plays a critical role in the occurrence of both ventricular and atrial arrhythmias by post-transcriptionally modulating ion channels and proteins related to cardiac electrical activity [18-20]. Based on these findings, the present study was conducted to investigate the two aspects. The first aim is to explore the effect of AE on HFD-induced QT prolongation. The second aim is to decipher the molecular mechanism of AE by regulating miR-1 and related ion channels. The current study enhances the understanding on the pharmacological role of AE, which may be useful for further development of novel anti-arrhythmic drug.

Materials and Methods

Animals

Animals were obtained from the Experimental Animal Center of Harbin Medical University and were maintained at a temperature of 22 °C–24 °C with a 12 h light-dark cycle. All of the animal experiments were conducted according to the Ethics Committee of Harbin Medical University, and the use of animals complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8th Edition, 2011). Male Wistar rats weighing 180–220 g (two months old) were randomly divided into control, HFD, and AE-treatment groups. Normal diet was given to rats in the control group, and high-fat diet was given to rats in the HFD and AE groups for a total of 10 weeks. First, HFD rats and AE-treatment rats were fed with high-fat diet for 4 weeks to establish the HFD model. Serum total cholesterol (TC) and triglyceride (TG) were detected to validate HFD model. Then, 100 mg/kg AE (98% purity, Tianfeng Biotechnology Co., Ltd., Xian, China) was dissolved in water and intragastrically administered daily for another 6 weeks.

Diets

Conventional food was obtained from the Animal Center of the Second Affiliated Hospital of Harbin Medical University. High-fat food comprises 10% lard, 10% egg yolk powder, 2% cholesterol, 0.2% bile salts, 0.2% methyl oxygen pyrimidine, and 77.6% basal feed. High-fat food was provided by HuaFuKang Biotechnology Co., Ltd. (Beijing, China).

Neonatal rat ventricular myocytes culture

The procedures for culturing neonatal rat ventricular myocytes (NRVMs) were according to previous work [21]. Briefly, the hearts of neonatal Wistar rats were rapidly removed, minced in serum-free Dulbecco's Modified Eagle Media (DMEM, HyClone, Logan, UT), and then digested in 0.25% trypsin solution. Dispersed cells were suspended in DMEM containing 10% fetal bovine serum and then centrifuged. The isolated cells were plated onto dishes at a density of 10^5 cells·cm⁻². Then, 0.1 mM bromodeoxyuridine was added into the medium to deplete nonmyocytes. Cardiomyocytes were cultured under a condition of 5% CO₂ at 37 °C. Cardiomyocytes were starved in serum-free medium for 24 h, and then transiently transfected with miR-1 (50 nM), miR-1 inhibitor (100 nM), or negative control (NC), using X-treme GENE siRNA transfection reagent (Roche, USA) according to the manufacturer's instructions. MiR-1 mimic (5'-UGGAAUGUAAAGAAGUGU GUAU-3'), miR-1 inhibitor (5'-ATACACACTTCTTTTACATTCCA-3'), and NC were synthesized by Guangzhou RiboBio (Guangzhou, China). Twenty four hours after transfection, cholesterol was prepared in ethanol and added into the medium at 10 µg/mL for 6 h. Cholesterol model was employed *in vitro* to mimic high-fat *in vivo*. After that, AE was prepared in DMSO and added into the medium at 90 µM for 24 h. The final concentration of ethanol or DMSO was less than 0.1%. In protocol 1, cells were randomly divided into control, cholesterol, cholesterol+miR-1 inhibitor, and cholesterol+NC groups. In protocol 2, cells were randomly divided into control, cholesterol, cholesterol+AE, cholesterol+AE+miR-1 mimic, cholesterol+AE+NC, and AE alone groups, respectively.

Measurement of serum lipid indexes

Blood samples (0.5-1 ml) were collected from the tail vein after high-fat diet for four weeks. The blood samples were placed on ice after 30 min and then centrifuged at 3,000 rpm for 10 min at 4 °C. Supernatants were extracted according to the kit instructions (Jiancheng Bioengineering Institute, Nanjing, China). Serum TC and TG contents were determined at 500 nm with an Infinite M200 microplate reader (Tecan, Salzburg, Austria).

Electrocardiogram analysis

Standard lead II electrocardiogram (ECG) was monitored using a BL-420F bio-functional experiment system (TME Technology Co., Ltd., Chengdu, China) after the rats were administrated AE for 0, 2, 4, and 6 weeks, respectively. Adult rats were anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg). The pedal withdrawal reflex (assessed by pinching the tail and the metacarpal region of the hind foot between the index finger and the thumb) was tested to assess the depth of anesthesia [22]. ECG recording was performed for 15 min every time. Six recordings were made for each group. The ECG waveforms averaged over five consecutive beats were used for analyses. The heart rate, PR interval, QRS duration and QT interval were measured from the ECG. The heart rate-corrected QT interval (QTc) was calculated by the Bazett formula: $QTc = QT/(RR/150)^{1/2}$, which was adjusted for rats [23]. The end of the T-wave was identified as the point at which the slow component of the biphasic T-wave returned to the isoelectric line. Five QRS complexes were analyzed for each rat. The surviving animals were then sacrificed, and the hearts were quickly excised, and used for the subsequent experiments.

Isolation of ventricular myocytes

The procedure for cardiomyocyte dissociation was described in a previous work [24]. Rat ventricular myocytes were briefly isolated by enzymatic dissociation. Rats were anaesthetized with sodium pentobarbital (40 mg/kg, ip). The hearts were excised rapidly and perfused through the aorta using Langendorff method for 5 min with Ca²⁺-free Tyrode's solution containing (in mM): NaCl 136.0, KCl 5.4, MgCl₂ 1.0, NaH₂PO₄ 0.33, glucose 10.0, and HEPES 10.0 (pH adjusted to 7.4 with NaOH). The hearts were then digested with Ca²⁺-free Tyrode's solution containing 0.2 mg/mL collagenase II (Worthington Biochemicals, Lakewood, NJ, USA) and 0.2 mg/mL bovine serum albumin (Gibco-BRL, Grand Island, NY, USA) for another 30-50 min. All of the solutions were oxygenated and warmed at 37 °C during perfusion. Single cardiomyocytes were

stored in Kraftbruhe solution containing (in mM): glutamic acid 70.0, taurine 15.0, KCl 30.0, HEPES 10.0, KH_2PO_4 10.0, MgCl_2 0.5, glucose 10, and EGTA 0.5 (pH adjusted to 7.4 with KOH). Single cell was obtained by gentle pipetting and stored at 4°C for 1 h before patch-clamp recording. Only rod-shaped and clear-texture cardiomyocytes were selected for further studies.

Whole-cell patch-clamp technique

Whole-cell patch-clamp technique was used to record the action potential (AP) and the inward rectifier K^+ current (I_{K1}) with an Axopatch™ 200B amplifier and analyzed using pCLAMP 10.0 software (Axon Instruments, Inc., Foster City, CA, USA). Cardiomyocytes were placed in the chamber at room temperature with Ca^{2+} -containing Tyrode's solution containing (mM): NaCl 136, KCl 5.4, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 0.33, CaCl_2 1.8, HEPES 10, and glucose 10 (pH adjusted to 7.4 with NaOH). Borosilicate glass electrodes with a tip resistance of 2–4 MΩ were used and filled with the pipette solution containing (in mM): KCl 20, Kaspargate 110, MgCl_2 1, HEPES 5, EGTA 10, and Na_2ATP 5 (pH adjusted to 7.2 with KOH). AP and I_{K1} were recorded by the current-clamp and voltage-clamp mode, respectively. I_{K1} was stimulated by using a holding potential of -40 mV and 300 ms pulses ranging from -120 mV to +50 mV with an increment of 10 mV.

Western blot analysis

Total protein samples were extracted from rat ventricular tissues or NRVMs as in previous studies [25]. Protein samples (~70 μg) were fractionated by SDS-PAGE (10% polyacrylamide gels) and transferred to nitrocellulose membrane. The membranes were suspended in 5% non-fat milk at room temperature for 2 h. The membranes were then incubated at 4°C overnight with the primary antibodies as follows: anti-Kir2.1, anti-Kv4.2, anti-Kv4.3 (1:200, Alomone Labs, Jerusalem, Israel), anti-Cx43 (1:500, Proteintech Group, Chicago, IL, USA), and anti-GAPDH (1:1,000, Kangcheng, Shanghai, China). The membranes were then washed thrice for 10 min each time with PBS-T, and incubated with secondary antibody for 1 h. Western blot bands were quantified by Odyssey CLx v2.1 software (LI-COR Biosciences, Lincoln, NE, USA).

Real-time PCR analysis

Total RNA samples from rat ventricles or NRVMs were extracted using TRIZOL reagent (Invitrogen, USA) according to the manufacturer's instructions. RNA concentration was assessed using the NanoDrop™ 8000 spectrophotometer (Thermo Scientific, France). cDNA was synthesized using a reverse transcription kit (Roche, USA). Quantitative real-time PCR was performed using SYBR Green PCR Master Mix on Light Cycler 480 RT-PCR system (Roche, USA). The sequences of primers were: miR-1 RT: GTCGTATCCAGTGCACCGAGGTATTTCGACTGGATACGACATACAC; forward: 5'-GCGGCGGTGGAATGTAAAGAAG-3' and reverse: 5'-ATC-CAGTGCAGGGTCCGAGG-3'; U6 RT: CGCTTCACGAATTTGCGTGTTCAT; forward: 5'-GCTTCGGCAGATATACTA-AAAT-3' and reverse: 5'-CGCTTCACGAATTTGCGTGTTCAT-3'. MiR-1 levels were quantified with the $2^{-\Delta\Delta\text{Ct}}$ relative quantification method that was normalized to U6.

Data analysis

All of the data were presented as mean ± SD. Two group comparisons were performed by *t*-test. One-way analysis of variance followed by Tukey's test was used for multiple comparisons. A two-tailed *P* < 0.05 indicated a statistically significant difference. Data were analyzed with GraphPad Prism 5.0.

Results

Effects of HFD on serum lipids

We validated HFD model successfully that serum levels of TC and TG were significantly increased in the group of rats fed with HFD (n=20) compared with control group (n=10). (Fig. 1B and C). Our results also showed that the body weight and the ratio of heart weight to body weight (HW/BW) were not changed in HFD rats compared with those in the control group, and were not changed after administration of AE for 6 weeks as compared with HFD rats (Fig. 2A and B).

Effect of AE on ECG in HFD rats

To explore the role of AE on the regulation of cardiac arrhythmia and electrophysiology, we set up an experimental model with AE-treatment in HFD rats. ECG was recorded after AE was administered for 0, 2, 4, and 6 weeks, respectively (Fig. 3A). The heart rate, PR interval, QRS duration, QT interval, and QTc were measured and calculated from the ECG (Fig. 3B to F). The heart rate was significantly slower with QT interval and QTc prolongation in HFD group as compared with that in the control group (Fig. 3B, E, and F), which were recovered after administration of AE for 6 weeks but not recovered after administration of AE for 0, 2, and 4 weeks. Clearly, AE-treatment for 6 weeks markedly shortened QTc (69.67 ± 4.56 ms), which was significantly prolonged by HFD (85.40 ± 14.62 ms) compared with that in the control group (68.37 ± 5.63 ms, Fig. 2F). No significant difference of PR interval and QRS duration were examined among these groups (Fig. 3C and D). These results indicated that AE prevented QTc prolongation induced by HFD.

Effect of AE on APD in ventricular cardiomyocytes of HFD rats

To confirm the effect of AE on cardiac electrical activity, we recorded the single-cell AP and compared the action potential duration (APD) by whole-cell patch-clamp in ventricular cardiomyocytes isolated from three different groups of rats (Fig. 4A). HFD markedly prolonged the action potential duration at 90% repolarization (APD_{90} , 149.20 ± 29.82 ms), compared with that in the control group (92.71 ± 19.19 ms, $P < 0.001$). AE markedly shortened the APD_{90} (105.80 ± 26.22 ms), compared with that in the HFD group ($P < 0.01$, Fig. 4B). In addition, the RMP of cardiac myocytes in the HFD group were significantly depolarized (-77.96 ± 8.64 mV), compared with that in the control group (-64.81 ± 6.42 mV, $P < 0.01$), which was recovered by AE (-73.72 ± 6.25 mV, Fig. 4C). These results indicated

Fig. 1. Chemical structure of AE and effects of high-fat diet (HFD) on serum lipids. (A) Chemical structure of AE. (B) Serum total cholesterol (TC) level. (C) Serum triglyceride (TG) level. Data are shown as mean $\pm$ SD, t-test analysis of variance (ANOVA). * $P < 0.05$, *** $P < 0.001$, versus Control. $n = 10$ rats in the control group, $n = 20$ rats in the HFD group.

Fig. 2. Comparisons of body weight and HW/BW of different groups. (A) Comparisons of body weight. (B) Comparisons of HW/BW. HW/BW: the ratio of heart weight to body weight. Data are shown as mean $\pm$ SD, one-way ANOVA. $n = 5$ rats/group.

Fig. 3. Effect of AE on ECG in HFD rats. (A) Representative traces of the ECG of rats after administration of AE for 0, 2, 4, and 6 weeks, respectively. (B) Heart rate. (C) PR interval. (D) QRS duration. (E) QT interval. (F) Heart rate-corrected QT interval (QTc). Data are shown as mean $\pm$ SD, one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, versus Control; # $P < 0.05$, versus HFD, $n = 6$ rats/group.

that AE could reverse the adverse electrophysiological changes manifested by abnormal APD prolongation and membrane depolarization in the HFD condition.

Effect of AE on I_{K1} in ventricular cardiomyocytes of HFD rats

I_{K1} plays an important role in the maintenance of RMP and is involved in the third period of action potential repolarization. Except for APD prolongation, HFD also depolarized RMP, suggesting that the current density of I_{K1} was down-regulated by HFD. Our results showed that I_{K1} current was significantly decreased in HFD rats (Fig. 5A and B). The current density of I_{K1} was significantly decreased from -18.99 ± 1.53 pA/pF of the control group to -30.78

Fig. 4. Effect of AE on APD in ventricular cardiomyocytes of HFD rats. (A) Representative traces of action potential. (B) APD_{90} of cardiomyocytes. (C) RMP of cardiomyocytes. Data are shown as mean $\pm$ SD, one-way ANOVA. ** $P < 0.01$, *** $P < 0.001$, versus Control; # $P < 0.05$, ## $P < 0.01$, versus HFD, $n = 8$ cells from 5 rats/group.

Fig. 5. Effect of AE on I_{K1} in ventricular cardiomyocytes of HFD rats. (A) Voltage protocol and representative current traces. (B) Averaged current density-voltage (I-V) relationships of I_{K1} . (C) Current densities of I_{K1} at a stimulus voltage of -120 mV. Data are shown as mean $\pm$ SD, one-way analysis of variance. *** $P < 0.001$, versus Control; ## $P < 0.01$, versus HFD, $n = 8$ cells from 5 rats/group.

± 2.35 of the HFD group, whereas the AE-treatment group reverted to -25.16 ± 0.97 pA/pF (Fig. 5C).

Effect of AE on Kir2.1 protein expression in the hearts of HFD rats

As the above mentioned results indicated, the I_{K1} densities increased as an ionic mechanism underlying the effect of AE to prevent or correct the HFD-induced QTc and APD

Fig. 6. Effects of AE on the expression of Kir2.1 protein and level of miR-1 in the hearts of HFD rats. (A) Relative level of Kir2.1 protein. $n = 6$ rats/group. (B) Relative level of miR-1. $n = 6$ rats/group. (C) Relative level of CX43 protein. $n = 5$ rats/group. Data are shown as mean $\pm$ SD, one-way ANOVA. ** $P < 0.01$, *** $P < 0.001$, versus Control; ## $P < 0.01$, ### $P < 0.001$, versus HFD.

prolongation. We inferred that AE might act by upregulating the expression of the genes encoding the ion channel protein of I_{K1} . Kir2.1 is the main K^+ channel subunit that mediates I_{K1} . Kir2.1 protein expression was detected by Western blot. Results showed that the protein expression of Kir2.1 in the HFD group was significantly decreased, as compared with that in the control group ($P < 0.001$). AE recovered Kir2.1 expression to normal levels (Fig. 6A). On the other hand, transient outward K^+ current (I_{to}), is known to be responsible for the initial phase of cardiac repolarization [26]. I_{to} is a current flowing through the K^+ channels composed of Kv4.2 and Kv4.3 as the main pore-forming α -subunits. A similar pattern of difference in Kv4.2 protein level was consistently observed in the HFD group but not in the AE group. The Kv4.3 protein level was not changed in the HFD and AE-treatment groups (Fig. 7A and B).

Effect of AE on the miR-1 level in the hearts of HFD rats

The inward rectifier potassium channel subunit Kir2.1 is encoded by the KCNJ2 gene, which is a target of miR-1. To verify the involvement of miRNAs in the regulation of Kir2.1 expression by AE, we further measured the level of miR-1 in this study. It was found that the level of miR-1 was increased (~ 2.73 -fold) in the hearts of HFD rat hearts as compared with that in the control group (Fig. 6B). Overexpression of miR-1 was reversed by AE. A validated and established target for miR-1 is also connexin 43. The connexin 43 protein level was down-regulated in HFD rats, but was not reversed by AE (Fig. 6C). Taken together, the results generally suggested that the expression of Kir2.1 protein was inhibited by the overexpression of miR-1 and AE may down-regulate the overexpression of miR-1 induced by long-term HFD, therefore recovering the decreased level of Kir2.1 protein in HFD rats.

Effects of AE on regulation of the level of miR-1 and Kir2.1 in vitro

To examine the effects of AE on cardiomyocytes suffering from HFD, NRVMs were cultured and subjected to cholesterol-enrichment to simulate HFD *in vitro*. Consistently, we

Fig. 7. Comparisons of the expressions of Kv4.2 and Kv4.3 proteins in the hearts of rats in different groups. (A) Relative level of Kv4.2 protein. $n = 5$ rats/group. (B) Relative level of Kv4.3 protein. $n = 4$ rats/group. Data are shown as mean $\pm$ SD, one-way ANOVA. ** $P < 0.01$, versus Control.

Fig. 8. Effects of AE on miR-1 and Kir2.1 on cholesterol-enriching neonatal rat ventricular myocytes (NRVMs). (A) Effect of cholesterol on miR-1 in NRVMs. $n = 5$ batches of cells. (B) Effect of cholesterol on Kir2.1 in NRVMs. $n = 5$ batches of cells. (C) Effect of AE on miR-1 in cholesterol-enriching NRVMs. $n = 4$ batches of cells. (D) Effect of AE on Kir2.1 in cholesterol-enriching NRVMs. $n = 4$ batches of cells. Data are shown as mean $\pm$ SD, one-way ANOVA. * $P < 0.05$, versus Control; # $P < 0.05$, ## $P < 0.01$, versus Cholesterol; ^ $P < 0.05$, ^^^ $P < 0.001$, versus Cholesterol+AE.

found that the miR-1 level was significantly higher with lower expression of Kir2.1 protein in cholesterol cells than in control cells. This alteration was markedly reversed by miR-1 inhibitor (Fig. 8A and B). Furthermore, the down-regulation of miR-1 and up-regulation

of the Kir2.1 protein in cholesterol+AE cells were restored by miR-1 mimic (Fig. 8C and D). These results strongly supported that AE prevented the downregulation of Kir2.1 by repressing miR-1 in the HFD condition.

Discussion

In this study, we examined QT-prolongation in HFD rats. To the best of our knowledge, we are the first to find that AE significantly inhibited HFD-induced QT-prolongation by repressing miR-1 and restoring I_{K1} /Kir2.1. Overall, our findings uncover a novel cardioprotective effect of AE against QT-prolongation.

A number of previous studies have indicated that emodin has a promising protective effect against several cardiac diseases. For example, Wu et al. found that emodin protected against diabetic cardiomyopathy by modulating Akt/GSK-3 β cascade in rats [27]. Emodin also inhibited acute cardiac ischemic injury through mitigation of local inflammation and apoptosis in mice [28]. Furthermore, emodin has hypoglycaemic and hypolipidaemic effects on dyslipidaemic-diabetic rats by upregulating L-type calcium channels in the pancreas and hearts [29]. By contrast, although the chemical structure of AE is similar to emodin, whether the application of AE has a cardioprotective effect is unknown. Accordingly, the current study was conducted to investigate the potential beneficial role of AE in HFD-induced QT-prolongation.

Previous studies have revealed that HFD promoted arrhythmic death and increased myocardial ischemia-reperfusion injury in rats [30]. Moreover, other studies also detected increased vulnerability of atrial arrhythmia and impaired conduction velocity in HFD mice [31]. Consistently, the current study found that HFD for 4 weeks caused a dysfunction in cardiac electrophysiology and increased QT-interval. Thus, the present study mainly focused on the relationship between AE and HFD-induced ventricular arrhythmias. Based on ECG recordings and patch-clamp data, our study demonstrated that AE significantly relieved HFD-induced QT prolongation by regulating inward rectifier potassium channel (I_{K1}).

Increasing evidences support that intracellular lipid content can regulate ion channels. In this study, we found that I_{K1} current and Kir2.1 expression was decreased in HFD rats. The electrical activity of the heart is an extraordinary arrangement by multiple categories of ion channels, and the movement of ions across the cytoplasmic membrane of cardiomyocytes is controlled by transmembrane proteins [32]. The membrane potential and the rate of membrane repolarization are managed by potassium (K^+) channels [33]. Among these channels, I_{K1} is a strong inward rectifier K^+ -selective current, which plays important roles in modulating the RMP and the final repolarization phase of action potential in cardiomyocytes [34]. I_{K1} also plays an important role in ventricular arrhythmias, highlighted by the Andersen-Tawil syndrome, associated with QT interval prolongation, and also mentioned as the LQT7, a form of the LQT syndrome [35, 36]. Down-regulation of I_{K1} may induce RMP depolarization and APD prolongation, resulting in QT prolongation. As a result of depolarization, the conductivity and automaticity of cardiomyocytes were inhibited, whereas the excitability of cardiomyocytes was increased. The dysfunction of I_{K1} in the hearts of HFD rats was caused by down-regulation of the protein expression of Kir2.1, which was the pore-forming subunit of the potassium channel to carry I_{K1} [37]. AE recovered the protein expression of Kir2.1. This result was in line with the QT interval shortening role of AE.

miR-1 is known to play an essential role in ventricular arrhythmias [38]. The I_{K1} subunit Kir2.1 (encoded by KCNJ2 gene) is a validated target of miR-1. In addition, previous studies have demonstrated that several agents, such as propranolol and Tanshinone effectively inhibited miR-1 expression to prevent ventricular arrhythmias [39, 40]. Lu et al. revealed that the beta-adrenoceptor-cAMP- PKA signaling pathway contributed to miR-1 expression, and serum response factor (SRF), which is known as one of the transcriptional enhancers of miR-1. Moreover, propranolol inhibited the beta-adrenoceptor-cAMP-PKA signaling pathway and suppressed SRF expression and partially produced beneficial effects by down-regulating

miR-1 [39]. Moreover, Shan et al. found that down-regulation of miR-1 and consequent recovery of Kir2.1 may partially account for the efficacy of Tanshinone IIA in suppressing ischemic arrhythmias and cardiac mortality [40]. Furthermore, Tanshinone IIA could inhibit miR-1 expression by inhibiting activated p38 MAPK and heart special transcription factors, SRF and MEF2, in ischemic and hypoxic cardiomyocytes [41]. Therefore, miR-1 was a key molecule in the development of ventricular arrhythmias, which was emphasized in the current study. Our results showed that both AE could down-regulate the level of miR-1 and up-regulate the expression of Kir2.1 protein *in vivo* and *in vitro*. However, the mechanism between AE and miR-1 is unclear and needs further investigation. Lin et al. found that AE inhibited the invasion of nasopharyngeal carcinoma cells by suppressing the expression of MMP-2 via the p38 MAPK-NF-kappaB signaling pathway [42]. In future work, we will propose potential mechanisms between Aloe-emodin and miR-1 expression from the perspective of the p38 MAPK signal pathway. We also hypothesize that the SRF, a transcriptional activator of the miR-1 gene was involved in the signal pathway. It should be noted that although AE significantly repressed miR-1 expression in HFD rats, other miRNAs could not be excluded in the pharmacological effect of AE.

Although we confirmed AE effects on high-fat diet induced QT prolongation, limitations still remain in this study, such as effects of AE on I_{Kr}/hERG. Because I_{Kr} current is difficult to capture in Wistar rats, we did not examine the change of I_{Kr}/hERG in this study. Thus, the contribution of I_{Kr} in HFD-induced QT prolongation should be considered when interpreting our results. Further studies are needed to examine the alteration of I_{Kr} in cardiomyocytes of guinea pigs. In conclusion, the present study provides insights into the new pharmacological role of AE against HFD-induced QT prolongation.

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Disclosure Statement

The authors declare no competing financial interests.

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