Betulinic acid ameliorates endothelium-dependent relaxation in L-NAME-induced hypertensive rats by reducing oxidative stress

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Abstract

Zizyphi Spinosi semen (ZSS) is one of the most widely used traditional Chinese herbs with protective effects on the cardiovascular system. It is not clear whether betulinic acid (BA), the key active constituent of ZSS, has beneficial cardiovascular effects on N\textsuperscript{o}-nitro-L-arginine methyl ester hydrochloride (L-NAME)-induced hypertensive rats. The objective of this study was to investigate the effect of BA on endothelium-dependent vasorelaxation in isolated aortic rings from L-NAME-induced hypertensive rats and its underlying mechanisms. Male Sprague–Dawley rats were injected with L-NAME (15 mg/kg/d, i.p.) for 4 weeks to induce hypertension. After treatment with L-NAME for 2 weeks, rats with mean blood pressure >120 mm Hg measured by tail-cuff method were considered hypertensive and then injected with BA (0.8, 4, 20 mg/kg/d, i.p.) for the last 2 weeks. The effect of BA on the tension of rat thoracic aortic rings was measured in an organ bath system. The levels of nitric oxide (NO), reactive oxygen species (ROS), and the activity of superoxide dismutase (SOD) and nitric oxide synthase (NOS) in aortas were assayed. We found that BA (0.1–100 \mu M) evoked a concentration-dependent vasorelaxation in endothelium-intact normal rat aortic rings, which was significantly attenuated by pretreatment with L-NAME (100 \mu M) or methylene blue (MB, 10 \mu M), but not by indomethacin (10 \mu M). Pretreatment with EC\textsubscript{50} (1.67 \mu M) concentration of BA enhanced the acetylcholine (ACh)-induced vasorelaxation, which was also markedly reversed by both L-NAME and MB. The blood pressure in hypertensive rats increased to 135.22 ± 5.38 mm Hg (P < 0.01 vs. control group), which was markedly attenuated by high dose of BA. The ACh-induced vasorelaxation in hypertensive rat aortic rings was impaired, which was markedly improved by chronic treatment with BA (20 mg/kg/d) for 2 weeks. The increase of ROS level and the decrease of NO level, SOD and eNOS activities in hypertensive rat aortas were all markedly inhibited by BA. These results indicate that BA decreased blood pressure and improved ACh-induced endothelium-dependent vasorelaxation in L-NAME-induced hypertension rats, which may be mediated by reducing oxidative stress and retaining the bioavailability of NO in the cardiovascular system.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide (AD, 1993) and hypertension is one of the most important risk factors for CVD (Gu et al., 2002). Hypertension affects more than 600 million populations and results in 13% of total deaths globally (Vasdev et al., 2006). It is estimated that there will be 29% of the world’s adult with hypertension by 2025 (Mittal and Singh, 2010). At present, in Chinese and Indian population, the prevalence of hypertension is approximately 20–30% (Mittal and Singh, 2010). The endothelium plays a crucial role in regulating vascular tone and structure by producing vasoactive mediators including nitric oxide (NO), prostacyclin (PGI\textsubscript{2}), and endothelin, and endothelial dysfunction is a potential initiating event for hypertension (Feletou and Vanhoutte, 2006; Gimbrone, 1995). Impaired endothelium-dependent relaxation (EDR) has been demonstrated in different models of hypertension (Boulanger, 1999; Demarco et al., 2010; Puzserova et al., 2010). The etiology of EDR impairment is multifactorial, among of which, overproduction of reactive oxygen species (ROS), alterations of endothelial nitric oxide synthase (eNOS) expression and activity, and decreased NO availability are widely accepted to be responsible for the endothelial dysfunction (Feletou and Vanhoutte, 2006; Palmer et al., 1987).

Down-regulation of NO bioavailability with both pharmacological NOS inhibitors and silence of eNOS expression caused significant...
peripheral vasoconstriction and elevation of blood pressure (Huang et al., 1995; Vrankova et al., 2010). In addition, low level of NO and low expression of eNOS were observed in spontaneously hypertensive rats (Kimoto-Kinoshita et al., 2000; Kumar et al., 2003). Furthermore, increased production of ROS in pathological conditions also contributes to vascular dysfunction (Forstermann, 2008). High level of superoxide readily reacts with NO, generating the highly reactive molecule peroxynitrite (ONOO−), to trigger a cascade of harmful events (Mollnau et al., 2002; Pryor and Squadrini, 1995). ONOO− not only decreases NO bioavailability, causing impaired vasorelaxation (Cai and Harrison, 2000; Rojas et al., 2006), but also causes uncoupling of NOS to produce superoxide instead of NO (Forstermann and Li, 2010). These results above indicate that ameliorating the anti-oxidative ability and the bioavailability of NO is potential to lessen endothelial dysfunction and hypertension (Forstermann and Li, 2010). However, the results of long-term clinical trials with oral delivery of acknowledged anti-oxidants such as vitamin C and vitamin E were ambiguous in treating cardiovascular diseases, including myocardial infarction, endothelial dysfunction, and hypertension (Forstermann, 2008; Lonn et al., 2005), which suggests the role of oxidative stress in hypertension is complex.

Zizyphi Spinosi semen (ZSS), a traditional Chinese herb for treating neurasthenia, is the dried seed of Ziziphus jujuba var spinosa (Bunge) Hu. Interestingly, modern pharmacological studies revealed that ZSS has some beneficial effects on the cardiovascular system, such as anti-arrhythmia and anti-hypertension, which suggested that ZSS may be mediated by betulinic acid, the key active constituent of ZSS, up-regulating eNOS and decreasing NADPH oxidase (Steinkamp-Fenske et al., 2007). Our previous study shows that betulinic acid ameliorated impairment of EDR induced by oxidative stress in rat aorta (Fu et al., 2010). We hypothesized that betulinic acid may attenuate endothelial dysfunction in hypertension via its modulation of the vascular NO pathway and oxidative stress.

Therefore, the aim of this study was to explore the effect of betulinic acid on acetylcholine (ACh)-induced vasorelaxation and blood pressure in hypertensive rats induced by NOS uncoupling to produce superoxide instead of NO (Steinkamp-Fenske et al., 2007). Our previous study shows that betulinic acid ameliorated impairment of EDR induced by oxidative stress in rat aorta (Fu et al., 2010). We hypothesized that betulinic acid may attenuate endothelial dysfunction in hypertension via its modulation of the vascular NO pathway and oxidative stress.

2. Materials and methods

2.1. Chemicals

Betulinic acid (BA) was from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China), and the purity was 98.0% by HPLC. ACh, phenylephrine (PE), L-NAME, methylene blue (MB) and indomethacin (Indo) were from Sigma–Aldrich, Inc. (Saint Louis, MO, USA). 3-Aminomethyl-2,7-difluorescein, diacetate (DAF-FM DA) and 2,7′-dichlorofluorescein-diacetate (DCFH-DA) were from Molecular Probes (Eugene, OR, USA). The kits for measurement of NOS and SOD were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Indo was prepared in distilled water containing 0.7% (wt./vol) sodium carbonate. Stock solutions of betulinic acid were prepared in dimethyl sulfoxide (DMSO) and diluted in Krebs’ solution, and the final concentration of DMSO was less than 0.03% (v/v). Preliminary experiments ascertained that none of the solvents at the final concentrations used had any effect on rat thoracic aortic rings. All other reagents were of analytical purity.

2.2. Animals

Male Sprague–Dawley rats (5 months old and weighing on 240–260 g) were obtained from the Experimental Animal Center of Zhejiang University. All procedures were performed according to protocols approved by the Institutional Committee for Use and Care of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The experiments were approved by the Ethics Committee for the Use of Experimental Animals in Zhejiang University.

2.3. Induction of hypertension

Animals were divided into eight groups (n = 5 in each group): normal control, BA control, DMSO control, hypertension treated with DMSO, and hypertension treated with low, medium, and high dose of BA. All the control groups were received saline, the solvent for L-NAME, intraperitoneally. Hypertension was induced by inject L-NAME (15 mg/kg/d, i.p.) for 4 weeks (Ambrozewicz et al., 2011). After the rat was treated with L-NAME for 2 weeks, the blood pressure of rats was measured by non-invasive rat caudal artery blood pressure measuring instrument (purchased from Chengdu Instrument Factory, China) and rats with average blood pressure >120 mm Hg (Zhou et al., 2009) were considered hypertensive.

2.4. Preparation of rat thoracic aortic rings and bioassay of vasoreactivity

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and killed by cervical dislocation. The descending thoracic aorta was rapidly dissected out and immersed in chilled Krebs’ solution, composed of (mM): NaCl 118; KCl 4.7; KH2PO4 1.2; MgSO4 1.2; NaHCO3 25; glucose 10; CaCl2 2.5; pH (7.4). After the perivascular tissue was carefully removed, aortic rings approximately 4 mm in length were cut. In some rings, the endothelium was mechanically removed by gentle rubbing with moistened cotton. For isometric force recording, aortic rings were mounted between two stainless steel hooks and suspended in a 10 ml organ bath containing Krebs’ solution at 37 °C bubbled with 95% O2 + 5% CO2 (pH 7.4). After equilibration under no tension for 30 min, the aortic rings were allowed to equilibrate for 1 h at a resting tension of 2 g. During the equilibration period, Krebs’ solution was changed every 15 min. Changes in tension were recorded by the isometric transducer connected to a data acquisition system (PowerLab, ADInstruments Shanghai Trading Co., Ltd., China). Before each experiment, rings were stimulated three times with 60 mM KCl until a reproducible contractile response was obtained. The presence of functional endothelium was verified by the ability of ACh (10 μM) to induce more than 80% relaxation of aortic rings pre-contracted by PE (1 μM) (Qian et al., 2006).

2.5. Assay of NOS and SOD activity

After treatment, the rings were incubated in normal Krebs’ solution (10 ml) in the presence of ACh (10 μM) for 15 min before measurement of biochemical parameters. The rings were blotted dry and weighed, and then made into a 10% tissue homogenate in ice-cold homogenizing medium containing 10 mM Tris–HCl, 0.1 mM EDTA–2Na, 10 mM sucrose, and 0.8% NaCl, pH 7.4. A supernatant was obtained from the homogenate by centrifugation (3000 rpm, 10 min, 4 °C). Following the commercial kit manual, in this supernatant, SOD activity was assayed by the xanthine–xanthine oxide method (Qian et al., 2006). Aortic total NOS (tNOS) (constitutive NOS [cNOS] + inducible NOS [iNOS]) and iNOS activity were assayed following the kit manual (Llorens and Salazar, 2005). The tNOS activity minus the iNOS activity gave the cNOS activity (in rat aortas the main cNOS is eNOS). NOS and SOD activity in serum of chronic treated animals were also determined according to the same kit manual.
above or serum from animals was obtained, it was pipetted into the supernatant was obtained from the aortic homogenate as follows: (1) normal control: rats intraperitoneally received saline for 4 weeks; (2) BA control: rats intraperitoneally received saline for 4 weeks and BA (20 mg/kg/d, dissolved in DMSO) for the last 2 weeks; (3) DMSO control: rats intraperitoneally received saline for 4 weeks and DMSO (the solvent of BA) for the last 2 weeks; (4) hypertension: rats intraperitoneally received l-NAME (15 mg/kg/d, dissolved in saline) for 4 weeks and DMSO for the last 2 weeks; (5) hypertension treated with BA: rats intraperitoneally received l-NAME for 4 weeks and low (0.8 mg/kg/d), medium (4 mg/kg/d), or high (20 mg/kg/d) dose of BA for the last 2 weeks.

2.8. Statistical analysis

All data are expressed as mean ± SD. The ACh/BA-induced maximal relaxation (P_max) in aortic rings was calculated as a percentage of the contraction in response to PE (1 µM). The half-maximum effective concentration (EC_{50}) was defined as the concentration of BA or ACh that induced 50% of maximum vasorelaxation of the contraction elicited by PE (1 µM) and was calculated from the concentration–response curve by nonlinear regression (curve fit) using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA). pD_2 is the negative logarithm of the EC_{50}. Statistical comparisons were made using one-way ANOVA followed by Newman–Keuls test. The P < 0.05 was considered to be statistically significant.

3. Results

3.1. Effect of BA on relaxation in aorta pre-contracted by PE

In the endothelium-denuded aortic rings pre-contracted by PE, BA (0.1–100 µM) did not evoke obvious vasorelaxation, the E_{max} reached 10.99 ± 7.29% (P > 0.05 vs. the endothelium-denuded control group, Fig. 1). However, BA (0.1–100 µM) evoked a concentration-dependent relaxation in endothelium-intact aortic rings pre-contracted by PE (Fig. 1); the E_{max} reached 74.06 ± 6.18%, and the pD_2 was 5.78 ± 0.79. EC_{50} was 1.67 µM. We chose the value of EC_{50} as the experimental concentration of BA for subsequent experiments. Pretreatment of the endothelium-intact rings with l-NAME (100 µM) or MB (10 µM) markedly attenuated the BA-induced vasorelaxation; the E_{max} fell to 17.75 ± 9.22% and 15.25 ± 3.28%, respectively. However, Indo (10 µM) did not influence the vasorelaxation induced by BA (Fig. 2).

3.2. Effect of BA on ACh-induced EDR in aorta pre-contracted by PE

ACh (0.001–10 µM) evoked a normal concentration-dependent relaxation (Fig. 3) and the E_{max} reached 70.74 ± 6.68%, which was abolished by pretreatment with l-NAME and the E_{max} fell to 14.10 ± 9.09% (Fig. 3A). BA alone enhanced the ACh-induced EDR, the E_{max} increased to 85.39 ± 7.16%, which was markedly reversed by pretreatment with both l-NAME, the inhibitor of NOS, and MB, a guanylyl cyclase inhibitor, and the E_{max} fell to 17.93 ± 3.70% and 29.33 ± 5.86%, respectively. However, the effect of BA was not significantly reduced by pretreatment with Indo, a cyclooxygenase inhibitor (Fig. 3B).
3.3. Effect of chronic treatment with BA on blood pressure of hypertensive rats

As shown in Fig. 4, the blood pressure in hypertensive rats induced by L-NAME increased to 135.22 ± 5.38 mm Hg (P < 0.01 vs. Con), which was not affected by DMSO, the solvent of BA (Fig. 4). The blood pressure in hypertensive rats treated with a high dose of BA decreased to 106.49 ± 7.28 mm Hg (P < 0.01 vs. hypertension). However, a low or medium dose of BA had no obvious effect on the blood pressure (P > 0.05 vs. Con, data not shown). Chronic BA or DMSO treatment had no significant effect on the blood pressure in normal control rats (P > 0.05 vs. Con).

3.4. Effect of chronic treatment with BA on ACh-induced EDR in aortas from hypertensive rats

The ACh-induced EDR in the aortic rings from hypertensive rats pre-contracted by PE was significantly impaired, and the E\text{max} fell to 23.66 ± 6.51% (P < 0.01 vs. Con, Fig. 5A). And the E\text{max} in hypertensive rats treated with DMSO fell to 25.27 ± 8.92% (P < 0.01, vs. Con, Fig. 5A). Though the E\text{max} in hypertensive rats treated with DMSO higher than that in hypertensive group, no significant difference was found between these two groups. Also, there were no obvious differences found among BA treatment alone group, DMSO group and normal control group (Fig. 5A).

As shown in Fig. 5B, chronic treatment with BA (20 mg/kg/d) for 2 weeks significantly improved the vasorelaxation, the E\text{max} of high dose BA treated groups reaching 46.47 ± 6.87% (P < 0.01 vs. hypertension) and the E\text{max} of medium dose BA (4 mg/kg/d) treated groups reaching 36.60 ± 9.16% (P < 0.05 vs. hypertension). However, the E\text{max} of low dose BA (0.8 mg/kg/d) treated group was not different from that of hypertensive group, with E\text{max} of 31.53 ± 4.41% (P > 0.05 vs. hypertension).

3.5. Effect of chronic treatment with BA on the level of ROS and NO, the activity of SOD and NOS in aortas from hypertensive rats

ROS level was markedly increased to 261.96% in hypertensive rat aortas compared with that in Con (P < 0.01), which was attenuated by chronic treatment with 20 mg/kg/d BA (P < 0.01 vs. hypertension, Fig. 6A). Similarly, the decrease of NO level in hypertensive rat aortas was markedly inhibited by BA (P < 0.01 vs. hypertension, Fig. 6B). As shown in Fig. 6C and D, chronic treatment with 20 mg/kg/d BA also inhibited the decrease of SOD and cNOS activities in hypertensive rat aortas (P < 0.01 vs. hypertension). Chronic treatment with BA alone had no significant effect on ROS level and SOD activity, but markedly increased NO level and cNOS activity in normal rat aortas compared with that in Con. The solvent of BA, DMSO, had no significant effect on NO level, ROS level, NOS and SOD activities in both normal and hypertensive rat aortas (Fig. 6).

4. Discussion

Zizyphi Spinosi semen (ZSS) is one of the most commonly used Chinese herbs. It is a sedative and hypnotic drug with additional effect on the cardiovascular system (Steinkamp-Fenske et al., 2007). Treatment of rats with ZSS could result in increased plasma levels of NO (Wang X, 2004). ZSS and one of its constituents, betulinic acid (BA), could up-regulated eNOS expression and down-regulated NADPH oxidase (Steinkamp-Fenske et al., 2007).
Also, BA inhibits high glucose-induced vascular smooth muscle cells proliferation and migration by multiple effects (Yoon et al., 2010b). BA treatment was found to show potent inhibitory effect on vascular inflammation process in human umbilical vein endothelial cells (Yoon et al., 2010a). Besides, in our previous study, we found BA ameliorates impairment of EDR induced by oxidative stress in rat aorta (Fu et al., 2010).

In the present study, we demonstrated for the first time the beneficial effect of BA on isolated rat aortas from hypertensive rats. We found that BA (0.1–100 μM) evoked a concentration-dependent relaxation in endothelium-intact aortic rings pre-contracted by PE. And functional removal of the endothelium markedly abolished the BA-induced relaxation in aortic rings pre-contracted by PE. These results indicated that the effect of BA on vasorelaxation was dose-dependent and endothelium played an important role in BA-induced relaxation. PGI2 and NO are two relaxing factors that involved in the endothelium-dependent modulation of vascular tone in rat thoracic aorta (Bryan et al., 2005; Woodman and Boujajaue, 2004). COX-2 synthesis (Chang et al., 1980) and NO is from l-arginine that is catalyzed by NOS. Pretreatment with l-NAME, an inhibitor of NOS, significantly reduced the BA-induced relaxation, while indomethacin, a COX inhibitor, did not influence the relaxation induced by BA. It indicates that EDR induced by BA may be mediated by NOS–NO pathway, but not by COX-PGI2 pathway.

There are two types of NOS isoforms (cNOS and iNOS) in vivo and each has a particular function (Wang and Marsden, 1995). Under normal conditions, the main cNOS in aorta is eNOS, which is the main source of NO under physiological conditions (Forstermann et al., 1998). When NO is formed in the endothelium by the activation of NOS, it diffuses out of the endothelium to the vascular smooth muscle where it binds to and activates soluble guanylyl cyclase to catalyze the conversion of GTP to cGMP. The rise of cGMP initiates the relaxation of the vascular smooth muscle (Marin and Rodriguez-Martinez, 1997; Vaandrager and de Jonge, 1996). Here, we found that MB, a guanylyl cyclase inhibitor, also significantly reduced the BA-induced EDR. Similarly, pretreatment with BA enhanced AC-heinduced EDR, which was abolished by l-NAME and BA. All the results above confirmed that BA promoted EDR in aortas by regulating NOS–NO–cGMP pathway. And it also implied that as the main bioactive constituent of ZSS, the activating NO pathway effect of BA may be the key of anti-hypertension induced by ZSS (Steinkamp-Fenske et al., 2007).

In 1992, chronic administration of l-NAME was first found to promote persistent hypertension and renal damage in rats (Baylis et al., 1992; Ribeiro et al., 1992). In the present study, intraperitoneal injection with l-NAME for 4 weeks induced high blood pressure in rats, decreased ACh-induced relaxation, accompanied by a decrease of ROS in aortas. Sustained blood pressure elevation is initiated by the decrease of NO bioavailability and loss of opposition to endogenous vasoconstrictors (Baylis et al., 1994; Deng et al., 1993). The blood pressure was increased obviously within the first 2 weeks after NO had been inhibited (Kuru et al., 2009); Even NO inhibition had been maintained for 1 week, however, correction of hypertension with acute l-arginine was only partial (Ribeiro et al., 1992). Moreover, the hypertension no longer depends exclusively on inactivation of the NOS–NO pathway once NO has been inhibited for more than a few days (Morton et al., 1993), structural alteration of the vascular walls (Gerova, 2000), as well as renal parenchymal injury such as glomerulosclerosis, glomerular collapse and

![Fig. 4. Effect of chronic treatment with betulinic acid (BA) on blood pressure in hypertensive rats induced by l-NAME. Con: age-matched normal control group; DMSO: normal control rats treated with the solvent of BA group (DMSO for 2 weeks); H-BA: high dose of BA control group (20 mg/kg/d BA for 2 weeks); hypertension: hypertensive group (15 mg/kg/d l-NAME for 4 weeks); hypertension + DMSO: hypertensive treated with the solvent of BA group (DMSO for 2 weeks); hypertension + H-BA: hypertensive treated with high dose of BA group (20 mg/kg/d BA for 2 weeks). All data were expressed as mean ± SD; n = 5. *P < 0.01 vs. control group (Con); **P < 0.01 vs. hypertensive group (hypertension).](image)

![Fig. 5. Effect of chronic treatment with betulinic acid (BA) on acetylcholine (ACh)-induced endothelium-dependent relaxation (A and B) in l-NAME-induced hypertensive rat aortic rings pre-contracted by 1 μM phenylephrine (PE). Tension was measured and calculated as a percentage of the contraction in response to PE. Con: age-matched normal control group; DMSO: normal control rats treated with the solvent of BA group (DMSO for 2 weeks); H-BA: high dose of BA control group (20 mg/kg/d BA for 2 weeks); hypertension: hypertensive group (15 mg/kg/d l-NAME for 4 weeks); hypertension + DMSO: hypertensive treated with the solvent of BA group (DMSO for 2 weeks); hypertension + L-BA, M-BA, H-BA: hypertensive group treated with low, medium, or high dose of BA groups (0.8, 4, or 20 mg/kg/d BA for 2 weeks). All data were expressed as mean ± SD; n = 5 rings from 5 rats per group. *P < 0.05, **P < 0.01 vs. control group (Con); ***P < 0.05, ****P < 0.01 vs. hypertensive group (hypertension).](image)
interstitial fibrosis (Tsuchiya et al., 2010), and abnormal activation of the renin-angiotensin system (Watson et al., 2002) may also be involved. Therefore, in our study, we choose BA to intervene after 2 weeks of L-NAME treatment; while the entire L-NAME treatment in hypertensive rats group was 4 weeks to maintain the consistent of hypertension.

Considerable evidences indicate that increased vascular oxidative stress plays an important role in endothelial dysfunction and the development of hypertension (Cai and Harrison, 2000; Thomas et al., 2003; Usui et al., 1999). The underlying mechanism is probably that excessive ROS not only quickly reacts with NO to generate ONOO−, but also uncouples NOS to produce more superoxide. Both the over-expression of NADPH oxidase (Paravicini and Touyz, 2008), and the deficiency of SOD in L-NAME-induced hypertensive rats result in the overproduction of ROS (Didion et al., 2002; Jung et al., 2003), which quenches NO and forms a vicious circle to damage the vasculature. Excessive ROS causes DNA damage, represses the activity of cellular enzymes, and induces cell death (Lum and Roebuck, 2001; Miao and St Clair, 2009). All the oxidative stress above contributes to the impairment of NO-induced EDR and hypertension (Channon, 2004; Forstermann and Munzel, 2006; Rojas et al., 2006). In our study, we found that pretreatment with BA for 2 weeks significantly inhibited the blood pressure elevation, attenuated the impairment of EDR, the increase of ROS level, the decrease of NO level, and the inhibition of eNOS and SOD activities in rat aortas induced by hypertension. It indicates that the anti-hypertensive effect of BA may attribute to its improvement of EDR, which at least mediated by reducing oxidative stress and enhancing the NOS–NO pathway.

In conclusion, we have shown that BA concentration-dependently evoked vasorelaxation in the endothelium-intact rat aortic rings, and decreased blood pressure, improved ACh-induced EDR in L-NAME-induced hypertension rats, which may be mediated by reducing oxidative stress and retaining the bioavailability of NO.

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References


Fig. 6. Effect of chronic treatment with betulinic acid (BA) on ROS level (A), NO level (B), activity of SOD (C) and constitutive nitric oxide (cNOS) (D) in aortic rings from L-NAME-induced hypertensive rats. Con: age-matched normal control group; DMSO: normal control rats treated with the solvent of BA group (0.1% DMSO for 2 weeks); H-BA: hypertensive group treated with high dose of BA (20 mg/kg/d BA for 2 weeks); hypertension: hypertensive group (15 mg/kg/d L-NAME for 4 weeks); hypertension + DMSO: hypertensive treated with the solvent of BA group (0.1% DMSO for 2 weeks); hypertension + H-BA: hypertensive treated with high dose of BA (20 mg/kg/d BA for 2 weeks). All data were expressed as mean ± SD; n = 5. *P < 0.05, **P < 0.01 vs. control group (Con); ***P < 0.01 vs. hypertensive group (hypertension).


