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Epigallocatechin-3-gallate exerts antihypertensive effects and improves endothelial function in spontaneously hypertensive rats

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ABSTRACT

Objective: To investigate the effect of epigallocatechin-3-gallate (EGCG) on endothelial dysfunction in spontaneously hypertensive rats (SHR).

Methods: Wistar-Kyoto (WKY) rats and SHR were divided into four groups; WKY control, SHR control and SHR treated with EGCG (50 mg/kg/day) or losartan (10 mg/kg/day). The treatment was given daily for 4 weeks by oral gavage and the blood pressure was monitored by tail-cuff method every 3 days. Acetylcholine-induced endothelium-dependent relaxations were assessed in isolated phenylephrine-precontracted aortic rings at the end of treatment. The vascular levels of reactive oxygen species, nitric oxide, tetrahydrobiopterin, and cyclic guanosine monophosphate were also measured. Moreover, the expression of angiotensin Ⅱ type 1 (AT_1) receptor protein was determined.

Results: The systolic blood pressure was significantly decreased in SHR treated with EGCG. The impaired endothelium-dependent relaxation was significantly improved in aortic ring isolated from the EGCG-treated SHR group. EGCG also significantly increased the levels of nitric oxide, tetrahydrobiopterin, and cyclic guanosine monophosphate, while decreasing the level of reactive oxygen species and the protein expression of AT_1 receptor in SHR.

Conclusions: EGCG attenuates endothelial dysfunction in SHR by decreasing oxidative stress and increasing vascular nitric oxide bioavailability, which may be modulated partly by inhibition of vascular AT_1 receptors. An increase in endothelium-dependent relaxation may contribute to a decrease in blood pressure in hypertensive animals.

KEYWORDS: Epigallocatechin-3-gallate; Vascular protection; Antihypertension; Tetrahydrobiopterin; Cyclic guanosine monophosphate; Angiotensin type Ⅰ receptor; Spontaneously hypertensive rats

1. Introduction

Cardiovascular diseases are a major public health problem that is approaching epidemic proportions globally[1]. Hypertension is a well-established risk factor for cardiovascular diseases and sooner will become a public health burden if left unattended due to increasing number of death and disability among people as the prevalence of hypertension goes higher[2]. One of the variables that contribute the increase in peripheral resistance are the abnormalities in the structure and function of the vasculature driving the event of endothelial dysfunction[3].

Endothelial dysfunction can be defined as an imbalance in production of vasodilator and vasoconstrictor molecules, hence resulting in the loss of its normal function[4]. It can be characterized by reduced release and/or the depletion of the bioavailability of

Significance

Epigallocatechin-3-gallate (EGCG) is a polyphenol that exhibits antihypertensive effects due to its vasoprotective effect. The current work demonstrated that treatment with EGCG attenuates endothelial dysfunction and the expression of vascular AT_1 receptors which may contribute to the vasoprotective effect of EGCG.

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nitric oxide (NO), a key regulator in the vasculature, leading to a compromised NO-cyclic GMP (cGMP) signaling pathway which causes vasodilation[5].

NO acts as an endothelium-derived hyperpolarizing factor (EDHF) which leads to vascular relaxation[6]. Endothelial nitric oxide synthase (eNOS) is the main source of NO and plays a crucial role in maintaining contraction and relaxation of the vascular tissues[7]. Tetrahydrobiopterin (BH_4) is one of the important cofactors for the proper functioning of eNOS to generate NO from its precursor *L*-arginine[8]. The produced NO is then released into vascular smooth muscle cells and activates the soluble guanylyl cyclase (sGC) by converting guanosine 5'-triphosphate to cyclic guanosine monophosphate (cGMP). The increased production of cGMP will induce smooth muscle relaxation, resulting in vasodilation[9].

Oxidative stress or a decrease in BH_4 level will disrupt the coupled state of eNOS[10]. The uncoupling of eNOS is linked to the enhanced oxidation of BH_4 by reactive oxygen species (ROS) which is derived from vasculature nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidases[11]. Under this condition, O_2^- scavenges NO to produce the toxic radical peroxynitrite ($ONOO^-$) which further decreases the bioavailability of NO and worsens endothelial dysfunction[12]. A previous study has shown endothelial dysfunction is associated with the inhibition of oxidative stress injury and the downregulation of the angiotensin Ⅱ/AT_1 receptor pathway in *D*-galactose-induced aging rats[13]. Since elevation of O_2^- is strongly associated with a decrease in BH_4 level, an increase in angiotensin Ⅱ type 1 (AT_1) receptors will in part lead to endothelial dysfunction partly due to eNOS uncoupling. Indeed, exposure to angiotensin Ⅱ has been demonstrated to cause endothelial dysfunction through NADPH-derived ROS production in several previous studies. An animal study also demonstrated angiotensin Ⅱ-induced endothelial dysfunction in mouse ophthalmic arteries *via* activation of AT_1 receptor and NOX_2-dependent ROS formation[14].

Tea obtained from *Camellia sinensis* plant is always used as a substance that promotes human health. The use of green tea is of great interest to researchers due to its good health effects and various bioactive compositions[15]. Epigallocatechin gallate (EGCG), the most abundant tea polyphenol found in green tea[16], has been demonstrated to improve vascular function and has antioxidant and anti-inflammatory properties which can reduce the risk of cardiovascular disease[17]. An *in vivo* study has demonstrated that green tea administration in spontaneously hypertensive rat (SHR) controlled the development of hypertension due to the antioxidative properties of catechins[18]. Further, Álvarez-Cilleros and colleagues have proven that EGCG improved endothelial dysfunction by decreasing the production of ROS and the activation of stress-related pathways in human umbilical vein endothelial cells[19]. In line with that, EGCG also alleviated endothelial dysfunction by downregulating HMGB1 in hypoxic trophoblast cells[20]. Besides, Mohd Sabri *et al*. reported that EGCG improved vascular function by reducing oxidative stress and eNOS uncoupling, thus increasing NO production in angiotensin Ⅱ-induced hypertensive mice[21].

The activation of NADPH oxidase due to stimulation of AT_1 receptor is one of the main mechanisms leading to oxidative stress. Although studies have shown that treatment with EGCG attenuates endothelium dysfunction *via* decreasing oxidative stress and eNOS uncoupling, the modulatory effect of EGCG on AT_1 receptors is yet to be elucidated. Therefore, this study aimed to investigate the effect of EGCG on the modulation of AT_1 receptors in improving endothelial dysfunction in a genetically hypertensive animal model.

2. Materials and methods

2.1. Drugs and chemicals

Phenylephrine (PE), acetylcholine (ACh), sodium nitroprusside (SNP), and Tween-20 were purchased from Sigma Chemicals (St Louis, MO, USA). EGCG was purchased from Cayman Chemicals. NaCl was purchased from Calbiochem® Merck (Darmstadt, Germany). $MgSO_4$, KCl, KH_2PO_2, glucose, and $CaCl_2$ were purchased from BDH Laboratory Supplies (Poole, UK). Bovine serum albumin was purchased from Santa Cruz (Dallas, Texas, USA). All chemicals were dissolved in deionized water.

2.2. Experimental animals and treatment

Briefly, 10-week-old male Wistar-Kyoto rats (WKY) (n=8) and SHR (n=24) were used for *in vivo* study. The experimental rats were purchased from the Animal Experimental Unit, Universiti Malaya, Malaysia. All animals were housed at the Animal Holding Facility, Universiti Tunku Abdul Rahman. They were maintained at a temperature of (24±1)℃, and a 12 h:12 h light/dark cycle and had free access to standard chow and water. Four weeks of treatment were given to the animals and they were randomly divided into four groups, namely WKY control, SHR control, SHR treated with EGCG (50 mg/kg/day; Cayman Chemical Company, Ann Arbor, MI, USA), and SHR treated with losartan (10 mg/kg/day; Sigma Company, St Louis, MO, USA).

2.3. Measurement of systolic blood pressure (SBP) by tail–cuff method

A modified tail-cuff method utilizing the CODA monitoring system (Torrington, CT, USA) was used to measure the SBP of the rats every three days starting from day 0 of the treatment period. All animals were trained for a week before the actual measurement of blood pressure to secure that they were adapted to the procedure. This minimized the stress associated with blood pressure measurements thus ensuring a more reproducible result. The animals

were restrained and pre-heated in a chamber for 30 min before the blood pressure measurements were performed. At least five to six successive measurements were recorded and the average values of all readings were calculated and reported.

2.4. Collection of samples

The animals were sacrificed by excessive inhalation of carbon dioxide. Blood samples of the animals were collected immediately *via* cardiac puncture. The thoracic aorta was isolated and placed immediately in ice-cold Krebs solution (in mM: NaCl 118.93, $NaHCO_3$ 25.00, $MgSO_4$ 1.18, KCl 4.69, KH_2PO_4 1.03, glucose 11.10, $CaCl_2$ 2.38). After that, the adhering connective tissues and adipose tissues were removed. The aorta was cut into approximately 5 mm segments for vascular function study while a portion of the isolated aortic tissues was placed in optimal cutting temperature compound for NO and ROS measurements or snapped-frozen into liquid nitrogen and stored in −80 ℃ for further processing.

2.5. Vascular function study (organ bath)

For the measurement of isometric tension, an aortic ring was suspended in an organ bath containing 5 mL of Krebs solution maintained at 37 ℃ and continuously aerated with 95% O_2 and 5% CO_2 throughout the experiment. The change in the isometric tension was recorded using a Powerlab recording system (AD Instrument, Sydney, NSW, Australia). The rings were stretched to their optimal basal tension for 1 h to maintain the equilibrium of the rings. After that, each ring was contracted with 60 mM KCl until a stable contraction was achieved to test its viability. The rings were then washed three times with Krebs solution before the endothelial function of the endothelium was investigated by cumulative addition of ACh (3 nM-10 μM) to PE (300 nM-1 μM)-contracted rings to induce endothelium-dependent relaxation. A cumulative concentration-response curve to SNP (10 nM-10 μM) was also obtained to investigate if there are any changes in the vascular smooth muscle sensitivity to NO.

2.6. Measurement of NO level

A segment of the thoracic aorta embedded in optimal cutting temperature (OCT) compound (Sakura Finetik, AJ Alphen aan den Rijn, Netherlands) was cut into cryostat sections 10 μm thick. The prepared glass slide was washed using phosphate-buffer saline (PBS) (Sigma, Aldrich, USA) three times every 5 minutes before incubation with 5 μM DAF-FM diacetate fluorescence stain (Invitrogen, Carlsbad, CA, USA) for 30 min at 37 ℃. After incubation, the aortic segments were washed again with PBS three times every 5 minutes to remove excessive staining dye. A cover slip was placed on the slides and the sections were observed by using a fluorescence microscope (ZIESS, Oberkochen, Germany) with excitation at 495 nm and emission at 515 nm for NO intensity detection.

2.7. Measurement of reactive oxygen species (ROS) level

A segment of the thoracic aorta embedded in OCT compound (Sakura Finetik, AJ Alphen aan den Rijn, Netherlands) was cut into cryostat sections 10 μm thick. The prepared glass slide was washed using PBS (Sigma, Aldrich, USA) three times every 5 min before incubation with 5 μM dihydroethidium (DHE) fluorescence stain (Invitrogen, Carlsbad, CA, USA) for 30 min at 37 ℃. After incubation, the aortic segments were washed again with PBS three times every 5 min to remove excessive staining dye. A cover slip was placed on the slides and the sections were observed by using a fluorescence microscope (ZIESS, Oberkochen, Germany) with excitation at 516 nm and emission at 606 nm for ROS intensity detection.

2.8. Measurement of cGMP

The frozen samples of isolated aortic tissues were homogenized in PBS and centrifuged at 15 000 ×*g* for 15 min at 4 ℃. The supernatant was collected for measurement of total cGMP using commercially available ELISA assay kits (Cayman Chemical Company, Ann Arbor, MI, USA). The plate was read at a wavelength of 405 nm using a plate reader (Hidex, Turku Finland, Sweden) immediately. The results are expressed as pg/mL and compared with the controls.

2.9. Measurement of BH_4 level

The frozen samples of isolated aortic tissues were homogenized in PBS and centrifuged at 15 000 ×*g* for 15 min at 4 ℃. The supernatant was collected for measurement of total vascular BH_4 level using a commercially available BH_4 ELISA assay kit (Elabscience, USA). The plate was read at a wavelength of 450 nm (Hidex, Turku Finland, Sweden) immediately. The results are expressed as pg/mL and compared with the controls.

2.10. Western blotting

Treated aortic tissues were homogenized in ice-cold radioimmunoprecipitation (RIPA) lysis buffer (1 μg/mL leupeptin, 5 μg/mL aprotinin, 100 μg/mL phenylmethanesulfonyl fluoride, 1 mM sodium orthovanadate, 1 mM egtazic acid, 1 mM ethylenediamine tetraacetic acid, 1 mM NAF, 2 mg/mL glycerol phosphate). The lysates were centrifuged and the supernatant was collected for Western blot. The protein concentration was determined by a modified Lowry assay (Bio-Rad Laboratories, Hercules, CA, USA). Twenty microgram protein concentrations for

each sample were loaded and separated in a 10% sodium dodecyl sulfate-polyacrylamide gel, then transferred to an immobilon-P polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA) at 110 V for 1 h 30 min. The blots were blocked with 5% bovine serum albumin at room temperature for 1 h. Then the blots were incubated with primary antibodies against AT_1 receptor (1:250, Santa Cruz Biotechnology; sc515884) and β-actin (1:10 000, Santa Cruz Biotechnology; sc-47778) at 4 ℃ overnight. The next day, the blots were washed three times with tris-buffered saline with 0.1% Tween® 20 detergent and incubated with respective HRP-conjugated secondary antibodies at room temperature for 2 h. The membranes were developed with an enhanced chemiluminescence detection system (Millipore, Billerica, MA, USA), and the protein expression images were captured under c600 Ultimate Western System (Azure biosystems) (Dublin, CA, USA). The protein expression was quantified using Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA). The targeted protein level was normalized to β-actin and then compared with the control.

2.11. Statistical analysis

All results were presented as mean ± standard error of mean (SEM). GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA) was used to analyze the concentration-response curves by non-linear regression fitting. One-way ANOVA followed by Bonferroni's multiple comparison tests (for more than two groups) was performed using the same statistical software. A P value of less than 0.05 was considered a statistically significant difference.

2.12. Ethical statement

All animal studies complied with the UK Animals (Scientific Procedures) Act 1986 which was agreed upon and approved by the Ethics Committee of Universiti Tunku Abdul Rahman (U/SERC/38/2020).

3. Results

3.1. Treatment with EGCG decreases SBP of SHR

Treatment with EGCG decreased SBP of SHR in a time-dependent manner. The basal SBP of SHR from different treatment groups was similar before treatment and was significantly higher than in WKY rats (P<0.05) (Figure 1). There was a sustained decrease in SBP of SHR treated with EGCG and losartan from day 3 onwards. The SBP of WKY and SHR control was not significantly altered before and after treatment.

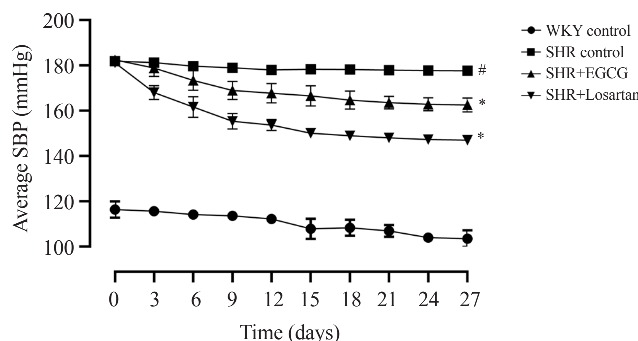

Figure 1. Average systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) treated with epigallocatechin-gallate (EGCG; 50 mg/kg/day) for four weeks. Data are expressed as mean ± SEM (n=4-6) and analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests. $^{\#}P$<0.05 compared to WKY control, $^{*}P$<0.05 compared to SHR control. WKY: Wistar Kyoto rats.

3.2. Treatment with EGCG improves vascular relaxation in SHR

There was a significant decrease in the relaxation to and the pEC_{50} of ACh in the aortic rings from SHR control compared to that of WKY control. Treatment with EGCG improved the relaxation to and the pEC_{50} of ACh in aortic rings of SHR compared to that of SHR control (Figure 2A-C and Table 1). The relaxation to SNP, an exogenous nitric oxide donor, was similar in the aorta of all experimental groups (Figure 2D and E).

3.3. Treatment with EGCG increases the level of vascular NO in SHR

The measurement of DAF fluorescence indicates that SHR exhibited a significantly lower level of vascular NO compared to the WKY control group (P<0.05) (Figure 3). The intensity of DAF fluorescence was significantly higher in aortic tissues of SHR treated with EGCG compared to that of SHR control (P<0.05), implying that four weeks of treatment with EGCG significantly increased the level of vascular NO in SHR.

Table 1. Sensitivity (pEC_{50}) to acetylcholine (ACh) and sodium nitroprusside (SNP) of aortic rings of spontaneously hypertensive rats (SHR) treated with epigallocatechin-gallate (EGCG) (Log M).

Groups	ACh	SNP
WKY control	−7.25 ± 0.22	−7.32 ± 0.30
SHR control	−6.62 ± 0.22#	−7.32 ± 0.40
SHR+EGCG	−6.83 ± 0.40	−7.10 ± 0.47
SHR+Losartan	−6.95 ± 0.12	−6.90 ± 0.26

Data are expressed as mean ± SEM (n=4-6) and analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests. $^{\#}P$<0.05 compared to WKY control.

3.4. Treatment with EGCG decreases the level of vascular ROS in SHR

The measurement of DHE fluorescence intensity showed that SHR exhibited a significantly higher level of vascular ROS compared to the WKY control group (P<0.05) (Figure 4). Treatment with EGCG for four weeks decreased the intensity of DHE fluorescence significantly (P<0.05), indicating a significant decrease in the level of ROS in SHR aorta.

3.5. Treatment with EGCG increases vascular BH_4 levels in SHR

The SHR control group exhibited a significantly lower level of vascular BH_4 compared to the WKY control group (P<0.05) (Figure 5). Treatment with EGCG for four weeks in SHR increased the level of BH_4 significantly compared to non-treated SHR (P<0.05).

3.6. Treatment with EGCG increases vascular cGMP level in SHR

A significantly lower level of cGMP was observed in aorta of the SHR control group compared to that of the WKY control group (P<0.05) (Figure 5). Treatment with EGCG significantly increased the level of cGMP in SHR aorta (P<0.05).

3.7. Treatment with EGCG decreases AT_1 receptor protein expression

The SHR control group had a slightly higher level of AT_1 protein compared to the WKY control group with no significant difference (P>0.05) (Figure 5). Four weeks of treatment with EGCG resulted in

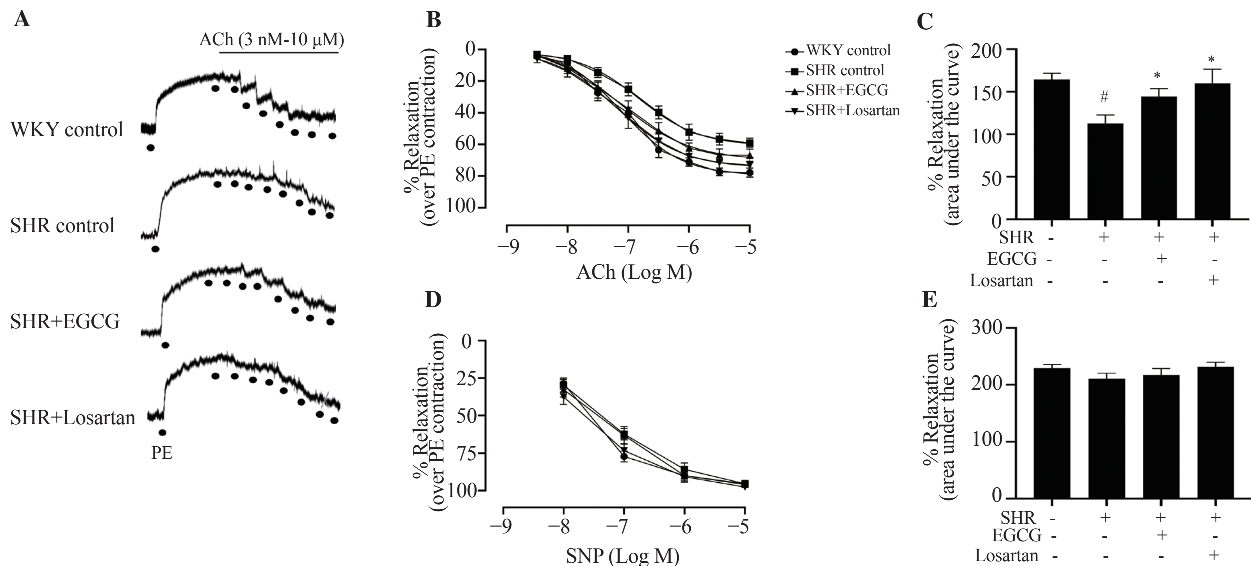

Figure 2. Relaxation curve to increasing concentration of acetylcholine (ACh) and sodium nitroprusside (SNP) of aortic rings from SHR treated with EGCG. (A) Representative tracings of ACh-induced relaxation in phenylephrine (PE)-contracted aortic rings of all experimental groups. (B) Relaxation curve and (C) area under the curve for percentage of relaxation to ACh of all experimental groups. (D) Relaxation curve and (E) area under the curve to SNP of all experimental groups. Data are expressed as mean ± SEM (n=4-6) and analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests. #P<0.05 compared to WKY control, *P<0.05 compared to SHR control.

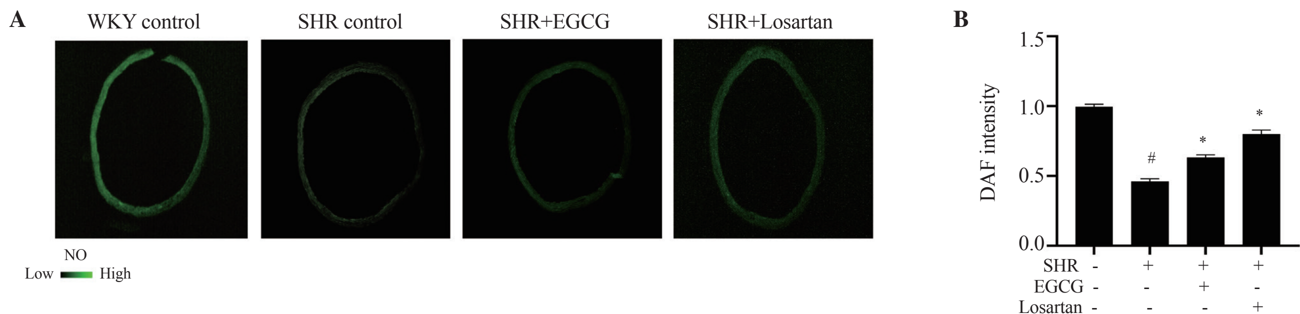

Figure 3. Level of 4-amino-5-methylamino-2',7'-dichlorofluorescein diacetate (DAF) fluorescence intensity in aortic rings of SHR after treatment with EGCG (50 mg/kg/day). (A) The representative images of DAF-stained aortic rings. (B) Quantified fluorescence intensity for all treatment groups. Data are expressed as mean ± SEM (n=4-6) and analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests. #P<0.05 compared to WKY control, *P<0.05 compared to SHR control.

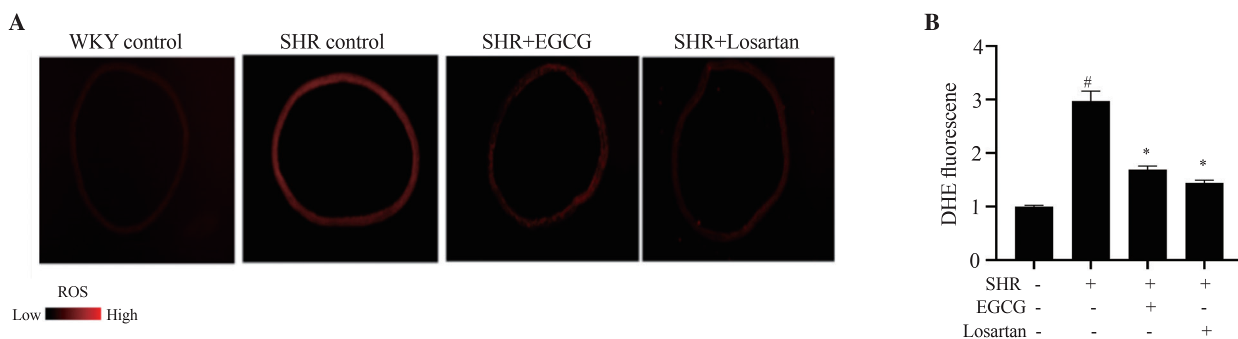

Figure 4. Level of reactive oxygen species (ROS) in aortic rings of SHR treated with 50 mg/kg EGCG for four weeks by dihydroethidium (DHE) fluorescence. (A) The representative images of DHE-stained aortic rings. (B) Quantified fluorescence intensity. Data are expressed as mean ± SEM (n=4-6) and analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests. $^{\#}P$<0.05 compared to WKY control, $^{*}P$<0.05 compared to SHR control.

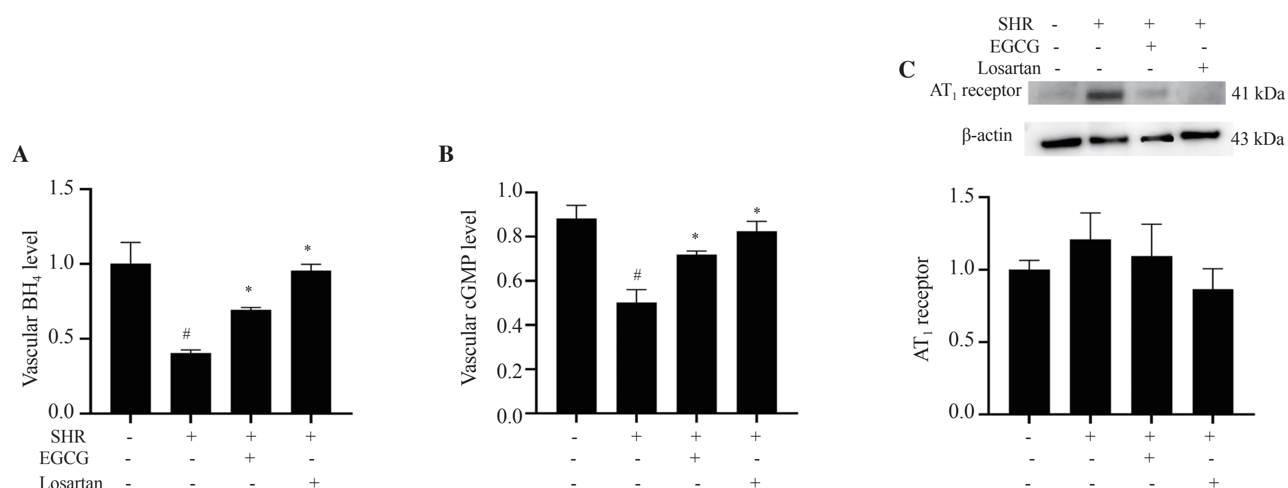

Figure 5. (A) Vascular tetrahydrobiopterin (BH_4) and (B) cyclic guanosine monophosphate (cGMP) levels as well as (C) angiotensin Ⅱ type 1 receptor (AT_1 receptor) protein level in aortic rings of SHR treated with 50 mg/kg EGCG. Data are expressed as mean ± SEM (n=4-6) and analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests. $^{\#}P$<0.05 compared to WKY control, $^{*}P$<0.05 compared to SHR control.

a decreasing trend of the AT_1 receptor in the aortic tissues of SHR.

4. Discussion

The present findings demonstrated that treatment with EGCG for four weeks significantly reduced SBP of hypertensive animals. This is followed by an improvement in endothelium-dependent relaxation in SHR aorta. Besides that, treatment with EGCG resulted in an increase in the level of total vascular NO and a decrease in the level of vascular ROS in SHR. In addition, an increase in the level of vascular BH_4 and cGMP was observed in the aortic tissues of SHR treated with EGCG. In line with these results, it is also observed that there is a decreasing trend in the protein expression of AT_1 receptor in SHR treated with EGCG. The current findings suggest that treatment with EGCG decreases the expression of AT_1 receptors, leading to a decrease in ROS level thus decreasing eNOS uncoupling *via* increasing the level of BH_4 which in turn increases the level of NO. This in part improves the vascular relaxation of SHR that contributes to a decrease in the blood pressure of hypertensive animals.

EGCG is a major catechin found in green tea. It has been demonstrated to have many beneficial effects on cardioprotection, antioxidant, and vascular remodeling[20]. Since EGCG is a major component in green tea, the antihypertensive effect of green tea may be attributed to the effect of EGCG in green tea. Indeed, four weeks of treatment with EGCG has lowered SBP in hypertensive animals in our study. This result is in line with a recent study that has shown that treatment with EGCG significantly reduced SBP in angiotensin Ⅱ-infused hypertensive mice[21] and SHR[22]. Although EGCG has been shown to exert an antihypertensive effect, its vascular protective mechanism is yet to be determined.

Hypertension is strongly associated with endothelial dysfunction[3]. A study done by Lau's team showed that SBP and body weight have

been normalized and improved endothelial-dependent relaxation in C57BL/6J tunicamycin-treated mice by 3′,4′-dihydroxyflavonol[23]. Another study also showed that a decrease in SBP is followed by an increase in endothelium-dependent relaxation[24]. Similarly, the present study demonstrated that a decrease in SBP of EGCG-treated SHR is accompanied by an improvement in the endothelium-dependent relaxation in SHR aorta. This is in line with a study done by Potenza and colleagues whereby 3 weeks of treatment with EGCG in SHR can improve vasodilation and insulin resistance by stimulating endothelial production of NO[25]. An experiment done by Mohd Sabri and colleagues has demonstrated that 14 days of treatment with EGCG significantly improved impaired vasodilation induced by ACh in angiotensin Ⅱ-infused mice[21]. These studies imply that the improvement in vascular function may partly contribute to the decrease in SBP of the treated hypertensive animals.

NO is a highly reactive molecule that plays an important role in vascular homeostasis and blood pressure regulation[26]. The release of NO leads to the activation of sGC to produce cGMP in vascular smooth muscle to induce vasodilation[27]. The DAF fluorescence staining on the vascular tissues of the animals in the current study has indicated that there is an increase in the level of vascular NO in EGCG-treated SHR compared to untreated SHR control. In addition to this, the current study has shown that there is an elevated level of BH_4 in SHR treated with EGCG. BH_4 is a cofactor of eNOS that is essential in the production of endogenous NO to maintain normal endothelial function[28]. Depletion of BH_4 resulted in the uncoupled state of eNOS and thus reduced NO bioavailability[29]. The elevated level of NO in SHR treated with EGCG is accompanied by an increase in the level of BH_4 in this current study which is tallying with the work done by Zhang and colleagues. Zhang *et al*. have demonstrated that 10-50 μM of EGCG treatment elevated the expression of BH_4 and eNOS (dimer) as well as increased NO production and attenuated ROS formation in high glucose-treated human umbilical vein endothelial cells[30]. Our current data imply that treatment with EGCG can increase the level of BH_4 in the aortic tissues of hypertensive animals, leading to increased eNOS coupling and thus increasing production of the NO level.

The NO produced in the endothelium diffuses into the underlying smooth muscle cells and binds to the heme moiety of sGC. This leads to the production of cGMP and thus activates the cGMP-dependent protein kinases and causes vascular relaxation[31]. In line with the increase in the level of vascular NO and BH_4, an elevated level of cGMP was observed in SHR treated with EGCG. A previous study has shown oral administration of EGCG (100 mg/kg) after 2 h increased the plasma cGMP level in both male and female C57BL/6J mice[32]. Hence, our current finding implies that treatment with EGCG improves endothelial dysfunction partly *via* stimulation of the NO-sGC-cGMP pathway.

Excessive production of ROS leads to oxidative stress and subsequently causes vascular cell damage[33]. The current study has demonstrated that there is a lower DHE fluorescence intensity in aortic ring of EGCG-treated SHR. This indicates that EGCG can scavenge free radicals and reduce the formation of ROS. This result is in line with the result obtained from DAF staining whereby there is an increase in NO level following treatment of EGCG. An increase in total vascular NO level accompanied by a decrease in the production of ROS cumulatively leads to decreased oxidative stress, thus contributing to the increase in vasodilation which partly reduces the blood pressure of SHR. The current finding is in agreement with the finding of the previous study in which two weeks of treatment with EGCG in angiotensin Ⅱ-infused mice successfully reduced the DHE fluorescence intensity, indicating a decrease in ROS formation and oxidative stress[21].

AT_1 receptor is a major bioactive peptide in the RAAS and its activation by angiotensin Ⅱ leads to the increased activation of NOx[34]. Activation of NOx through the stimulation of AT_1 receptor leads to the production of O_2^- thus elevating the level of ROS and oxidative stress in vasculature[35]. Han showed the protective effect of EGCG in vascular endothelial cells *via* decreasing NOx expression[36]. However, there have been no studies on the effect of EGCG on the upstream of NOx activation. The current study showed treatment with EGCG reduced the expression of AT_1 receptor, albeit statistically insignificant, in aortic tissues of SHR compared to the untreated hypertensive animals. Hence, EGCG may also partly improve the endothelial function of SHR by attenuating AT_1 receptor-dependent signaling pathway.

EGCG has been demonstrated to inhibit transcriptional activity of NF-κB-p65 in normal human bronchial epithelial cells[37]. It also suppresses inflammation by inhibiting NF-κB in human coronary artery endothelial cells[38]. Since binding of angiotensin Ⅱ to AT_1 receptor activates NF-κB which leads to increased level of oxidative stress, the beneficial effect of EGCG observed in this study may be attributed to NF-κB suppression induced by EGCG. Further study involving the measurement of the level of NF-κB will help to verify if treatment with EGCG suppresses NF-κB and thus improve endothelial function, leading to attenuation of elevated blood pressure.

Further, angiotensin Ⅱ-AT_1 receptor binding activates the pro-inflammatory transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which is mediated by NOX-derived ROS production and MAPK activation.

In summary, the findings from this study showed that EGCG improved endothelial dysfunction in hypertensive animals, accompanied by a decrease in oxidative stress and BH_4-eNOS uncoupling and an increase in NO bioavailability. Besides, treatment

with EGCG also caused a decrease in the protein expression of vascular AT_1 receptors.

Despite mixed outcomes from clinical trials of green tea and EGCG, the good safety profile of EGCG and its vasoprotective effect should provide new impetus to conduct preliminary investigations of the effectiveness of the polyphenol for treatment and prevention of hypertension. Besides, longer treatment durations of EGCG should be studied to investigate its long-term effect. Finally, other potential studies with EGCG should also examine other mechanisms including potential anti-inflammatory and the central actions of the polyphenol.

Conflict of interest statement

The authors declare no conflict of interest.

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Authors' contributions

YYYK and WCL performed the research and analyzed the data. WCL, SKL, and DDM designed the research study. WCL, SKL, and DDM contributed to essential reagents and tools. All authors wrote and reviewed the manuscript.

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