

## Original Article

# Sesamin ameliorates arterial dysfunction in spontaneously hypertensive rats via downregulation of NADPH oxidase subunits and upregulation of eNOS expression

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**Aim:** Sesamin is one of the major lignans in sesame seeds with antihyperlipidemic, antioxidative and antihypertensive activities. The aim of this study was to examine the effects of sesamin on arterial function in spontaneously hypertensive rats (SHRs).

**Methods:** SHRs were orally administered sesamin (40, 80 and 160 mg·kg<sup>-1</sup>·d<sup>-1</sup>) for 16 weeks. After the rats were killed, thoracic aortas were dissected out. The vasorelaxation responses of aortic rings to ACh and nitroprusside were measured. The expression of eNOS and NADPH oxidase subunits p47<sup>phox</sup> and p22<sup>phox</sup> in aortas were detected using Western blotting and immunohistochemistry. Aortic nitrotyrosine was measured with ELISA. The total antioxidant capacity (T-AOC) and MDA levels in aortas were also determined.

**Results:** The aortic rings of SHRs showed significantly smaller ACh-induced and nitroprusside-induced relaxation than those of control rats. Treatment of SHRs with sesamin increased both the endothelium-dependent and endothelium-independent relaxation of aortic rings in a dose-dependent manner. In aortas of SHRs, the level of T-AOC and the expression of nitrotyrosine, p22<sup>phox</sup> and p47<sup>phox</sup> proteins were markedly increased, while the level of MDA and the expression of eNOS protein were significantly decreased. Treatment of SHRs with sesamin dose-dependently reversed these biochemical and molecular abnormalities in aortas.

**Conclusion:** Long-term treatment with sesamin improves arterial function in SHR through the upregulation of eNOS expression and downregulation of p22<sup>phox</sup> and p47<sup>phox</sup> expression.

**Keywords:** sesamin; spontaneously hypertensive rat; aorta; vasorelaxation; endothelium; eNOS; NADPH oxidase; malondialdehyde; ACh; nitroprusside

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## Introduction

The endothelial nitric oxide synthase (eNOS) within normal endothelium contributes to the regulation of systolic blood pressure by the production of nitric oxide (NO) from *L*-arginine. Spontaneously hypertensive rats (SHR) are characterized by arterial dysfunction resulting from the increased generation of reactive oxygen species (ROS), which subsequently causes NO breakdown and the suppression of eNOS expression<sup>[1–5]</sup>. Several studies have shown that eNOS expression is significantly decreased in dysfunctional arteries from SHR<sup>[6, 7]</sup>. An exaggerated production of the superoxide anion ( $\cdot\text{O}_2^-$ ) by the vasculature has been observed in SHR<sup>[8]</sup>, which is predomi-

nantly derived from NADPH oxidase<sup>[9]</sup>. NO can be scavenged by  $\cdot\text{O}_2^-$  to form peroxynitrite, effectively reducing the bioavailability of endothelium-derived NO<sup>[10]</sup>. Thus, diminished NO has been linked to decreased eNOS expression and increased  $\cdot\text{O}_2^-$  in hypertension and may lead to arterial dysfunction<sup>[11–14]</sup>.

Sesamin (Ses), a major lignan in sesame seeds, has antihyperlipidemic, antioxidative and antihypertensive pharmacological properties in different murine models<sup>[15–23]</sup>. Recent studies have demonstrated that sesamin metabolites induce an endothelial NO-dependent vasorelaxation through their antioxidative property-independent mechanisms<sup>[22, 24]</sup>. Furthermore, sesamin feeding leads to enhanced endothelium-dependent relaxation by suppressing aortic NADPH oxidase in deoxycorticosterone acetate (DOCA)-salt hypertensive rats<sup>[25]</sup>. Kong<sup>[18]</sup> *et al* and Wu<sup>[26]</sup> *et al* reported that sesamin can improve endothelial function and exert reno-protective effects

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by enhancing NO bioactivity in renovascular hypertensive rats fed with a high-fat, high-sucrose diet. However, no study to date has reported the effects of sesamin on the aortas from SHR.

Therefore, the purpose of this study was to evaluate the arterio-protective effects of sesamin in SHR. Additionally, we examined the roles of p22<sup>phox</sup>, p47<sup>phox</sup>, eNOS and nitrotyrosine (the footprint of NO interaction with  $\cdot\text{O}_2^-$ ) expression to explore the mechanisms behind sesamin-mediated arterial protection.

## Materials and methods

### Drugs and reagents

Sesamin (Figure 1, >94%) was provided by Tianyi Lvbao Technology Co, Ltd (Chinese invention patent number ZL 03113181.6 Wuhu, China). Norepinephrine, phenylephrine, acetylcholine (ACh, an endothelium-dependent vasodilator), and nitroprusside (an endothelium-independent vasodilator) were purchased from Sigma (St Louis, MO, USA). Krebs solution was made from the following reagents (in mmol/L): 118.3 NaCl, 25 NaHCO<sub>3</sub>, 4.7 KCl, 2.5 CaCl<sub>2</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 1.2 MgSO<sub>4</sub>, and 11.1 glucose.

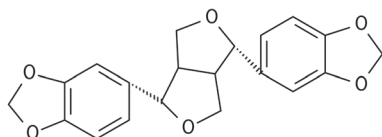


Figure 1. The structure of sesamin.

### Experimental animals, grouping and treatment

The experiments were performed on aortas from age- (16 weeks) and weight-matched male SHR and Wistar-Kyoto rats (WKY) [SCXK (Shanghai) 2007-0005, Shanghai SLAC Laboratory Animal Co, Ltd]. Twenty-eight SHR were randomly assigned to a model group (SHR-untreated,  $n=7$ ), three treatment groups (SHR-ses 160 mg/kg, SHR-ses 80 mg/kg, SHR-ses 40 mg/kg,  $n=7$  each) or a control group (WKY-untreated,  $n=7$ ). Animals had free access to a standardized diet and tap water. Sesamin was suspended in 0.5% carboxymethylcellulose sodium and orally administered between 5:00 and 6:00 PM every day for 16 weeks in the three treatment groups. Untreated groups received an equal volume of carboxymethylcellulose sodium as a control. All animals were housed in a room with standardized temperature ( $21\pm 1^\circ\text{C}$ ) and exposed to a 12 h dark-light cycle.

### Sample collection

At the end of the experiment, the rats were fasted overnight and anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg, ip). Thoracic aortas from the rats were carefully removed, cleaned of adhering tissue and divided into two parts. One part contained the descending thoracic aorta, which was cut into two transverse rings (3–4

mm in length), was used for the vascular reactivity experiments. Portions of the arcus aortae were either used for immunohistochemistry or were quickly frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  for future processing.

### Vascular reactivity studies

The thoracic aorta was quickly removed and cut into rings 3 mm in length. Two rings were taken from every rat aorta and were suspended between two triangular-shaped stainless steel stirrups in two jacketed organ chambers (A and B, Model ALC-M, Alcott Biotech Co, Ltd, Shanghai, China) containing 10 mL of Krebs solution at  $37^\circ\text{C}$  that was continuously oxygenated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Pre-load (2 g) was applied to the rings, and the vessels were allowed to equilibrate for 60 min (with 4 washes). Changes in isometric tension were detected by force transducers and recorded via a MAP2000 polygraph (Alcott Biotech Co, Ltd, Shanghai, China). The rings were stimulated with norepinephrine ( $3\times 10^{-7}$  mol/L) to evaluate their viability and were serially washed back to baseline levels and equilibrated once again. In chambers A or B, the concentration-relaxation response curves to acetylcholine ( $10^{-8}$  to  $10^{-5}$  mol/L) or nitroprusside ( $10^{-9}$  to  $10^{-6}$  mol/L) were performed in aortic rings, which were precontracted by phenylephrine ( $10^{-6}$  mol/L), respectively.

### Malondialdehyde determination

Malondialdehyde (MDA), a degradation product of lipid peroxidation, is a class of thiobarbituric acid reactive substances. Aortic MDA levels were measured by a thiobarbituric acid assay proposed by Rodriguez-Martinez and Ruiz-Torres<sup>[27]</sup> and modified by Manso<sup>[28]</sup> *et al*.

### Total antioxidant capacity assays

Total antioxidant capacity (T-AOC) was determined in rat aortic homogenate using commercial kits from the Beyotime Institute of Biotechnology (Haimen, China). The frozen aortas were homogenized in 10 mmol/L Tris buffer (pH 7.4) containing 1% Triton X-100, 1 mmol/L EDTA, 1 mmol/L phenylmethyl sulfonyl fluoride (PMSF), 10 mg/mL aprotinin and 10 mg/mL leupeptin. T-AOC was determined by the ferric-reducing ability of plasma (FRAP) method<sup>[29]</sup>. Protein concentration was determined by the bicinchoninic acid assay (Beyotime Institute of Biotechnology, Haimen, China).

### ELISA

Nitrotyrosine is a stable end product of peroxynitrite-mediated oxidation/nitration, which can be used as a footprint of *in vivo* peroxynitrite (ONOO<sup>-</sup>) formation and a surrogate index of *in vivo*-uncoupled eNOS-dependent damage<sup>[30]</sup>. Peroxynitrite is a strong oxidant formed in the reaction between NO and superoxide ( $\cdot\text{O}_2^-$ ) and the subsequent reaction of peroxynitrite with protein, which results in the formation of nitrotyrosine. Aortic nitrotyrosine was measured using the NWLSS nitrotyrosine ELISA kit (Northwest Life Science Specialties, LLC) according to the manufacturer's protocol. The protein concentration was determined by the bicinchoninic acid assay (Beyotime Institute

of Biotechnology, Haimen, China).

### Immunohistochemistry

Immunostaining for p22<sup>phox</sup>, p47<sup>phox</sup>, and eNOS were performed on 4% paraformaldehyde-fixed rat aorta sections according to previously described protocols<sup>[31–33]</sup>. Thoracic aortas embedded in paraffin were cut into 5- $\mu$ m sections. Slides were incubated in methanol containing 0.3 % H<sub>2</sub>O<sub>2</sub> for 30 min and blocked with normal goat serum in PBS (containing 0.1% BSA and 0.01% Tween 20) for 30 min. Primary antibodies against p22<sup>phox</sup>, p47<sup>phox</sup> (Santa Cruz Biotechnology, Santa Cruz, USA), or eNOS (Boster Bio-engineering, Wuhan, China) diluted in PBS containing 0.1% BSA and 0.01% Tween 20 were applied to slides for 12 h at 4°C. After two washes with PBS containing 0.01% Tween 20, slides were processed using the ABC and DAB staining kit (Vector Laboratories, Burlingame, CA, USA). After counterstaining with hematoxylin, slides were dehydrated and permanently mounted. Sections (five microscopic fields per slice with one slice/animal) were digitized using an Olympus BX51 microscope (Olympus Optical Co, Ltd, Tokyo, Japan). Digital images were processed using Image-Pro Plus 6.0 software (Media Cybernetics Inc, Maryland, USA), which was used to determine the integral optical density (IOD) and average optical density (AOD, AOD=positive area $\times$ OD/total area) of p22<sup>phox</sup>, p47<sup>phox</sup>, and eNOS as described previously<sup>[34, 35]</sup>.

### Western blotting analysis

P22<sup>phox</sup>, p47<sup>phox</sup>, and eNOS protein levels in the aortas were detected by western blotting as previously described<sup>[36–38]</sup>. Aorta samples were homogenized in ice-cold RIPA buffer (Beyotime Institute of Biotechnology, Haimen, China). The protein concentrations were determined using a BCA protein assay kit (Beyotime Institute of Biotechnology, Jiangsu and China). Aortic extracts (30  $\mu$ g of protein per lane) were mixed with sample loading buffer and separated on a 12% SDS-polyacrylamide gel. Proteins were electro-transferred on PVDF membranes (0.45  $\mu$ m, Beyotime Institute of Biotechnology, Haimen, China). The membranes were blocked in TBST (1 $\times$ TBS, 0.1% Tween-20) containing 5% nonfat dry milk for one hour at room temperature and incubated with the following antibodies: p22<sup>phox</sup>, p47<sup>phox</sup> (Santa Cruz Biotechnology, Santa Cruz, USA), eNOS (Boster Bio-engineering, Wuhan, China) and  $\beta$ -actin (Bioss Biotechnology, Beijing, China) at 4°C overnight. The bands were detected using a chemiluminescence assay (ECL Plus, Beyotime Institute of Biotechnology, Haimen, China) and evaluated by densitometry using Image-Pro Plus 6.0 software (Media Cybernetics Inc, USA). The relative density values of each band normalized to  $\beta$ -actin were presented.

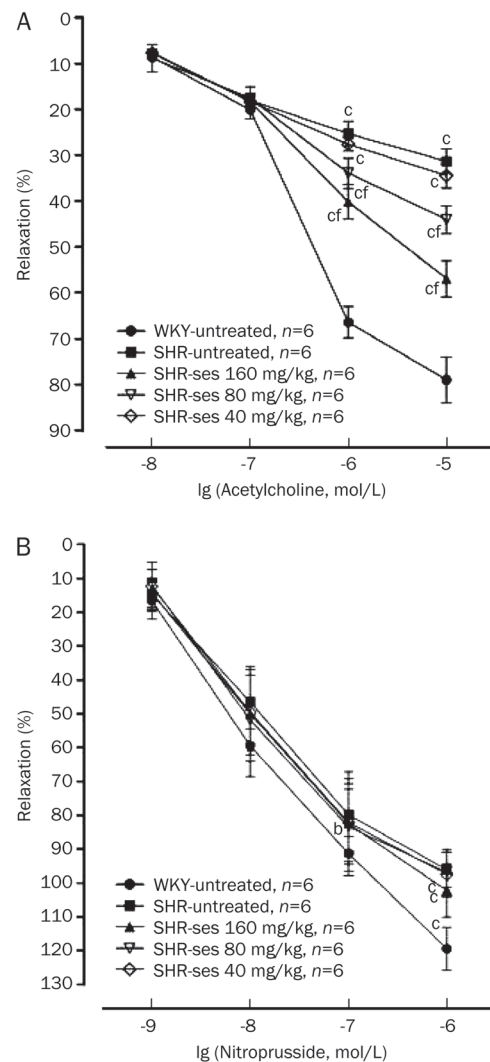
### Statistical analysis

Data were expressed as the mean $\pm$ SD. For statistical analysis, one-way ANOVA was used followed by Newman-Keuls tests. A *P*-value of *P*<0.05 was considered to be statistically significant.

## Results

### Vascular activity *ex vivo*

Acetylcholine elicited a concentration-dependent relaxation in phenylephrine precontracted aortic rings in all experimental groups. Acetylcholine-induced vasorelaxation was significantly decreased (by 25.27%, 31.34% for 10<sup>-6</sup>, 10<sup>-5</sup> mol/L acetylcholine, respectively; *P*<0.01) in the SHR-untreated group compared with the WKY-untreated group. The aortic rings from the SHR-ses 160 mg/kg and SHR-ses 80 mg/kg groups showed significant increases (by 25.57% and 12.73% for 10<sup>-5</sup> mol/L acetylcholine, respectively; *P*<0.01) in vasodilatation induced by acetylcholine as compared to those from the SHR-untreated group (Figure 2A). The endothelium-independent vasorelaxation induced by sodium nitroprusside was significantly decreased (by 11.50%, 23.42% for 10<sup>-7</sup>, 10<sup>-6</sup> mol/L sodium nitroprusside, respectively; *P*<0.01) in the SHR-untreated group compared with the WKY-untreated

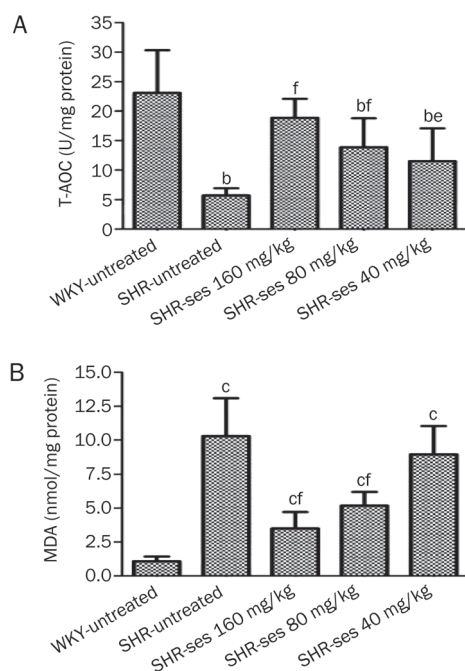


**Figure 2.** Effects of sesamin on the aortic relaxant responses. (A) ACh induced relaxation responses. (B) SNP induced relaxation responses. <sup>b</sup>*P*<0.05, <sup>c</sup>*P*<0.01 vs WKY-untreated. <sup>f</sup>*P*<0.01 vs SHR-untreated.

group. The vasorelaxation induced by sodium nitroprusside was increased in SHR-ses 160 mg/kg and SHR-ses 80 mg/kg groups (by 6.13%, 5.83% for  $10^{-6}$  mol/L sodium nitroprusside, respectively;  $P>0.05$ ) compared with the SHR-untreated group (Figure 2B). These results indicated that sesamin may significantly improve endothelium-dependent dysfunction and weakly increase endothelium-independent relaxation.

#### Measurement of aortic T-AOC and MDA

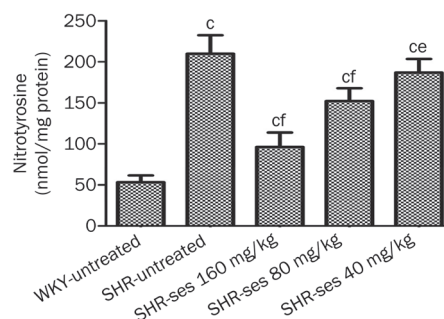
As shown in Figure 3, the decrease of aortic T-AOC content and increase in aortic MDA content confirmed that oxidative damage had been induced in untreated SHR ( $P<0.01$  vs WKY-untreated). The level of aortic T-AOC in the SHR-ses 160 mg/kg, SHR-ses 80 mg/kg, and SHR-ses 40 mg/kg groups were significantly higher than those in the SHR-untreated group ( $P<0.01$  or  $P<0.05$ ). The level of aortic MDA in SHR-ses 160 mg/kg and SHR-ses 80 mg/kg groups was significantly lower than that in the SHR-untreated group ( $P<0.01$ ). These results confirmed previous evidence, which demonstrated that sesamin has antioxidative properties.



**Figure 3.** Effect of sesamin on T-AOC and MDA level in aortas.  $n=7$ . <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs WKY-untreated. <sup>e</sup> $P<0.05$ , <sup>f</sup> $P<0.01$  vs SHR-untreated.

#### Measurement of aortic nitrotyrosine

Aortic nitrotyrosine was significantly higher in the SHR-untreated group compared to the WKY-untreated group (Figure 4). Aortic nitrotyrosine levels were significantly lower in SHR-ses 160 mg/kg, SHR-ses 80 mg/kg, and SHR-ses 40 mg/kg groups. These results demonstrate that sesamin treatment decreases peroxynitrite production by reducing the reaction between superoxide and NO, thereby increasing NO bioavailability.



**Figure 4.** Effect of sesamin on vascular nitrotyrosine content measured by ELISA.  $n=7$ . <sup>c</sup> $P<0.01$  vs WKY-untreated. <sup>f</sup> $P<0.01$  vs SHR-untreated.

#### Expression of eNOS by immunohistochemistry

SHR exhibited a significant reduction of eNOS protein expression in aortic endothelium when compared with WKY. Treatment with sesamin was able to enhance protein expression of aortic eNOS (Figures 5, 6).

#### Expression of p22<sup>phox</sup> by immunohistochemistry

As shown in Figures 7 and 8, p22<sup>phox</sup> protein expressions in aortic tissues were significantly higher in the SHR-untreated group when compared with the WKY-untreated group. These abnormalities were essentially reversed by treatment with 160 mg/kg and 80 mg/kg sesamin for 16 weeks.

#### Expression of p47<sup>phox</sup> by immunohistochemistry

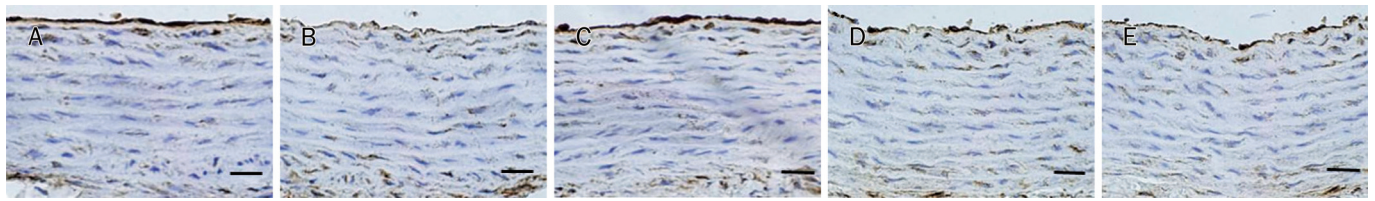
The SHR-untreated group exhibited a notable rise of p47<sup>phox</sup> protein expression ( $P<0.01$  vs WKY-untreated) in Figures 9 and 10. Abnormal p47<sup>phox</sup> protein expression levels were reversed by treatment with 160 mg/kg and 80 mg/kg of sesamin for 16 weeks.

#### Expression of p22<sup>phox</sup>, p47<sup>phox</sup>, and eNOS by Western blotting

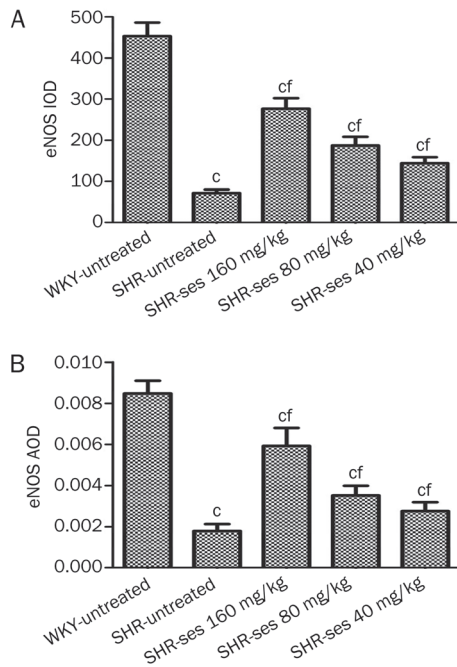
The aortas from the SHR-untreated group exhibited a significant reduction in eNOS protein expression with a notable rise in p22<sup>phox</sup> and p47<sup>phox</sup> protein expression ( $P<0.01$  vs WKY-untreated). The abnormal expression of p22<sup>phox</sup>, p47<sup>phox</sup>, and eNOS were reversed by treatment with sesamin for 16 weeks (Figure 11).

#### Discussion

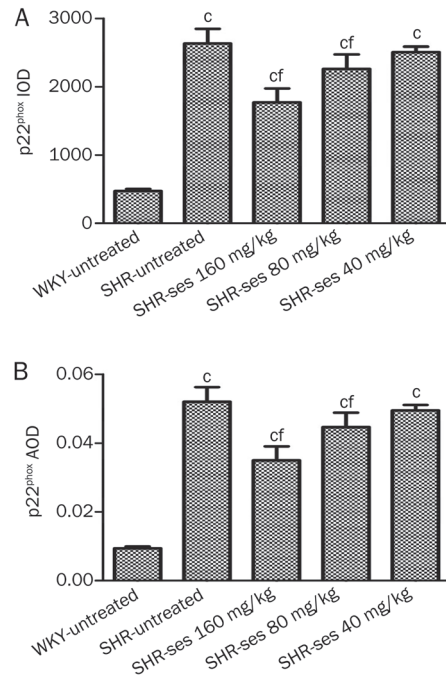
In the present study, we observed that acetylcholine-induced endothelium-dependent vasorelaxation and nitroprusside-induced endothelium-independent vasorelaxation were significantly decreased in the aortas from untreated SHR. These data indicated that there was arterial dysfunction in 32-week-old male SHR. These results were consistent with previous reports<sup>[39, 40]</sup>. After 16 weeks of treatment with sesamin, the endothelium-dependent dysfunction had improved, and the endothelium-independent relaxation was weakly increased. These findings suggest that chronic treatment with sesamin significantly improves arterial dysfunction in SHR. Thus, it is important to ascertain how sesamin could produce



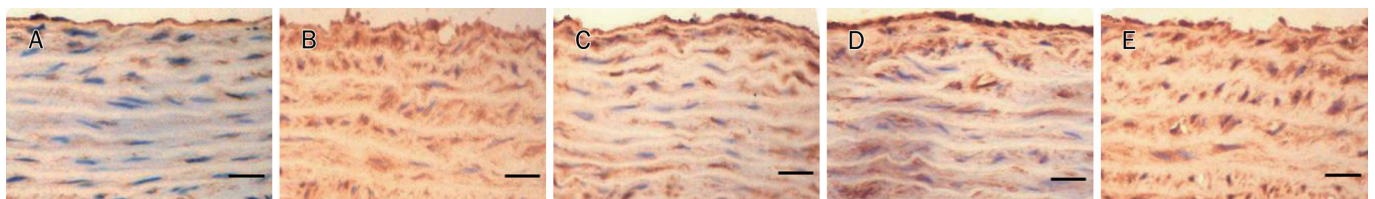
**Figure 5.** Effect of sesamin on the protein expression of eNOS in aortic endothelium of SHRs. (A) WKY-untreated. (B) SHR-untreated. (C) SHR-ses 160 mg/kg. (D) SHR-ses 80 mg/kg. (E) SHR-ses 40 mg/kg. Brown staining in aortic endothelium represents eNOS in the aortas.  $\times 400$ . Bar=50  $\mu\text{m}$ .



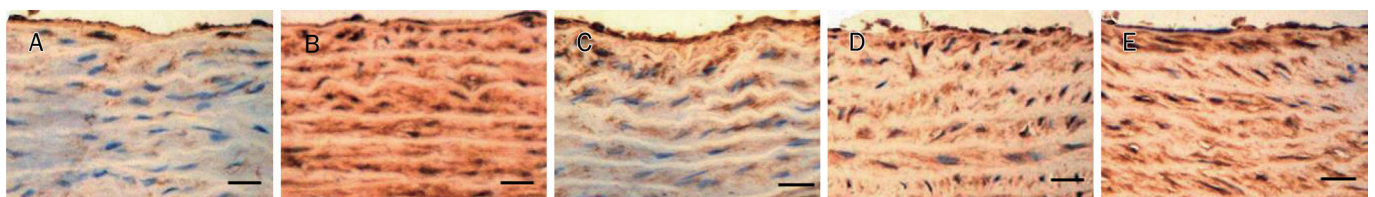
**Figure 6.** Effect of sesamin on the integral optical density (IOD, A) and average optical density (AOD, B) of eNOS in aortic endothelium. Mean  $\pm$  SEM.  $n=7$ .  $^{\circ}P<0.01$  vs WKY-untreated.  $^{\dagger}P<0.01$  vs SHR-untreated.



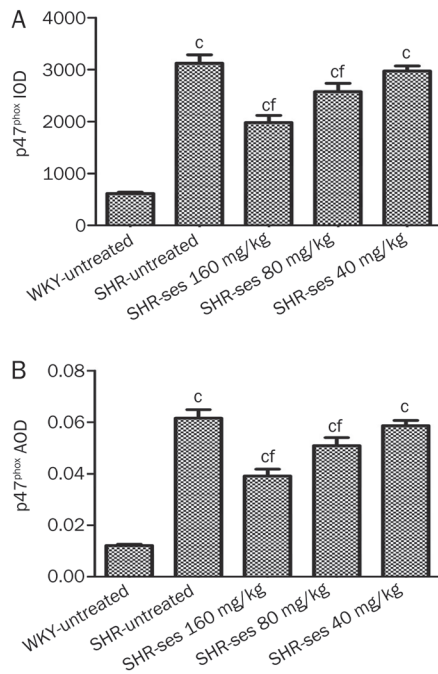
**Figure 8.** Effect of sesamin on the IOD (A) and AOD (B) of p22<sup>phox</sup> in aortas. Data are presented as mean  $\pm$  SEM.  $n=7$ .  $^{\circ}P<0.01$  vs WKY-untreated.  $^{\dagger}P<0.01$  vs SHR-untreated.



**Figure 7.** Effect of sesamin on the protein expression of p22<sup>phox</sup> in aortas. (A) WKY-untreated. (B) SHR-untreated. (C) SHR-ses 160 mg/kg. (D) SHR-ses 80 mg/kg. (E) SHR-ses 40 mg/kg. Brown staining represents p22<sup>phox</sup> in the aortas.  $\times 400$ . Bar=50  $\mu\text{m}$ .



**Figure 9.** Effect of sesamin on the protein expression of p47<sup>phox</sup> in aortas. (A) WKY-untreated. (B) SHR-untreated. (C) SHR-ses 160 mg/kg. (D) SHR-ses 80 mg/kg. (E) SHR-ses 40 mg/kg. Brown staining represents p47<sup>phox</sup> in the aortas.  $\times 400$ . Bar=50  $\mu\text{m}$ .

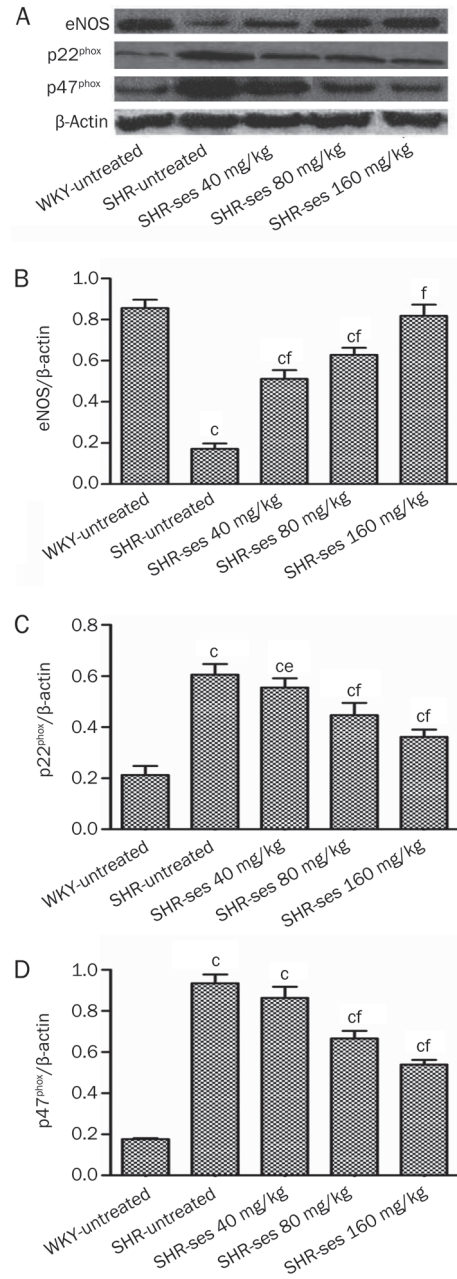


**Figure 10.** Effect of sesamin on the IOD (A) and AOD (B) of p47<sup>phox</sup> in aortas. Data are presented as mean±SEM. *n*=7. <sup>c</sup>*P*<0.01 vs WKY-untreated. <sup>f</sup>*P*<0.01 vs SHR-untreated.

these effects in SHR.

The eNOS within the normal endothelium contributes to the regulation of systolic blood pressure by the production of NO from *L*-arginine. Endothelium-derived NO is an important endogenous vasodilator, which plays a key role in the regulation of vascular tone in addition to other various endothelium-derived molecules<sup>[11, 41]</sup>. NO has opposing, dose-dependent effects on endothelial cell apoptosis; low concentrations of NO protect endothelial cells from apoptosis-inducing stimuli and high concentrations of NO can induce apoptosis<sup>[42, 43]</sup>. Importantly, the diminished capacity to produce NO by the endothelium in hypertension has been linked to decreased eNOS expression<sup>[11, 44]</sup>. We found that sesamin treatment improved the aortic arterial function by increasing eNOS expression in the endothelium of SHR. Several recent findings have supported this conclusion: sesamin enhanced eNOS expression in the aorta of 2K1C rats fed with a HFS; sesamin metabolites induced NO-dependent vasorelaxation; sesamin feeding had no antihypertensive action in chronically *L*-NOARG-treated rats or DOCA-salt-treated mice; sesamin induced eNOS mRNA and protein expression and enhanced NOS activity in human umbilical vein endothelial cells<sup>[18, 24, 45]</sup>. The present experiment demonstrated that sesamin enhanced eNOS expression in the aortic endothelium of SHR and subsequently increased NO levels. Consequently, arterial function was improved. These results confirm and expand upon previous studies demonstrating sesamin mediated improvement in endothelial function<sup>[18, 22]</sup>.

In addition, vascular oxidative stress plays an important



**Figure 11.** Effect of sesamin on the protein expression of eNOS (B), p22<sup>phox</sup> (C), and p47<sup>phox</sup> (D) in aortas by Western blotting. Histograms represent densitometric values normalized to the corresponding β-actin. *n*=7. <sup>b</sup>*P*<0.05, <sup>c</sup>*P*<0.01 vs WKY-untreated. <sup>e</sup>*P*<0.05, <sup>f</sup>*P*<0.01 vs SHR-untreated.

pathophysiological role in the development of arterial dysfunction<sup>[46–49]</sup>. Oxidative stress occurs when the generation of ROS overwhelms antioxidant defense systems<sup>[50, 51]</sup>. There are several parameters of oxidative stress including MDA and T-AOC. MDA, the product of lipid peroxidation, is the most frequently used biomarker for assessing oxidative stress<sup>[52]</sup>. T-AOC provides an overview of the biological interactions between individual antioxidant species, which is composed of some enzymes, such as SOD, GSH-Px, CAT, and non-enzyme-

matic antioxidants<sup>[53]</sup>. In this study, we showed that sesamin effectively increased T-AOC and decreased MDA levels in the aortas from SHR. Therefore, the antioxidative effect of sesamin may account for the improvement in arterial function.

The NADPH oxidases appear to be critical sources of hyper-tensive ROS<sup>[54]</sup>. Vascular  $\cdot\text{O}_2^-$  is produced predominantly from multisubunit NADPH oxidase in the endothelium, VSMCs and the adventitia<sup>[55-64]</sup>. Genetic models of hypertension, such as SHR, exhibit enhanced NADPH oxidase-mediated  $\cdot\text{O}_2^-$  generation in the aorta<sup>[8]</sup>. These processes are associated with increased expression of NADPH oxidase subunits, particularly p22<sup>phox</sup> and p47<sup>phox</sup>, and increased activity of the enzyme<sup>[64]</sup>. NO can interact with  $\cdot\text{O}_2^-$  to form the highly reactive peroxynitrite<sup>[65-67]</sup>. Peroxynitrite is a weak vasodilator compared with NO and a powerful oxidant. Furthermore, it can suppress eNOS expression via activation of RhoA<sup>[5, 68]</sup> and lead to eNOS uncoupling (inactivation of eNOS), which results in reduced NO production, increased  $\cdot\text{O}_2^-$  by the enzyme<sup>[69-71]</sup> and, ultimately, vascular dysfunction.

In our experimental conditions, we found the elevation of aortic nitrotyrosine and upregulation of p22<sup>phox</sup> and p47<sup>phox</sup> protein expression in the vascular tissues of untreated SHR. These results were consistent with previous reports<sup>[64, 72]</sup>. Indeed, the increased nitrotyrosine protein expression from the untreated SHR group was tangible evidence for the presence of oxidative stress leading to enhance NO inactivation. The increased p22<sup>phox</sup> and p47<sup>phox</sup> expression could increase  $\cdot\text{O}_2^-$  production and contribute to the elevation of nitrotyrosine expression and NO inactivation in SHR. Several reports have recently suggested that long-term sesamin treatment decreased aortic nitrotyrosine and p47<sup>phox</sup> protein expression in renovascular hypertensive rats that were fed with a high-fat, high-sucrose diet<sup>[18]</sup>, and sesamin feeding decreased aortic superoxide production and mRNA expression of NADPH oxidase subunits (p22<sup>phox</sup>, gp91<sup>phox</sup>, Nox1, and Nox4) in DOCA-salt hypertensive rats<sup>[22, 25]</sup>. In the present study, we showed that sesamin effectively reduced the expression of nitrotyrosine, p22<sup>phox</sup> and p47<sup>phox</sup> in the aortas from SHR. These results demonstrated that the antioxidative effects of sesamin were due to reduced p22<sup>phox</sup> and p47<sup>phox</sup> expression. Subsequently, the reaction between NADPH oxidase-mediated  $\cdot\text{O}_2^-$  and NO was suppressed, and eNOS expression was increased because of reduced peroxynitrite. Consequently, these physiological changes led to enhanced levels and bioactivity of NO. Ultimately, these findings demonstrated that sesamin improved arterial function, which may be attributed to decreased p22<sup>phox</sup> and p47<sup>phox</sup> expression and increased eNOS expression.

### Acknowledgements

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### Author contribution

Jie-ren YANG and Jun-xiu ZHANG designed research; Jun-xiu ZHANG, Wen-xing LI, Li-juan TANG and Hui YANG performed research; Guo-xiang CHEN and Xiang KONG contributed new analytical tools and reagents; Jun-xiu ZHANG and Jie-ren YANG analyzed data; Jun-xiu ZHANG wrote the paper.

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