Blueberry anthocyanin-enriched extract ameliorates transverse aortic constriction-induced myocardial dysfunction via the DDAH1/ADMA/NO signaling pathway in mice

WEIQING HU[1], WENYUE WANG[2], QING MA[3], TAO LIU[1], JIEFENG ZHANG[1] and JICUN ZHANG[1]

[1]Department of Vascular Surgery, The People' Hospital of Weifang City, Weifang, Shandong 261000; Departments of [2]Obstetrics and Gynecology, and [3]Pharmacy, Weifang Hospital of Traditional Chinese Medicine, Weifang, Shandong 261041, P.R. China

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Abstract. Blueberry anthocyanin-enriched extract (BAE) has been demonstrated to protect against cardiovascular diseases by activating multiple target genes. The present study investigated the effects of BAE on transverse aortic constriction (TAC)-induced myocardial dysfunction in mice and explored its possible molecular mechanisms. A total of 30 male mice were divided randomly into control, TAC and TAC + BAE groups. Mice in the TAC + BAE groups were administered BAE by oral gavage for 6 consecutive weeks. Myocardial dysfunction was assessed using echocardiogram, histopathology, TUNEL assay, immunofluorescence staining, reverse transcription-quantitative PCR and western blot analysis. The results demonstrated that BAE treatment significantly ameliorated heart weight, left ventricular weight, myocardial dysfunction, left ventricular hypertrophy and fibrosis. In addition, BAE treatment alleviated TAC-induced inflammation, oxidative stress and apoptosis. Notably, BAE treatment markedly reduced asymmetric dimethylarginine (ADMA) concentration and significantly increased dimethylarginine dimethylaminohydrolase 1 (DDAH1) expression and nitric oxide (NO) production. The present data indicated that BAE treatment ameliorated TAC-induced myocardial dysfunction, oxidative stress, inflammatory response and apoptosis via the DDAH1/ADMA/NO signaling pathway.

Correspondence to: Professor Jicun Zhang, Department of Vascular Surgery, The People' Hospital of Weifang City, 151 Guangwen Street, Kuiwen, Weifang, Shandong 261000, P.R. China
E-mail: jicunzhang8@126.com

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Introduction

Transverse aortic constriction (TAC) causes compensatory hypertrophy, chronic maladaptive hemodynamic overload, cardiac dilatation and heart failure over time (1). TAC is often used as an experimental method to produce pressure overload, cardiac hypertrophy and heart failure in mice (2). Although it has been well demonstrated that TAC-induced myocardial dysfunction is associated with inflammation, oxidative stress and cardiomyocyte apoptosis (3-6), the molecular signaling pathways involved in TAC-induced myocardial dysfunction have yet to be elucidated. Therefore, it is important to investigate possible target genes and to identify possible treatments for TAC-induced myocardial dysfunction.

Asymmetric dimethylarginine (ADMA) is reported to be an endogenous nitric oxide synthase (NOS) inhibitor, and its accumulation is associated with various cardiovascular diseases, including hypertension, diabetes and cardiac dysfunction (7-10). ADMA attenuates endothelial NOS (eNOS) activity to reduce nitric oxide (NO) production and induce NOS uncoupling to generate reactive oxygen species (ROS) (8,11). Dimethylarginine dimethylaminohydrolase 1 (DDAH1) serves an important role in regulating vascular endothelial injury repair and angiogenesis, as well as functions in degrading ADMA to maintain NO signaling (12,13). Overexpression of the DDAH1 gene reduces inflammatory infiltration through decreasing ADMA expression (14). Serum ADMA concentration was slightly higher when left ventricle DDAH1 expression was low, while DDAH1 knock out (KO) exacerbated left ventricle hypertrophy, fibrosis and dysfunction in mice after TAC (15). These findings suggest that DDAH1 may protect cardiac hypertrophy and ventricular remodeling against stress conditions.

Berry anthocyanins have recently drawn widespread scientific interest due to their diverse health benefits, including antioxidant, anti-inflammatory, antihypertensive, anti-atherosclerotic, antimicrobial, anticancer and neuroprotective properties (16). According to National Health and Nutrition Education Survey (NHANES), an average of 12.5 mg of anthocyanins are consumed per day by people in the United States (17). *In vitro* and *in vivo* studies have shown that blueberry

anthocyanins activate cellular antioxidant systems and inhibit inflammatory infiltration, and protect against inflammation and oxidative stress, through activation of multiple target genes. The present study investigated the effects and potential mechanisms of blueberry anthocyanin-enriched extract (BAE) on TAC-induced myocardial dysfunction.

Materials and methods

Experimental animals. A total of 30 male C57BL/6 mice (age, 6-8 weeks; weight, 20-25 g) were obtained from the Experimental Animal Center of the General Hospital of Shenyang Military Command. All animals were housed at 20±2˚C and 55-60% humidity under a 12-h light/dark cycle, were provided standard laboratory animal feed and water *ad libitum*, and were allowed to acclimate for 7 days before the study. All experimental animal procedures were approved by the Animal Ethics Committee of the People's Hospital of Weifang City.

Materials and reagents. BAE was provided by the Food College of Shenyang Agricultural University (18). The total anthocyanin content was ~25.7/100 g of extract. The composition of BAE, as measured by high-performance liquid chromatography/mass spectrometry, was: Malvidin 3-galactoside (28.11%), malvidin 3-arabinoside (16.18%), malvidin 3-glucoside (14.08%), malvidin 3-(6''-acetyl) glucoside (8.49%), malvidin 3-(6''-acetyl) galactoside (5.50%), petunidin 3-galactoside (5.44%), petunidin 3-glucoside (5.26%), peonidin3-glucoside (5.22%), cyanidin3-galactoside (2.96%) and delphinidin 3-glucoside (1.41%).

Experimental protocol. After acclimation, the 30 mice were divided randomly into control, TAC and BAE groups. The mice in the control group did not undergo surgery. The mice in the BAE group were modelled for TAC and, following a recovery period of 24 h after the surgery, they were administered with 0.5 g/kg BAE daily by oral gavage for 6 consecutive weeks. Mice in the control and TAC groups were given the same volume of distilled water.

TAC model. TAC models were created as described previously (19). Briefly, mice were anesthetized in an induction chamber with 2% isoflurane mixed with 0.5-1.0 l/min 100% O_2. Mice were fixed in a supine position on top of a heating pad to maintain body temperature. Partial thoracotomy at the second rib was performed under a surgical microscope and the sternum was retracted using a chest retractor. Fine tip 45˚ angled forceps were used to gently separate the thymus and fat tissue from the aortic arch. Following identification of the transverse aorta, a small piece of a 6.0 silk suture was placed between the innominate and left carotid arteries. Two loose knots were tied around the transverse aorta and a small piece of a gauge blunt needle was placed parallel to the transverse aorta. The first knot was quickly tied against the needle, followed by the second one, and the needle was promptly removed to yield a constriction of 0.4 mm in diameter. The chest retractor was removed and the outflow of the ventilator pinched off for 2 sec to re-inflate the lungs. The rib cage was closed using a 6.0 prolene suture with an interrupted suture pattern. The skin was closed using a 6.0 prolene suture with a continuous suture pattern.

Echocardiogram. All animals were anesthetized with 1.6% isoflurane and were assessed by echocardiogram (ECHO; Vevo 770, a 12 MHz transducer; VisualSonics Inc.) according to previous literature (20). Briefly, to ensure that the mitral and aortic valves and the apex were visualized, parasternal long axis views were obtained and recorded. Short axis views were recorded at the mid-papillary muscle level. To calculate the end-systolic left ventricular (LV) area, endocardial area tracings were obtained using 2D mode from digital images captured on cine-loop. All measurements were made by a single observer and were averaged over 3-5 consecutive cardiac cycles. The reproducibility of measurements was assessed in two sets of baseline measurements in 10 randomly selected rats. Repeated measure variability did not exceed 65%.

Sample collection. Briefly, mice were anesthetized by intraperitoneal injection of 2% pentobarbital sodium 50 mg/kg of body weight (1.5 ml/kg; cat. no. 57-33-0; year 2016; Shanghai Haohai Biological Technology Co., Ltd.) and then sacrificed. Tissues and blood samples were obtained from abdominal aortas and stored for analysis. Heart and lung tissues were also collected. Following weighing on an electronic balance, tissues were either fixed in 10% formaldehyde at room temperature for 3-5 days or immediately frozen in liquid nitrogen and then transferred to a -80˚C freezer.

ELISA. Levels of inflammatory factors, including interleukin (IL)-1β and tumor necrosis factor (TNF)-α were measured with ELISA kits (cat. nos. ab197742 and ab208348, respectively; Abcam). The concentrations of the heart ADMA (cat. no. CEB301Ge, Cloud-Clone Corp.) and NO (cat. no. BH3702; Shanghai Kaibo Biology Technology Co., Ltd.) were measured with ELISA kits, according to the manufacturers' directions. The absorbance was measured at 450 nm using an Enzyme Labeled Instrument (Bio-Rad Laboratories, Inc.), and the concentration of NO and ADMA in the samples was calculated by standard curve.

Histopathological analysis. Samples for histological analysis were immersed in 10% formalin buffer at room temperature for 3-5 days, embedded in paraffin using a Leica Microsystem tissue processor (ASP 300S), and sliced into 3-4 μm sections using a Leica Microsystem microtome (model RM 226; Leica Microsystems GmbH). These sections were stained with hematoxylin and eosin (H&E) at room temperature for 10 min. LV fibrosis was estimated by staining with modified Masson's Trichrome Stain kit (Sigma-Aldrich; Merck KGaA).

Reverse transcription-quantitative (RT-q) PCR. Total RNA was extracted from heart tissue with TRIzol (Takara Bio, Inc.) and reverse-transcribed using the SuperScript™ Double-Stranded cDNA Synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.), following the suppliers' protocols. Reactions were performed in an RT-qPCR thermocycler (Bio-Rad Laboratories, Inc.) using SYBR Green PCR Master Mix (Takara Bio Inc.). The thermocycling conditions were: 94˚C for 5 min, followed by

35 cycles of 94˚C for 35 sec (denaturation), 57.3˚C for 35 sec (annealing), and 72˚C for 50 sec (extension). Primer sequences were: Atrial natriuretic peptide (ANP), forward 5'-AGCGAG CAGACCGATGAAG-3' and reverse 5'-AGCCCTCAGTTT GCTTTTCA-3'; brain natriuretic peptide (BNP), forward 5'-TGATTCTGCTCCTGCTTTC-3' and reverse 5'-GTGGAT TGTTCTGGAGACTG-3'; myosin heavy chain β (β-MHC), forward 5'-AGAGCAAAAGCAAAGGGTTTC-3' and reverse 5'-GTGATGGTACGAGATGGGCTA-3'; IL-1β, forward 5'-CCTGTTCTTTGAAGTTGACGG-3' and reverse 5'-AGC TTCTCCACAGCCACAAT-3'; TNF-α, forward 5'-CCACCA CGCTCTTCTGTCTA-3' and reverse 5'-GAGAGGGAGGCC ATTTGGGA-3'; DDAH1, forward 5'-AGGTGCTGAAAT CTTGGCTG-3' and reverse 5'-GCAGATTCGCTGGACCCT AT-3'; melanoma differentiation-associated protein 5 (MDA5), forward 5'-AGTGTCTCCACTTGCTGACC-3' and reverse 5'-CAGCAGCTCTCTTACACCTGA-3'; inositol-requiring enzyme α (IREα), forward 5'-AGCACAGTTACACTGCCT GAG-3' and reverse 5'-CTTCCACGTGTGTTGGGACCT-3'; superoxide dismutase-1 (SOD-1), forward 5'-GAGCATTCC ATCATTGGCCG-3' and reverse 5'-GGCAATCCCAATCAC ACCAC-3'; Bax, forward 5'-TCCACCAAGAAGCTGAGC GA-3' and reverse 5'-TTGAAGTTGCCATCAGCAAACA-3'; Caspase-3, forward 5'-ATGGGAGCAAGTCAGTGGAC-3' and reverse 5'-GTCCACATCCGTACCAGAGC-3'; Bcl-2, forward 5'-TGAGTACCTGAACCGGCATC-3' and reverse 5'-AAGCCCAGACTCATTCAACCA-3'; Caspase-8, forward 5'-CCTAGACTGCAACCGAGAGG-3' and reverse 5'-TCC AACTCGCTCACTTCTTCTG-3'; and GAPDH, forward 5'-CGGATTTGGTCGTATTGGG-3' and reverse 5'-CTG GAAGATGGTGATGGGATT-3'. The mRNA expression of the target genes was normalized to GAPDH as an internal control, and relative fold changes in mRNA expression were calculated using the formula $2^{-\Delta\Delta Cq}$ (21).

Western blot analysis. Western blotting was performed as previously described (22). Briefly, heart tissues were lysed in radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP40, 0.1% sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride and 1X protease inhibitor cocktail; Roche Diagnostics) and homogenized by Sonic Dismembrator 100 (Thermo Fisher Scientific, Inc.). The protein concentration of tissue homogenates was measured using a Bio-Rad Protein Assay (Bio-Rad Laboratories, Inc.). A total of 30 μg soluble protein was separated on 10% polyacrylamide gels and transferred into nitrocellulose membrane. The membrane was blocked with 5% skimmed milk powder at room temperature for 1 h. The following primary antibodies were used: TNF-α (1:2,000; ab8348; Abcam), IL-6 (1:1,000; ab83053; Abcam), IREα (1:2,000; 3294; Abcam), MDA5 (1:200; ab69983; Abcam), ANP (1:500; ab251006; Abcam), BNP (1:1,000; ab239510; Abcam), β-MHC (1:200; ab23990; Abcam), SOD-1 (1:1,000; sc-11407; Santa Cruz Biotechnology, Inc.), Bax (1:2,000; sc-526; Santa Cruz Biotechnology, Inc.), Caspase 3 (1:500; sc-7148; Santa Cruz Biotechnology, Inc.), Bcl-2 (1:1,000; sc-7382; Santa Cruz Biotechnology, Inc.), Caspase 8 (1:100; sc-5263; Santa Cruz Biotechnology, Inc.), DDAH1 (1:500; sc-5268; Santa Cruz Biotechnology, Inc.) and GAPDH (1:5,000; sc-32233; Santa Cruz Biotechnology, Inc.). The secondary antibodies were: Horseradish peroxidase (HRP)-labeled goat anti-mouse secondary antibody (ab6789; 1:4,000; Abcam), goat anti-rabbit HRP-labeled secondary antibody (ab6721; 1:4,000; Abcam) and goat anti-rat HRP-labeled secondary antibody (ab7097; 1:2,000; Abcam). Secondary antibodies were incubated for 1.5 h at room temperature. Proteins were visualized using a ClarityTM Western ECL Substrate (cat. no. 170-5061; Bio-Rad Laboratories, Inc.) and a Tanon 5200 full automatic chemiluminescence image analysis system (Tanon Science and Technology Co., Ltd.).

Table I. Cardiac function parameters in the experimental groups.

Parameters	Control (n=10)	TAC (n=10)	BAE (n=10)
LVPW (mm)	2.39±0.36	12.01±0.27[a]	5.71±0.38[b]
LVEDd (mm)	3.25±0.53	4.91±0.47[a]	3.91±0.32[b]
LVESd (mm)	1.72±0.47	3.75±0.71[a]	2.28±0.53[b]
LVEDV (μl)	47.25±6.04	89.54±9.53[a]	68.45±4.53[b]
LVESV (μl)	18.05±7.31	55.34±6.93[a]	31.13±2.05[b]
LVEF (%)	72.32±5.95	31.42±3.18[a]	51.34±0.34[b]
LVFS (%)	42.64±4.42	23.65±3.08[a]	32.32±6.38[b]

Data are presented as mean ± standard error of the mean. [a]P<0.05 vs. control group; [b]P<0.05 vs. TAC group. TAC, transverse aortic constriction; BAE, blueberry anthocyanin-enriched extract; LV, left ventricle; LVPW, LV wall thickness; LVEDd, LV end-diastolic diameter; LVESd, LV end-systolic diameter; LVEDV, LV end-diastolic volume; LVEF, LV ejection fraction; LVFS, LV fractional shorting.

Statistical analysis. Statistics were performed using SPSS 20.0 statistical software (IBM Corp.). Data were expressed as means ± standard error of the mean and were analyzed using unpaired t-test and one-way ANOVA followed by a Bonferroni correction post hoc test for differences among >2 groups. P<0.05 was considered to indicate a statistically significant difference.

Results

BAE ameliorates TAC-induced cardiac hypertrophy in mice. Compared with the control group, the TAC group had significantly higher heart weight and LV weight/heart weight ratio, but these effects were significantly attenuated by BAE treatment (Fig. 1A and B; P<0.05). Additionally, compared with the control group, the TAC group had significantly increased protein and mRNA expression levels of ANP, BNP and β-MHC in their heart LV tissues, and these were significantly ameliorated following BAE treatment (Fig. 1C-I; P<0.05).

BAE ameliorates TAC-induced LV dysfunction in mice. Compared with the control group, the TAC group demonstrated reduced LV fractional shorting and LV ejection fraction, and increased LV end-systolic diameter, LV end-diastolic diameter, and LV wall thickness. BAE treatment markedly elevated the LV ejection fraction and LV fractional shorting and decreased the LV end-diastolic and end-systolic diameters compared with the TAC group (Table I; P<0.05).

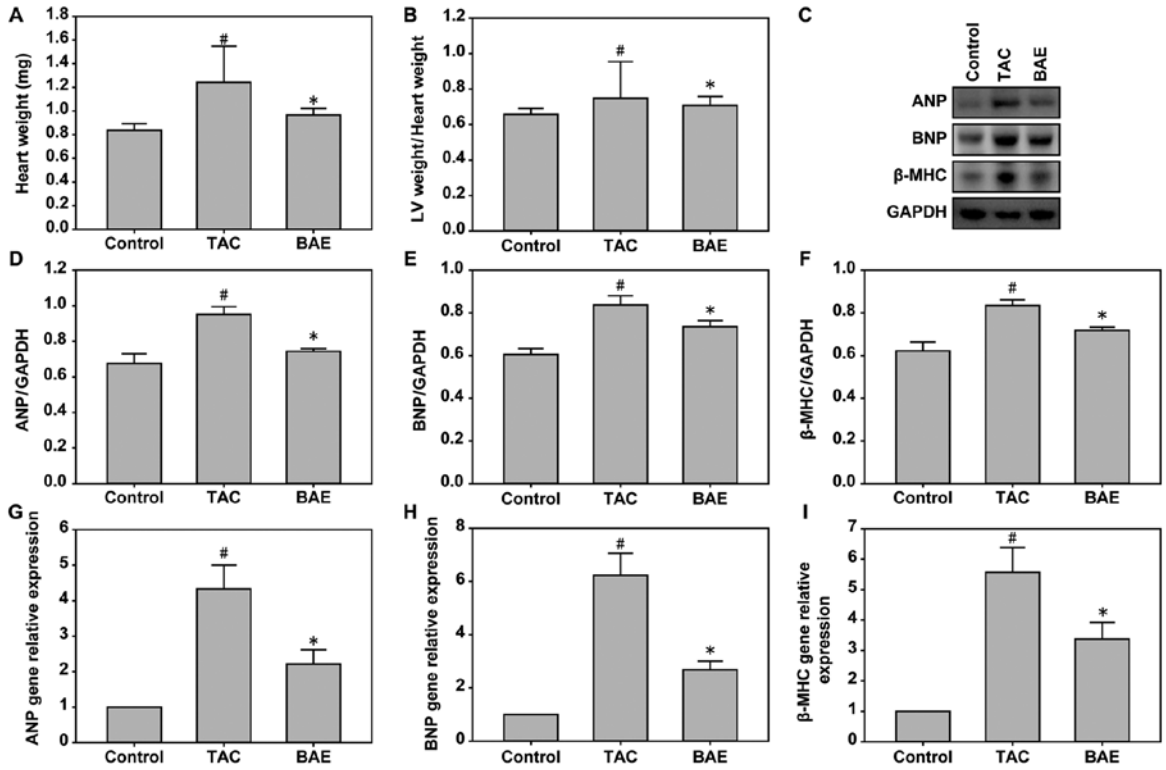

Figure 1. BAE ameliorates TAC-induced cardiac hypertrophy. (A) Heart weight (mg). (B) LV weight/heart weight ratio. (C) Representative western blot images and quantitative analysis of (D) ANP, (E) BNP and (F) β-MHC, with GAPDH used as loading control. (G) Reverse transcription-quantitative PCR analysis of ANP, (H) BNP and (I) β-MHC. All experiments were repeated at least three times. Data are presented as mean ± standard error of the mean. #P<0.05 vs. control group; *P<0.05 vs. TAC group. BAE, blueberry anthocyanin-enriched extract; TAC, transverse aortic constriction; LV, left ventricle; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; β-MHC, myosin heavy chain β.

BAE ameliorates TAC-induced LV inflammation and fibrosis in mice. Compared with the control group, the TAC group experienced heart hypertrophy, edema, inflammatory cell infiltration, as well as a significant increase in the levels of pro-inflammatory cytokines IL-1β and TNF-α (Fig. 2). BAE treatment significantly reversed the TAC-induced pro-inflammatory factor secretion (Fig. 2; P<0.05). In addition, TAC induced significant LV fibrosis compared with the control group, and this effect was ameliorated following BAE treatment (Fig. 2; P<0.05).

BAE ameliorates TAC-induced oxidative stress in mice. To assess the effect of BAE on TAC-induced oxidative stress, the protein and mRNA expression levels of MDA5 and IREα (markers of oxidative stress), and of SOD-1 (antioxidant status marker), were assessed in the heart tissues of the experimental mice. The data demonstrated that TAC significantly increased MDA5 and IREα expression, but decreased SOD-1 expression. By contrast, BAE treatment enhanced the expression of MDA5 and IREα and reduced the expression of SOD-1 (Fig. 3; P<0.05).

BAE ameliorates TAC-induced heart tissue apoptosis in mice. The present data demonstrated that TAC significantly increased Bax and Caspase-3 expression, but decreased Bcl-2 and Caspase-8 expression, compared with the control group, at both the mRNA and protein levels. By contrast, BAE treatment significantly reversed the upregulation of Bax and Caspase-3 and the downregulation of Bcl-2 and Caspase-8 induced by TAC (Fig. 4; P<0.05).

BAE ameliorates TAC-induced myocardial dysfunction via DDAH1/ADMA/NO signaling in mice. To investigate whether DDAH1/ADMA/NO signaling was involved in TAC-induced myocardial dysfunction, the protein expression levels of DDAH1 and the concentrations of ADMA and NO were measured in heart tissues by western blot analysis, RT-qPCR and ELISA. Compared with the control group, TAC significantly increased ADMA concentration (Fig. 5E; P<0.05), but decreased DDAH1 expression (Fig. 5A-C; P<0.05) and NO production (Fig. 5D; P<0.05). Notably, BAE treatment significantly reversed these effects (Fig. 5; P<0.05).

Discussion

Cardiovascular risk factors, including diabetes, smoking, hypertension, dyslipidemia, aging, and obesity, are associated with ROS, oxidative stress, inflammatory response and apoptosis (23). TAC increases free oxygen radicals, inflammation and apoptosis in heart tissues. The present study demonstrated that BAE ameliorated cardiac dysfunction, LV hypertrophy and fibrosis induced by TAC. The present data also demonstrated that BAE treatment attenuated TAC-induced LV leukocyte infiltration, inflammatory cytokine expression, oxidative stress and apoptosis. Furthermore, BAE significantly increased DDAH1 expression and NO production, and decreased ADMA concentration. These results suggested that TAC-induced myocardial dysfunction may be ameliorated by BAE treatment via the DDAH1/ADMA/NO signaling pathway.

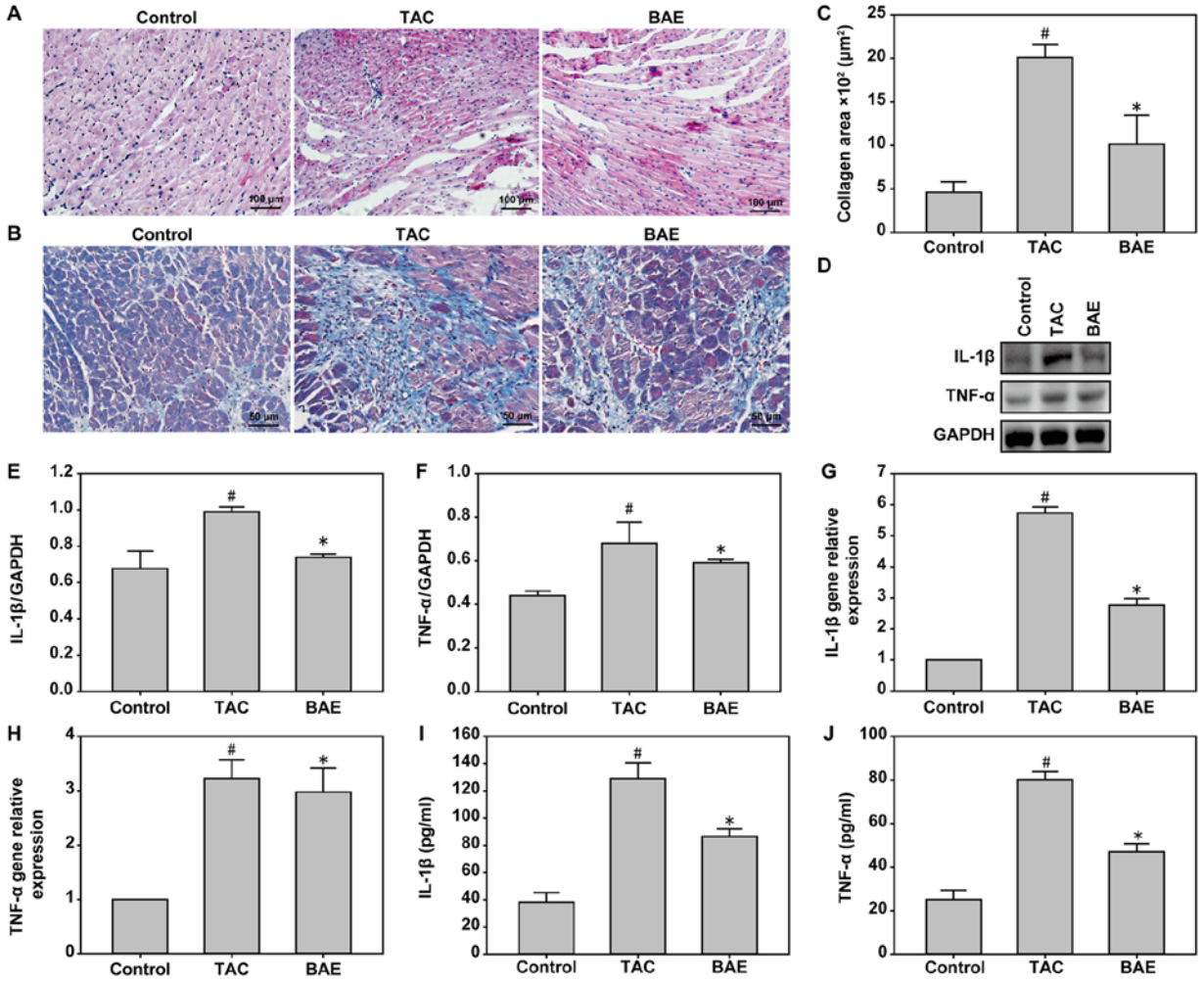

Figure 2. BAE ameliorates TAC-induced LV inflammation and fibrosis in mice. (A) H&E and (B) Masson trichrome-stained sections of LV tissues. (C) Quantification of cardiac fibrosis area from Masson trichrome-stained sections. (D-F) Levels of inflammatory factors IL-1β and TNF-α were measured by western blotting, (G and H) reverse transcription-quantitative PCR and (I and J) ELISA. All experiments were repeated at least three times. Data are presented as mean ± standard error of the mean. #P<0.05 vs. control; *P<0.05 vs. TAC group. BAE, blueberry anthocyanin-enriched extract; TAC, transverse aortic constriction; LV, left ventricle; IL, interleukin; TNF, tumor necrosis factor.

A previous study demonstrated that 20 and 80 mg/kg BAE treatment attenuated cyclophosphamide-induced cardiac dysfunction, left ventricular hypertrophy and fibrosis (24). In addition, BAE significantly reduced systolic blood pressure and increased aortic vessel relaxation in response to acetylcholine in high fat/high cholesterol diet-fed rats (25,26). Oxidative stress activates several intracellular signaling pathways and upregulates the expression of a large number of pro-inflammatory cytokines (27). A previous study demonstrated that a long-term blueberry-enriched diet decreases blood pressure and attenuates oxidative status in the kidneys of spontaneously hypertensive rats (28). BAE significantly inhibits hydrogen peroxide-induced human retinal pigment epithelial cell apoptosis, decreased vascular endothelial growth factor levels and activates protein kinase B (Act)-signal pathways (29). Song *et al* (30) demonstrated that BAE protects retinal cells against diabetes-induced oxidative stress and inflammation. In addition to the antioxidant effects, a previous study demonstrated that blueberry supplementation decreases serum inflammatory markers, including TNF-α, IL-6 and C-reactive protein (31). Freeze-dried blueberries markedly reduce pro-inflammatory cytokines TNF-α and IL-6 production in macrophages of apolipoprotein E KO mice by inhibiting nuclear factor (NF)-κB activation and the mitogen-activated protein kinase pathway (32). BAE attenuates C-C motif chemokine ligand 4-induced liver fibrosis, associated with reducing sources of ROS generation and associated oxidative damage, decreasing the influence of pro-inflammatory cytokines (33). Blackberry and blueberry anthocyanin supplementation significantly reduces serum and hepatic lipid levels, markedly elevates hepatic SOD and glutathione peroxidase activities, and reduces the expression of TNF-α, IL-6 and NF-κB genes in high-fat diet fed C57BL/6 mice (34). Additionally, blueberry suppresses cell cycle progression and induces mitochondrial-mediated cell apoptosis by abolishing the Janus kinase/STAT3 pathway (35). In brief, the present data, in agreement with the previous literature, suggested that BAE may have a protective effect against TAC-induced LV inflammation, oxidative stress and apoptosis.

In vivo and *in vitro* experiments have demonstrated that the DDAH1/ADMA/NO signaling pathway is closely

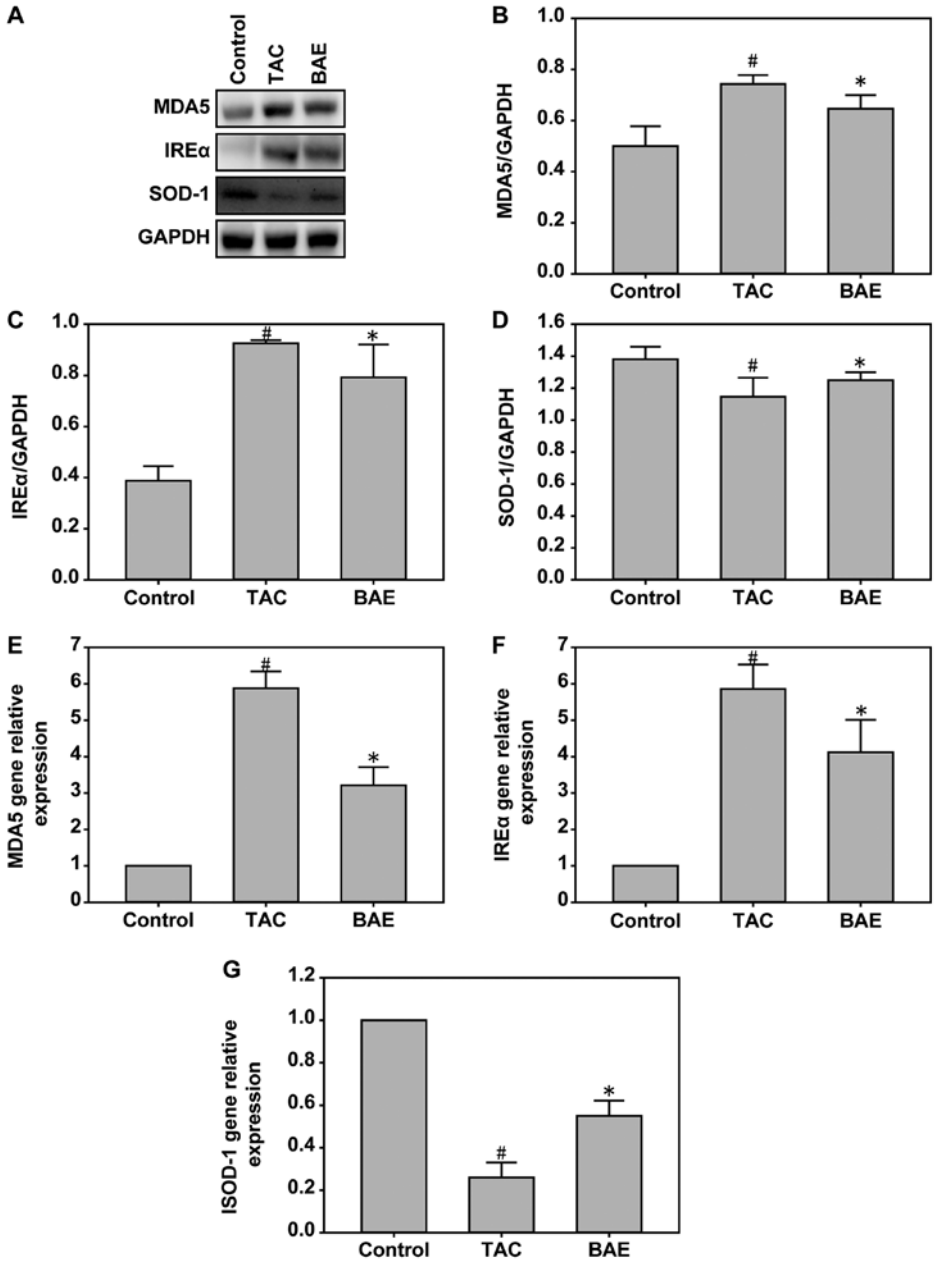

Figure 3. BAE ameliorates TAC-induced oxidative stress in mice. Heart tissues were isolated and the protein and mRNA expression levels of MDA5, IREα and SOD-1 were detected by western blotting and reverse transcription-quantitative PCR, respectively. (A) Representative blot images. (B-D) Quantification of western blotting results. (E-G) mRNA expression levels. All experiments were repeated at least three times. Data are presented as mean ± standard error of the mean. $^{\#}$P<0.05 vs. control group; *P<0.05 vs. TAC group. BAE, blueberry anthocyanin-enriched extract; TAC, transverse aortic constriction; MDA5, melanoma differentiation-associated protein 5; IREα, inositol-requiring enzyme α; SOD-1, superoxide dismutase-1.

associated with cardiovascular disease (10,12), cancer (13,36), liver diseases (37), preeclampsia (38,39) and ischemic and reperfusion injury (40). The present study demonstrated that TAC-induced a significant increase in ADMA concentration and a decrease in DDAH1 expression and NO production, which were ameliorated by BAE treatment. Previous studies have demonstrated that dietary blueberries have vascular beneficial effects by activating multiple targets including eNOS/NO/cyclic guanosine monophosphate (cGMP), redox and inflammatory signaling pathways (41-43). The production of NO by eNOS involves multiple steps and can be activated in different ways (44). In addition to vasodilation, NO exerts many beneficial vascular effects through anti-inflammation, antiplatelet, antiproliferation and antimigration activity (45). A randomized, double-blinded, placebo-controlled clinical trial study found that daily blueberry supplement reduces blood pressure and arterial stiffness, which may be related to increase NO production (46). In addition, pterostilbene, an active constituent of blueberries, causes eNOS phosphorylation and subsequent NO production through activation of the PI3K/Akt pathway (47); this blueberry supplement may change the intestinal microbiota and the anti-hypertensive effect of blueberries may be regulated by the NO-dependent pathway (48). Collectively, these studies suggest that the vascular effects of blueberries could occur via eNOS/NO/cGMP signaling, but the direct effect of

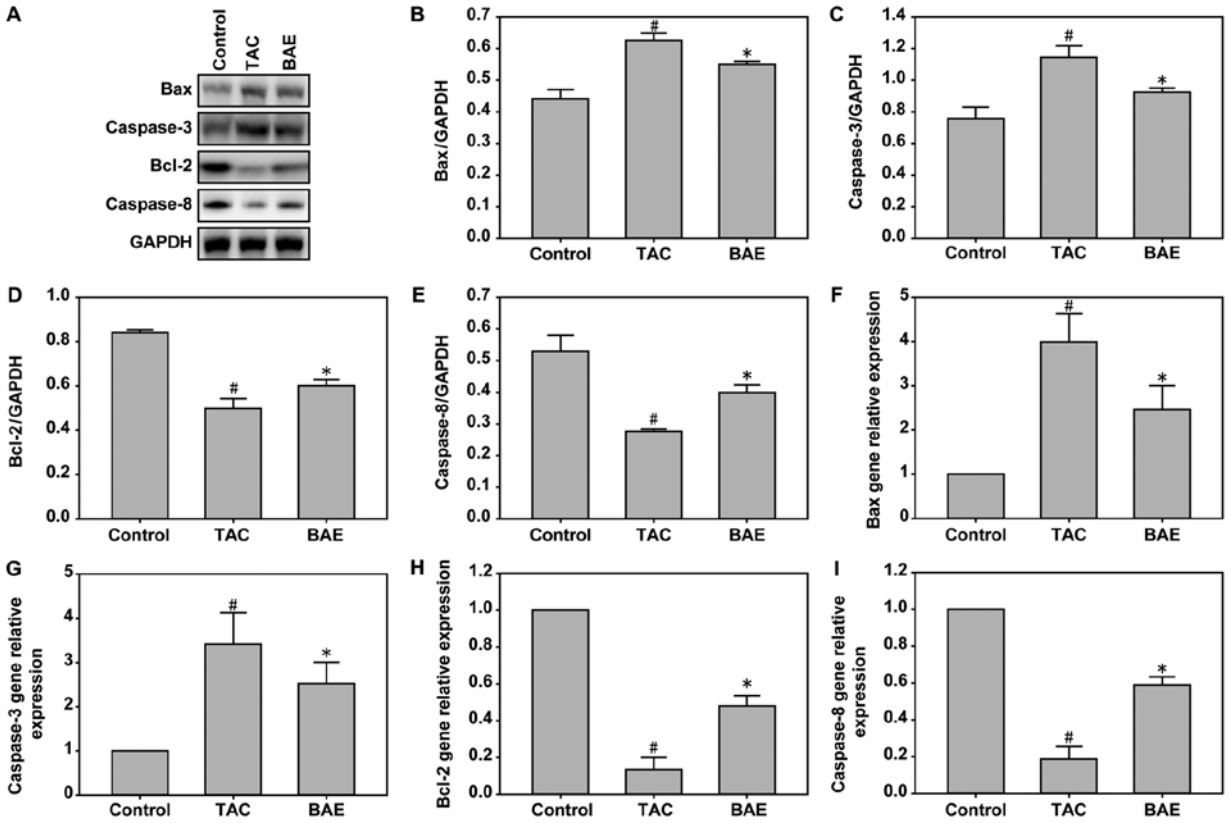

Figure 4. BAE ameliorates TAC-induced heart tissue apoptosis in mice. The protein and mRNA expression levels of Bax, Caspase-3, Bcl-2 and Caspase-8 were detected in heart tissues by western blotting reverse transcription-quantitative PCR, respectively. (A) Representative blot images. (B-E) Quantification of western blotting results. (F-I) mRNA expression levels. All experiments were repeated at least three times. Data are presented as mean ± standard error of the mean. #P<0.05 vs. control group; *P<0.05 vs. TAC group. BAE, blueberry anthocyanin-enriched extract; TAC, transverse aortic constriction.

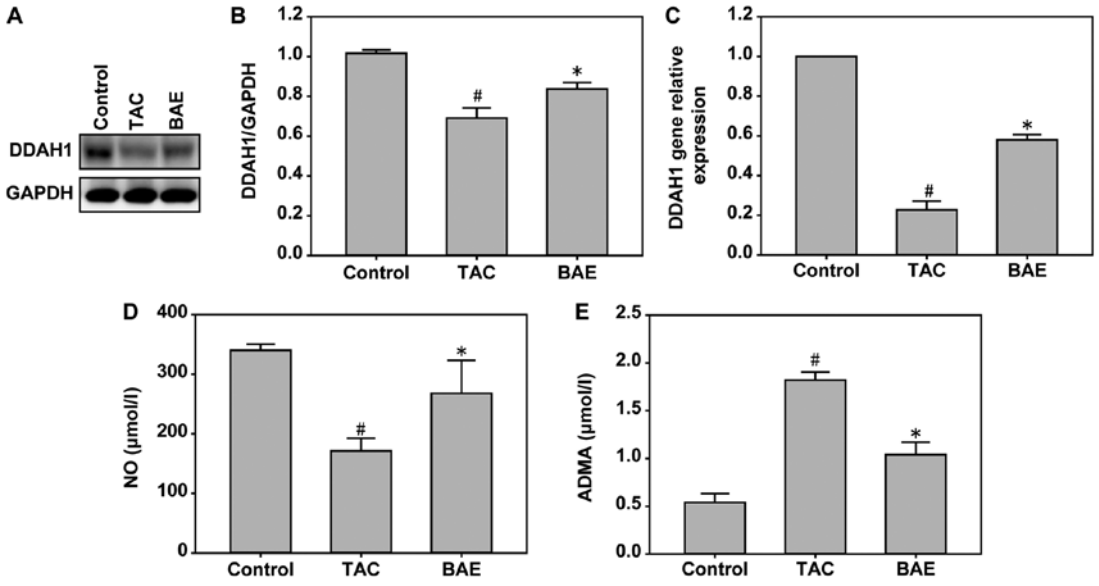

Figure 5. BAE ameliorates TAC-induced myocardial dysfunction through DDAH1/ADMA/NO signaling in mice. (A) The protein expression levels of DDAH1 in heart tissues were detected by western blotting. Representative blot images and (B) quantification are shown. (C) mRNA expression levels of DDAH1 were measured by reverse transcription-quantitative PCR. (D) ADMA concentration and (E) NO production were detected by ELISA. All experiments were repeated at least three times. Data are presented as mean ± standard error of the mean. #P<0.05 vs. control group; *P<0.05 vs. TAC group. BAE, blueberry anthocyanin-enriched extract; TAC, transverse aortic constriction; DDAH1, dimethylarginine dimethylaminohydrolase 1; ADMA, asymmetric dimethylarginine; NO, nitric oxide.

blueberry anthocyanins on eNOS activation remains to be elucidated.

In conclusion, the data form the present study suggested that BAE exerted potential beneficial vascular effects against

TAC-induced LV inflammation, oxidative stress and apoptosis, by decreasing ADMA concentration and by elevating DDAH1 expression and NO production. BAE treatment ameliorated the TAC-induced myocardial dysfunction, oxidative stress and inflammatory response and apoptosis, potentially via the DDAH1/ADMA/NO signaling pathway.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

WW, QM, TL and JFZ performed the experiments, analyzed and interpreted data. WH and JCZ made substantial contributions to the conception and design of the present study, and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and were approved by the Animal Ethics Committee of the People' Hospital of Weifang City (Shandong, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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