

Cardioprotective and hepatoprotective effects of ellagitannins from European oak bark (*Quercus petraea* L.) extract in rats

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Received: 18 May 2011 / Accepted: 3 November 2011 / Published online: 22 November 2011
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Abstract

Background Red wine contains many potentially bioactive polyphenols including resveratrol, catechins, anthocyanins and flavonoids as well as tannins derived from oak during maturation. This study examined the effects of a mixture of ellagitannins from oak bark (*Quercus petraea* L.) on cardiovascular, metabolic and liver changes in high-carbohydrate, high-fat diet-fed rats and in Spontaneously Hypertensive Rats (SHR).

Methods First, 8-week-old male Wistar rats were divided into four groups and given either cornstarch diet, cornstarch diet + oak bark extract (0.5 mL/kg food), high-carbohydrate, high-fat diet or high-carbohydrate, high-fat diet + oak bark extract (0.5 mL/kg food) for 16 weeks. Oak bark extract was added to the diets for last 8 weeks of the feeding period. Secondly, SHR aged 42 weeks fed on standard chow diet were divided into two groups with and without oak bark extract treatment for 12 weeks (0.5 mL/kg food).

Results The high-carbohydrate, high-fat diet induced signs of metabolic syndrome along with cardiovascular remodelling and non-alcoholic steatohepatitis. Oak bark extract attenuated the signs of metabolic syndrome in high-

carbohydrate, high-fat diet-fed rats and improved the structure and function of the heart and the liver. SHR after oak bark extract treatment for 12 weeks showed lower systolic blood pressure, lower cardiac fibrosis and cardiac stiffness and improved vascular reactivity.

Conclusions Oak bark extract containing ellagitannins improved cardiovascular, metabolic and liver parameters in these rat models of human disease, suggesting that part of the benefits attributed to red wine may be produced by these ellagitannins.

Keywords Obesity · Cardiovascular disease · Dyslipidaemia · Ellagitannins · Hypertension

Introduction

Oak has been used in winemaking for more than two millennia, starting in Europe during the Roman Empire. It was later discovered that wine stored in oak barrels had improved characteristics including better taste. The ellagitannins, extracted from oak barrels into the wine during this maturation process [1, 2], are complex naturally occurring polyphenolic compounds present as monomers or oligomers [3, 4]. These polyphenols are hexahydroxydiphenyl esters of carbohydrates with more than 500 naturally occurring tannins having been identified [4, 5]. Oak is one of the major sources of ellagitannins [6] with the European oak (*Quercus petraea* L.) containing vescalagin, castalagin, grandinin and roburin E as the major ellagitannins [7]. The ellagitannins, although present in red wine, have received little attention as potential bioactive compounds. In contrast, the mechanisms for the cardiovascular benefits from other polyphenols in red wine such as resveratrol have been extensively studied [8].

Electronic supplementary material The online version of this article (doi:10.1007/s00394-011-0277-1) contains supplementary material, which is available to authorized users.

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Other dietary sources of ellagitannins include pomegranates, chestnuts, raspberries, strawberries, blackberries and walnuts [5, 6, 9, 10]. Ellagitannins from pomegranates have been studied for their cardioprotective effects in laboratory animals [11]. Pomegranate juice attenuated isoproterenol-induced cardiac necrosis [12] and reduced angiotensin-induced hypertension, glucosuria and proteinuria in streptozotocin-induced diabetic rats [13]. Pomegranate fruit extract containing punicalagin as the major ellagitannin improved vascular endothelial function without affecting the plasma lipid profile in obese Zucker rats [14, 15].

Cardiovascular disease, including hypertension, remains a major cause of morbidity and mortality around the world [16]. Together with obesity and insulin resistance, hypertension is a key criterion for diagnosis of the metabolic syndrome, a major risk factor for cardiovascular disease and type 2 diabetes [17] as well as the development of non-alcoholic fatty liver disease [18]. Rats are widely used to mimic human disease states, especially cardiovascular and endocrine diseases [19–22]. Signs of human metabolic syndrome can be induced by high-carbohydrate, high-fat feeding in male Wistar rats [20]. The Spontaneously Hypertensive Rat (SHR) is the most common rat model of human hypertension [19].

This study has characterised the cardioprotective and hepatoprotective effects of oak bark extract (OBE) containing ellagitannins. Studies were performed on high-carbohydrate, high-fat diet-fed male Wistar rats (H) or the control cornstarch diet-fed rats (C). Subgroups of H and C rats were treated with OBE for 8 weeks (HO and CO rats, respectively). Further, adult male SHR with stable and high systolic blood pressure (S rats) were treated with OBE for 12 weeks (SO rats). Cardiovascular, hepatic and metabolic parameters were studied to evaluate whether OBE was effective in attenuating the complications observed in these rat models of human disease.

Materials and methods

Extract preparation and characterisation

European oak bark (*Quercus petraea* L.) was converted into chips and extracted for 6 months at room temperature in brandy containing 70% alcohol. OBE was filtered to remove the solid material and de-alcoholised before being analysed by liquid chromatography/mass spectrometry (LC/MS) employing electrospray mass spectrometry and ultraviolet detection. The analysis was performed on a Micromass Quattro micro tandem quadrupole mass spectrometer (Waters, Manchester, UK). LC separation was provided by a Waters liquid chromatograph (Waters,

Milford, USA), consisting of a 2,695 separation module and 2,487 dual-wavelength ultraviolet detector. Data were acquired by the Masslynx data system for both the MS and ultraviolet data. For LC, a flow rate of 1 mL/min was used with 0.1% aqueous formic acid and methanol as solvent and injection volume of 20 μ L. For UV detection, 254 and 280 nm wavelengths were used. Castalagin,¹ vescalagin (see footnote 1), grandinin (see footnote 1), roburin E (see footnote 1) and ellagic acid (MP Bio-medicals, Seven Hills, NSW, Australia) were used as standards in this procedure. There were 20% uncertainties in the measurements of ellagitannins derived from the contributions of the uncertainties in the preparation and analysis of the standards and samples.

Rats, diets and treatment with OBE

All experimental protocols were approved by The University of Queensland Animal Experimentation Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia. Male Wistar rats (8 weeks old, weighing 328 ± 2 g, $n = 40$) and male SHR (42 weeks old, weighing 422 ± 6 g, $n = 20$) were supplied by The University of Queensland Biological Resources facility and The Prince Charles Hospital, Brisbane, respectively.

Male Wistar rats were randomly divided into four experimental groups and were fed with either cornstarch diet (C; $n = 10$), cornstarch diet + OBE (0.5 mL/kg food; CO; $n = 10$), high-carbohydrate, high-fat diet (H; $n = 10$) or high-carbohydrate, high-fat diet + OBE (0.5 mL/kg food; HO; $n = 10$) for 16 weeks. CO and HO rats were fed with respective diets for the first 8 weeks without OBE; OBE was supplemented in the diets of CO and HO rats for the last 8 weeks of the protocol. Compositions of H and C diets used in this study have been described previously [20, 23]. Male adult SHR with high and stable systolic blood pressure were divided into two groups of 10 rats each, one without treatment (S) and one with OBE treatment in diet (SO; 0.5 mL/kg in food) for 12 weeks. All SHR were fed on standard powdered chow diet (Specialty Feeds, Glen Forest, WA, Australia). All rats were given ad libitum access to food and water and were individually housed in temperature-controlled 12-h light-dark conditions. Energy intakes were calculated as described previously [20, 23].

¹ Pure castalagin, vescalagin, grandinin and roburin E for use as standards were kindly provided by Professor Stéphane Quideau, European Institute of Chemistry and Biology, University of Bordeaux I, France.

Physiological and metabolic parameters

All rats were monitored daily for body weight, food intake and water intake. Abdominal circumference and body length of rats were measured at the end of protocol using a standard measuring tape under light anaesthesia with Zoletil (tiletamine, 10 mg/kg; zolazepam, 10 mg/kg, i.p.; Virbac, Peakhurst, NSW, Australia). Body mass index (BMI) and feed efficiency were calculated as previously described [20].

At the end of the protocol, rats were food-deprived for 12 h and oral glucose tolerance tests were performed as previously described [20, 23]. Blood glucose concentrations obtained from oral glucose tolerance tests were used to calculate area under the curve (AUC). Plasma concentrations of total cholesterol and triglycerides were determined using kits and controls supplied by Olympus using an Olympus analyser (AU 400, Tokyo, Japan) [20, 23]. Non-esterified fatty acids (NEFA) in plasma were determined using a commercial kit (Wako, Osaka, Japan) [20, 23]. During terminal experiments, abdominal fat pads were removed, weighed and normalised to tibial length at the time of fat removal.

Assessment of cardiovascular structure and function

Systolic blood pressure measurements

Systolic blood pressure of rats was measured under light anaesthesia with Zoletil, using an MLT1010 Piezo-Electric Pulse Transducer (ADInstruments, Sydney, Australia), an inflatable tail-cuff connected to a MLT844 Physiological Pressure Transducer (ADInstruments, Sydney, Australia) and PowerLab data acquisition unit (ADInstruments, Sydney, Australia) [20, 23]. These measurements were taken every fourth week for C, CO, H and HO groups starting at initiation of feeding period and every second week for S and SO groups starting 2 weeks before the initiation of protocol.

Echocardiography

Echocardiographic examinations (Phillips iE33, 12 MHz transducer) were performed to assess the cardiovascular structure and function in all the rats at the end of protocol as previously described [20].

Isolated Langendorff heart preparation

Rats were killed by injection with Lethobarb (pentobarbitone sodium, 100 mg/kg, i.p.; Virbac, Peakhurst, NSW, Australia), and heparin (200 IU; Sigma-Aldrich Australia, Sydney, Australia) was injected through the right femoral

vein. The abdomen was then opened and blood (~5 mL) was withdrawn from the abdominal aorta, collected into heparinised tubes and centrifuged at $5,000\times g$ for 15 min to obtain plasma. Plasma was stored at $-20\text{ }^{\circ}\text{C}$ before further biochemical analysis. Hearts were removed and used as isolated Langendorff heart preparations to assess left ventricular function ($n = 8$ in each group) as previously described [20, 23]. End-diastolic pressures were obtained for the calculation of diastolic stiffness constant (κ , dimensionless). After performing Langendorff heart perfusion studies, the heart was separated into right ventricle and left ventricle (with septum) and weighed.

Vascular reactivity

Thoracic aortic rings (~4 mm in length) from rats ($n = 10$ from each group) were suspended in an organ bath filled with Tyrode physiological salt solution bubbled with 95% O_2 –5% CO_2 , maintained at $35\text{ }^{\circ}\text{C}$ and allowed to stabilise at a resting tension of approximately 10 mN. Cumulative concentration–response curves (contraction) were obtained for noradrenaline (Sigma-Aldrich Australia, Sydney, Australia) and cumulative concentration–response curves (relaxation) were obtained for acetylcholine (Sigma-Aldrich Australia, Sydney, Australia) and sodium nitroprusside (Sigma-Aldrich Australia, Sydney, Australia) following submaximal (70%) contraction to noradrenaline [20, 23].

Histology of the heart

Hearts were removed from the rats ($n = 2$ from each group) soon after death and were processed for histological assessments for inflammatory cells and collagen deposition [20].

Assessment of hepatic structure and function

Livers ($n = 8$ from each group) from C, CO, H and HO rats were isolated and weighed. Liver portions were isolated ($n = 2$ from each group) and fixed in 10% neutral buffered formalin for three days. These tissue samples were dehydrated and then embedded in paraffin wax. Thin sections (5 μm) of these tissues were cut and stained with haematoxylin and eosin for the determination of inflammatory cell infiltration (20 \times) and for determining the presence of fat vacuoles (40 \times) in the liver. Liver sections were also stained with Milligan's Trichrome stain to determine portal fibrosis (20 \times) [20, 23].

Plasma activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) and plasma concentrations of albumin, total bilirubin, urea and uric acid were

determined using kits and controls supplied by Olympus using an Olympus analyser (AU 400, Tokyo, Japan) in C, CO, H and HO rats [20, 23].

Oxidative stress and inflammatory markers

Plasma concentrations of malondialdehyde were determined by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) as previously described [24]. Plasma glutathione peroxidase activity was measured using an automated spectrophotometer (Cobas Mira) as previously described [25]. Plasma C-reactive protein concentrations were estimated using commercial kits (BD Bioscience, Franklin Lakes, NJ) according to manufacturer-provided standards and protocols. Heart and liver samples isolated from rats ($n = 3$ from each group) were stored at -80°C . These samples were thawed and sonicated after adding cell lysis buffer. These samples were then ultracentrifuged at $100,000\times g$ for 30 min at 4°C . Supernatants were used to measure the protein concentration in each sample by the bicinchoninic acid method (Thermo Scientific). The expression of Nrf2 and NF- κ B was studied in these supernatants at equal protein amounts (40 μg) by Western blot analysis.

Statistical analysis

All data are mean \pm SEM. Four groups of Wistar rats (C, CO, H and HO) were tested for homogenous variance using Bartlett's test, and variables that were not normally distributed were transformed (using log 10 function) prior to statistical analyses. These four groups were tested for effects of diet, treatment and their interactions by two-way ANOVA. When interaction and/or the main effects were significant, means were compared using Newman–Keuls multiple comparison post-test. Two SHR groups (S and SO) were compared using Student's t test. $P < 0.05$ was considered significant. All statistical analyses were performed using GraphPad Prism version 5.00 for Windows (San Diego, CA, USA).

Results

Chemical profile of OBE and daily intake of ellagitannins

OBE contained vescalagin (1.2 mg/mL), castalagin (0.8 mg/mL), roburin E (0.8 mg/mL) and grandinin (2 mg/mL) as major ellagitannins as well as ellagic acid (0.1 mg/mL). Daily intakes of individual ellagitannins have been calculated from these values for CO, HO and SO rats (Table 1).

Effects of OBE on H-induced metabolic syndrome

Physiological and metabolic parameters

Body weights of H rats were higher than those of C rats after 16 weeks and HO rats had lower body weight (Table 2). Water intake was lower in H rats than in C rats. HO rats had lower water intake when compared to H rats (Table 2). H rats consumed less food than C rats, although the energy intake was higher for H rats. OBE did not change food intake or energy intake in CO and HO rats (Table 2). H rats showed abdominal obesity measured as higher abdominal circumferences and increased abdominal fat pads than C rats. These signs of obesity were attenuated with OBE treatment in HO rats (Table 2). BMI and feed efficiency were higher in H rats than in C rats, and both parameters were lower in CO and HO rats when compared to C rats and H rats, respectively (Table 2). H rats showed higher basal blood glucose concentrations when compared to C rats after 16 weeks, whereas HO rats had lower basal blood glucose concentrations (Table 2). H feeding impaired oral glucose tolerance, while OBE treatment improved it (Fig. 1a). Plasma concentrations of triglycerides, total cholesterol and NEFA were higher in H rats than in C rats. HO rats had lower plasma concentrations of these lipid components (Table 2). H rats showed lower plasma urea concentrations and higher plasma uric acid concentrations than C rats. Plasma urea concentrations were higher and plasma uric

Table 1 Daily intake of ellagitannins in OBE-treated rats

Components from OBE	CO	HO	SO
OBE (μL)	16.41 \pm 0.37	10.66 \pm 0.48	15.21 \pm 0.23
Vescalagin (μg)	19.70 \pm 0.44	12.79 \pm 0.57	18.25 \pm 0.28
Castalagin (μg)	13.13 \pm 0.30	8.52 \pm 0.38	12.17 \pm 0.19
Grandinin (μg)	32.83 \pm 0.74	21.31 \pm 0.95	30.42 \pm 0.46
Roburin E (μg)	13.13 \pm 0.30	8.52 \pm 0.38	12.17 \pm 0.19
Total ellagitannin intake (μg)	78.79 \pm 1.77	51.15 \pm 2.29	73.00 \pm 1.11
Ellagic acid (μg)	1.64 \pm 0.04	1.07 \pm 0.05	1.52 \pm 0.02

Values are mean \pm SEM and $n = 10$ for each group. Daily intake of OBE and individual ellagitannins from extract was calculated based on the daily intake of food

CO cornstarch diet + OBE-fed rats, HO high-carbohydrate, high-fat diet + OBE-fed rats, SO SHR supplemented with OBE

Table 2 Effects of OBE on H-induced physiological, metabolic and oxidative stress variables in C, CO, H and HO rats

Variables	C	CO	H	HO	P value		
					Diet	OBE	Diet × OBE
Physiological variables							
Initial body weight (g)	334 ± 7	329 ± 8	332 ± 9	336 ± 6	>0.05	>0.05	>0.05
Final body weight (g)	424 ± 11 ^{bc}	401 ± 6 ^c	510 ± 10 ^a	440 ± 8 ^b	<0.001	<0.001	<0.05
Water intake (mL/day)	30.2 ± 0.6 ^a	31.5 ± 0.4 ^a	20.1 ± 0.4 ^b	18.6 ± 0.5 ^c	<0.001	>0.05	<0.01
Food intake (g/day)	30.7 ± 0.7 ^a	32.8 ± 0.8 ^a	22.5 ± 0.8 ^b	22.3 ± 1.0 ^b	<0.001	>0.05	>0.05
Energy intake (kJ/day)	345 ± 10 ^b	365 ± 11 ^b	479 ± 15 ^a	476 ± 16 ^a	<0.001	>0.05	>0.05
BMI (g/cm ²)	0.70 ± 0.01 ^b	0.65 ± 0.01 ^c	0.78 ± 0.01 ^a	0.69 ± 0.01 ^b	<0.001	<0.001	>0.05
Feed efficiency (g/kJ)	0.26 ± 0.02 ^b	0.20 ± 0.01 ^c	0.37 ± 0.02 ^a	0.22 ± 0.01 ^{bc}	<0.001	<0.001	<0.05
Abdominal circumference (cm)	21.4 ± 0.3 ^b	19.6 ± 0.2 ^c	23.8 ± 0.3 ^a	20.0 ± 0.2 ^c	<0.001	<0.001	<0.001
Abdominal fat pads (mg/mm tibial length)	407 ± 48 ^b	206 ± 31 ^c	805 ± 53 ^a	415 ± 47 ^b	<0.001	<0.001	<0.05
Metabolic variables							
Basal blood glucose (mmol/L)	4.2 ± 0.2 ^b	3.8 ± 0.2 ^{bc}	4.9 ± 0.2 ^a	3.3 ± 0.2 ^c	>0.05	<0.001	<0.01
AUC (mmol/L/min)	688 ± 13 ^b	626 ± 18 ^c	768 ± 12 ^a	664 ± 16 ^{bc}	<0.001	<0.001	>0.05
Plasma total cholesterol (mmol/L)	1.5 ± 0.1 ^b	1.3 ± 0.1 ^b	2.0 ± 0.1 ^a	1.4 ± 0.1 ^b	<0.01	<0.001	>0.05
Plasma triglycerides (mmol/L)	0.4 ± 0.1 ^b	0.4 ± 0.1 ^b	0.9 ± 0.1 ^a	0.5 ± 0.1 ^b	<0.01	>0.05	>0.05
Plasma non-esterified fatty acids (mmol/L)	1.3 ± 0.1 ^b	1.1 ± 0.1 ^b	2.6 ± 0.2 ^a	1.4 ± 0.1 ^b	<0.001	<0.001	<0.001
Plasma urea (mmol/L)	5.5 ± 0.3 ^{ab}	6.1 ± 0.3 ^a	3.4 ± 0.2 ^c	4.8 ± 0.3 ^b	<0.001	<0.01	>0.05
Plasma uric acid (μmol/L)	35.2 ± 1.8 ^b	37.2 ± 2.1 ^b	52.6 ± 2.6 ^a	33.1 ± 1.9 ^b	<0.01	<0.001	<0.001
Oxidative stress and inflammatory markers							
Plasma malondialdehyde (μmol/L)	27.1 ± 1.1 ^b	27.5 ± 1.0 ^b	31.5 ± 1.2 ^a	28.0 ± 1.2 ^b	<0.05	>0.05	>0.05
Plasma glutathione peroxidase activity (U/L)	1,165 ± 39 ^a	1,135 ± 26 ^a	880 ± 27 ^b	1,063 ± 27 ^a	<0.001	<0.05	<0.01
Plasma C-reactive protein (μmol/L)	2.60 ± 0.13 ^{bc}	3.27 ± 0.17 ^a	2.76 ± 0.08 ^b	2.36 ± 0.10 ^c	<0.01	>0.05	<0.001

Values are mean ± SEM and $n = 8-10$ for each group. Means without a common letter differ, $P < 0.05$

C cornstarch diet-fed rats, CO cornstarch diet + OBE-fed rats, H high-carbohydrate, high-fat diet-fed rats, HO high-carbohydrate, high-fat diet + OBE-fed rats

acid concentrations were lower in HO rats when compared to H rats (Table 2).

Cardiovascular structure and function

Systolic blood pressure was higher in H rats than in C rats (Fig. 1b). Left ventricular internal diameter during diastole (LVIDd), left ventricular wall thickness during diastole (LVPWd) and systolic volume were higher, whereas fractional shortening and ejection fraction were lower in H rats than in C rats after 16 weeks (Table 3). HO rats had lower systolic blood pressure, LVIDd and systolic volume without any change in LVPWd (Fig. 1b and Table 3). HO rats had higher fractional shortening and ejection fraction than H rats (Table 3). Left ventricular masses were higher in H rats than C rats, whereas HO rats showed lower left ventricular masses (Table 3). Infiltration of inflammatory cells was observed in the hearts of H rats after 16 weeks (Fig. 2c); this was not observed in HO rats (Fig. 2d).

Left ventricular diastolic stiffness (Table 3) and fibrosis (Fig. 2g) were higher in H rats than in C rats (Table 3 and Fig. 2e) after 16 weeks. HO rats had lower left ventricular diastolic stiffness (Table 3) and fibrosis (Fig. 2h). Impairment in vascular contraction with noradrenaline (Fig. 3a) and vascular relaxation with acetylcholine and sodium nitroprusside (Fig. 3b and c) induced by H feeding were attenuated in HO rats.

Hepatic structure and function

Livers from H rats were higher in wet weight than those from C rats after 16 weeks, whereas liver wet weights were lower in HO rats (Table 3). H feeding for 16 weeks caused hepatic steatosis (Fig. 4c) and fibrosis (Fig. 4k) with infiltration of inflammatory cells in liver (Fig. 4g). These changes in the liver were attenuated in HO rats (Fig. 4d, l, and h). H rats showed higher plasma activities of ALT, AST, ALP and LDH when compared to C rats. HO rats had

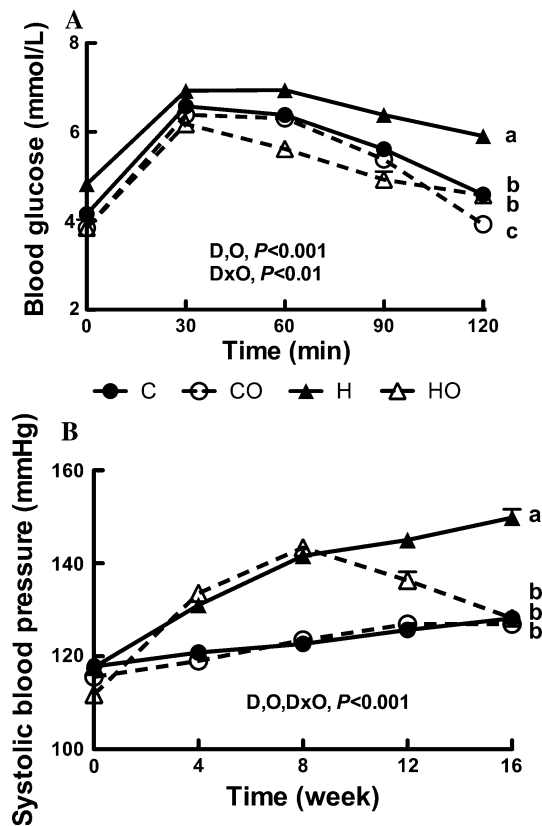


Fig. 1 Effects of OBE on oral glucose tolerance (a) and systolic blood pressure (b). Values are mean \pm SEM and $n = 10$ for each group. End-point means without a common letter differ, $P < 0.05$. *D*, *O* and *D* \times *O* represent effects of diet, OBE and interaction of diet and OBE. *C* cornstarch diet-fed rats, *CO* cornstarch diet + OBE-fed rats, *H* high-carbohydrate, high-fat diet-fed rats, *HO* high-carbohydrate, high-fat diet + OBE-fed rats

lower plasma activities of ALT, AST, ALP and LDH than H rats (Table 3). Plasma bilirubin concentrations were higher in H rats than in C rats, whereas HO rats had lower plasma bilirubin concentrations than H rats (Table 3). No changes were observed in plasma concentrations of albumin between the groups (Table 3).

Oxidative stress and inflammatory markers

H rats had higher plasma malondialdehyde concentrations and lower plasma glutathione peroxidase activities than C rats. HO rats showed lower plasma malondialdehyde concentrations and higher plasma glutathione peroxidase activities than H rats (Table 2). There was no difference between the plasma C-reactive concentrations of C and H rats. C-reactive protein concentrations in plasma were lower in HR and higher in CR rats when compared to H and C rats, respectively (Table 2). The heart and the liver from H rats showed up-regulation of NF- κ B expression and down-regulation of Nrf2 expression. These changes in the expression of NF- κ B and Nrf2 were normalised in HO rats (Fig. 5).

Effects of OBE in SHR

Physiological and metabolic parameters

OBE extract did not affect body weight, water, food and energy intake and plasma lipid components in SO rats (Online Resource 1). Basal blood glucose concentrations, abdominal obesity and plasma malondialdehyde concentrations were lower in SO rats when compared to S rats (Online Resource 1). Oral glucose tolerance was improved in SO rats (Online Resource 2).

Cardiovascular structure and function

SO rats had lower systolic blood pressure after 6 weeks of treatment, and systolic blood pressure was lower in SO rats than in S rats until 12 weeks of treatment (Fig. 6a). LVIDd and systolic volumes were lower in SO rats than in S rats without affecting LVPWd (Table 4). Fractional shortening and ejection fraction were higher, whereas left ventricular masses were lower in SO rats than in S rats (Table 4). Left ventricular diastolic stiffness was lower in SO rats when compared to S rats as shown by lower diastolic stiffness constant (Table 4). Vascular responses to noradrenaline (Fig. 6b), acetylcholine (Fig. 6c) and sodium nitroprusside (Fig. 6d) were improved in SO rats when compared with S rats. Infiltration of inflammatory cells was not observed in SO rats, and fibrosis was lower in SO rats than in S rats (Online Resource 3).

Oxidative stress and inflammatory markers

SO rats had lower plasma malondialdehyde concentrations and higher plasma glutathione peroxidase activities than S rats (Online Resource 1). Plasma C-reactive protein concentrations were lower in SO rats when compared to S rats (Online Resource 1).

Discussion

Red wine contains many polyphenols with potential biological activities [26], especially resveratrol and catechins [8, 26, 27], but few other compounds from red wine have been evaluated. This study has shown that an ellagitannin mixture extracted from European oak bark used in red wine maturation produces both cardiac and liver protection as well as improved metabolic profile in high-carbohydrate, high-fat diet-fed Wistar rats. In addition, OBE improved cardiovascular structure and function in the SHR.

Limited studies with related ellagitannins from other sources, such as punicalagin from pomegranates, have shown cardiovascular improvement in rat models of

Table 3 Effects of OBE on cardiovascular and hepatic variables in H-induced metabolic syndrome in C, CO, H and HO rats

Variables	C	CO	H	HO	P value		
					Diet	OBE	Diet × OBE
Cardiovascular variables							
LVIDd (mm)	6.46 ± 0.16 ^b	6.40 ± 0.15 ^b	7.21 ± 0.20 ^a	6.56 ± 0.18 ^b	<0.05	<0.05	>0.05
LVPWd (mm)	1.58 ± 0.03 ^b	1.60 ± 0.04 ^b	1.75 ± 0.05 ^a	1.66 ± 0.05 ^{ab}	<0.05	>0.05	>0.05
Systolic volume (μL)	54.6 ± 5.1 ^b	50.2 ± 4.0 ^b	115.1 ± 4.8 ^a	58.9 ± 4.1 ^b	<0.001	<0.001	<0.001
Relative wall thickness	0.49 ± 0.01	0.48 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	>0.05	>0.05	>0.05
Fractional shortening (%)	53.2 ± 1.3 ^a	52.2 ± 1.4 ^a	42.4 ± 1.0 ^b	51.0 ± 1.5 ^a	<0.001	<0.01	<0.001
Ejection fraction (%)	85.1 ± 1.2 ^a	82.6 ± 1.4 ^a	73.0 ± 1.1 ^b	82.1 ± 1.4 ^a	<0.001	<0.05	<0.001
Estimated left ventricular mass (g)	0.69 ± 0.02 ^b	0.68 ± 0.02 ^b	0.82 ± 0.03 ^a	0.71 ± 0.02 ^b	<0.01	<0.05	<0.05
Left ventricular + septum wet weight (mg/mm tibial length)	20.1 ± 0.8 ^b	19.4 ± 0.7 ^b	22.5 ± 0.8 ^a	19.9 ± 0.6 ^b	>0.05	<0.05	>0.05
Right ventricular weight (mg/mm tibial length)	4.1 ± 0.3	4.3 ± 0.3	4.5 ± 0.4	4.2 ± 0.3	>0.05	>0.05	>0.05
Left ventricular diastolic stiffness constant, κ	19.1 ± 0.8 ^c	18.4 ± 0.9 ^c	28.2 ± 1.1 ^a	22.8 ± 0.8 ^b	<0.001	<0.01	<0.05
Hepatic variables							
Liver wet weight (mg/mm tibial length)	265 ± 10 ^b	232 ± 11 ^b	297 ± 9 ^a	256 ± 10 ^b	<0.01	<0.001	>0.05
Plasma ALT (U/L)	35.6 ± 0.7 ^b	32.5 ± 0.6 ^c	48.2 ± 0.6 ^a	36.9 ± 0.9 ^b	<0.001	<0.001	<0.001
Plasma AST (U/L)	75.1 ± 4.8 ^b	77.8 ± 5.3 ^b	101.2 ± 6.6 ^a	82.3 ± 5.9 ^b	<0.05	>0.05	>0.05
Plasma ALP (U/L)	165 ± 15 ^b	172 ± 15 ^b	257 ± 20 ^a	192 ± 18 ^b	<0.01	>0.05	<0.05
Plasma LDH (U/L)	220 ± 20 ^b	206 ± 22 ^b	451 ± 28 ^a	262 ± 25 ^b	<0.001	<0.001	<0.001
Plasma albumin (g/L)	27.9 ± 0.3	28.1 ± 0.3	28.5 ± 0.3	28.0 ± 0.3	>0.05	>0.05	>0.05
Plasma total bilirubin (μmol/L)	2.0 ± 0.1 ^b	1.9 ± 0.1 ^b	2.5 ± 0.1 ^a	2.0 ± 0.1 ^b	<0.01	<0.01	>0.05

Values are mean ± SEM and *n* = 8–10 for each group. Means without a common letter differ, *P* < 0.05

C cornstarch diet-fed rats, CO cornstarch diet + OBE-fed rats, H high-carbohydrate, high-fat diet-fed rats, HO high-carbohydrate, high-fat diet + OBE-fed rats

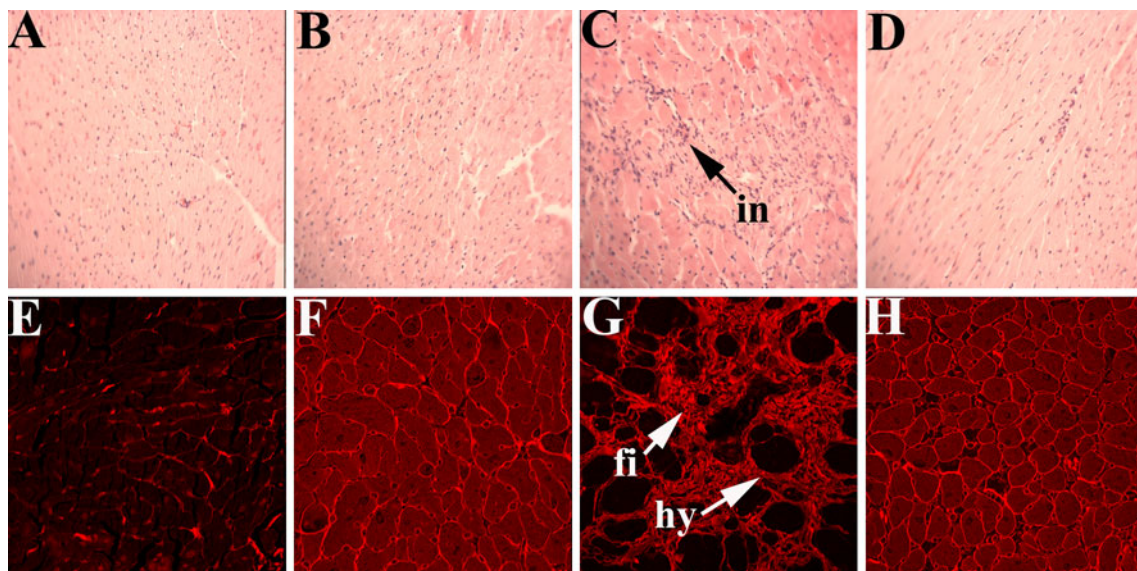


Fig. 2 Effects of OBE on inflammation and fibrosis in the heart induced by H feeding. Haematoxylin and eosin staining of left ventricle showing infiltration of inflammatory cells (a–d, inflammatory cells as dark spots surrounding the myocytes marked as *in*) (×40) from cornstarch diet-fed rats (a), cornstarch diet + OBE-fed rats (b), high-carbohydrate, high-fat diet-fed rats (c) and high-carbohydrate,

high-fat diet + OBE-fed rats (d). Picosirius red staining of left ventricle showing collagen deposition (e–h, fibrosis marked as *fi* and hypertrophied cardiomyocytes as *hy*) (×40) from cornstarch diet-fed rats (e), cornstarch diet + OBE-fed rats (f), high-carbohydrate, high-fat diet-fed rats (g) and high-carbohydrate, high-fat diet + OBE-fed rats (h)

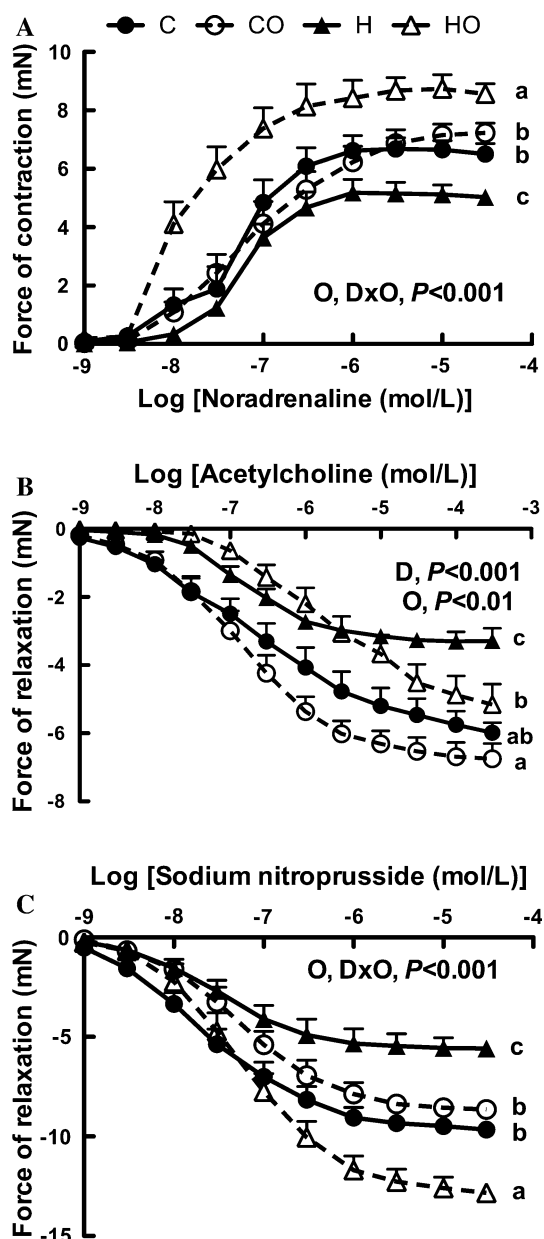


Fig. 3 Effects of OBE on vascular responses in rats. Noradrenaline-induced contraction (a), acetylcholine-induced relaxation (b) and sodium nitroprusside-induced relaxation (c) in thoracic aortic rings from C, CO, H and HO rats. Values are mean \pm SEM and $n = 10$ for each group. End-point means without a common letter differ, $P < 0.05$. D, O and DxO represent effects of diet, OBE and interaction of diet and OBE. C cornstarch diet-fed rats, CO cornstarch diet + OBE-fed rats, H high-carbohydrate, high-fat diet-fed rats, HO high-carbohydrate, high-fat diet + OBE-fed rats

isoproterenol-induced cardiac necrosis and angiotensin-induced hypertension [12, 13] with no adverse effects even at high doses [28]. The pharmacokinetics of ellagitannins from pomegranate have been studied in humans as well as in rats [29, 30]. Ellagitannins are not absorbed directly due to their large molecular size [29, 30]. Earlier studies

showed that urolithins, as metabolites of ellagitannins, were found in the urine of human subjects even 48 h after pomegranate juice consumption [9]. Ellagic acid, the hydrolysis product of ellagitannins, was not found in the blood within a few hours after pomegranate juice consumption [29]. Thus, the bioactive metabolites from ellagitannins are likely to be urolithins rather than ellagic acid.

H feeding in rats leads to the development of signs of metabolic syndrome and associated end-organ damage [20]. H rats developed abdominal obesity, hypertension, dyslipidaemia and impaired glucose tolerance. These changes were accompanied by cardiovascular remodelling and non-alcoholic steatohepatitis [20]. Thus, rats fed with H diet are suitable as a model to demonstrate the major changes found in human metabolic syndrome [20, 21]. We have previously shown that these diet-induced signs of the metabolic syndrome can be reversed by natural products with antioxidant and anti-inflammatory properties including rutin, olive leaf, purple carrots and chia seeds [23, 31–33]. Ellagitannins from oak bark showed similar biological activity to these other natural products.

The protective effects mediated by the oak-derived ellagitannins could relate to free radical scavenging and anti-inflammatory properties as with pomegranate-derived ellagitannins [34, 35]. The reduced plasma malondialdehyde concentrations and increased plasma glutathione peroxidase activity along with increased expression of Nrf2 in both the heart and the liver strongly support an antioxidant mechanism. The antioxidant activity of ellagitannins may lead to higher NO bioavailability by removal of superoxide, leading to reduction in blood pressure. Increased expression of eNOS, as shown with punicalagin in hypercholesterolaemic mice [36], would also increase NO bioavailability. Further, the improvement in vascular relaxation responses of OBE-treated rats is supportive of a reduction in blood pressure, especially the improvement in acetylcholine-induced relaxation, by a response dependent on endothelium-derived NO. In our study, the anti-inflammatory mechanism is supported by the lower expression of NF- κ B, lower infiltration of inflammatory cells in the heart and lower collagen deposition in OBE-treated rats. These outcomes are associated with lower ventricular stiffness, possibly improving ventricular function. Similar anti-inflammatory activities of ellagitannins from pomegranate have been reported in a model of rheumatoid arthritis and in a colon inflammation model [35, 37]. The hepatoprotective responses with oak-derived ellagitannins are shown by reduced plasma activities of transaminases, attenuation of fat deposition and fibrosis, and inhibition of infiltration of inflammatory cells in the liver. The altered expression of NF- κ B and Nrf2 in the liver confirms the antioxidative and anti-inflammatory effects of

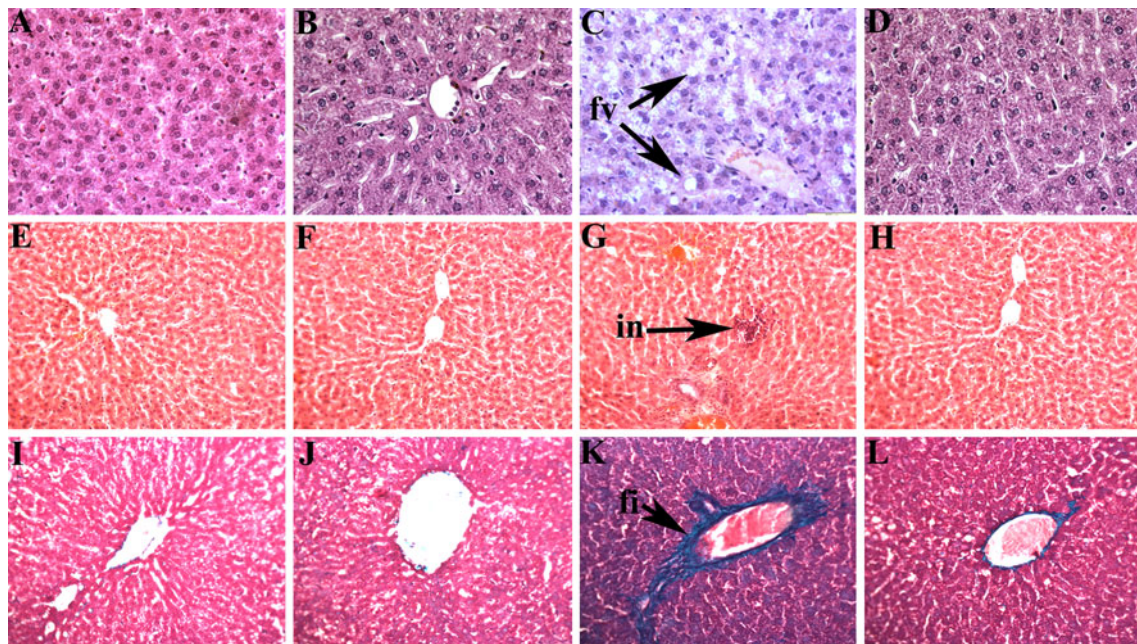
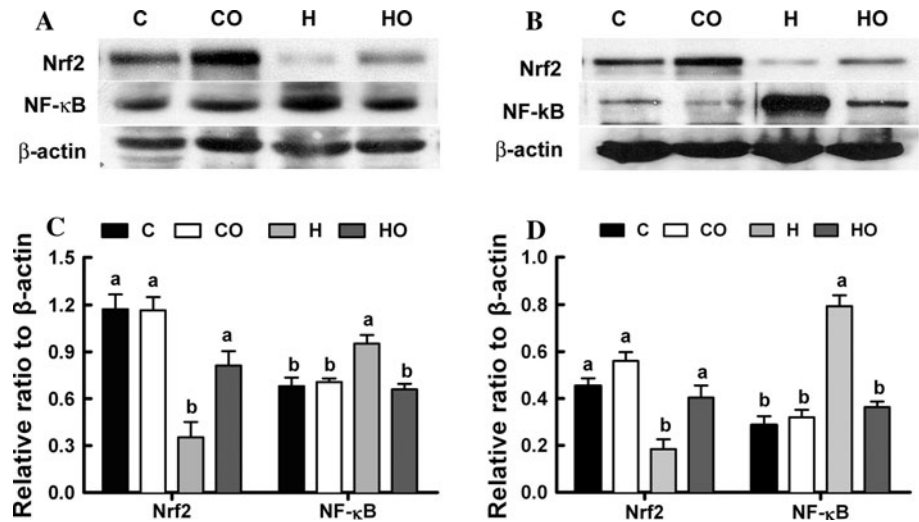


Fig. 4 Effects of OBE on fat deposition, inflammation and fibrosis in rat livers. Haematoxylin and eosin staining of liver showing enlarged fat vacuoles (a–d, marked as *fv*) ($\times 40$) and inflammatory cells (e–h, marked as *in*) ($\times 20$) from cornstarch diet–fed rats (a, e), cornstarch diet + OBE–fed rats (b, f), high-carbohydrate, high-fat diet–fed rats

(c, g) and high-carbohydrate, high-fat diet + OBE–fed rats (d, h) rats. Milligan’s Trichrome staining of hepatic portal regions showing fibrosis (i–l, marked as *fi*) ($\times 20$) from cornstarch diet–fed rats (i), cornstarch diet + OBE–fed rats (j), high-carbohydrate, high-fat diet–fed rats (k) and high-carbohydrate, high-fat diet + OBE–fed rats (l)

Fig. 5 Effects of OBE on expression of Nrf2 and NF- κ B in the heart (a) and the liver (b). For quantitative analysis, the expression of these proteins was normalised against the expression of β -actin in the heart (c) and the liver (d). Values are mean \pm SEM, $n = 3$. Means without a common letter differ, $P < 0.05$. C cornstarch diet–fed rats, CO cornstarch diet + OBE–fed rats, H high-carbohydrate, high-fat diet–fed rats, HO high-carbohydrate, high-fat diet + OBE–fed rats



ellagitannins from OBE. Thus, ellagitannins derived from oak bark ameliorated the changes associated with diet-induced cardiovascular remodelling and non-alcoholic fatty liver disease probably by both antioxidant and anti-inflammatory mechanisms.

OBE-treated rats presented improved metabolic parameters including lower abdominal fat deposition and improved glucose tolerance, as well as protection of the heart and the liver. Tannins from pomegranate leaf produced antiobesity effects by inhibiting energy intake in high-fat diet–fed mice [38], unlike the unchanged energy

intake in our study. Decreased oxidative stress and inflammation may also be the mechanism responsible for improving metabolic parameters [39].

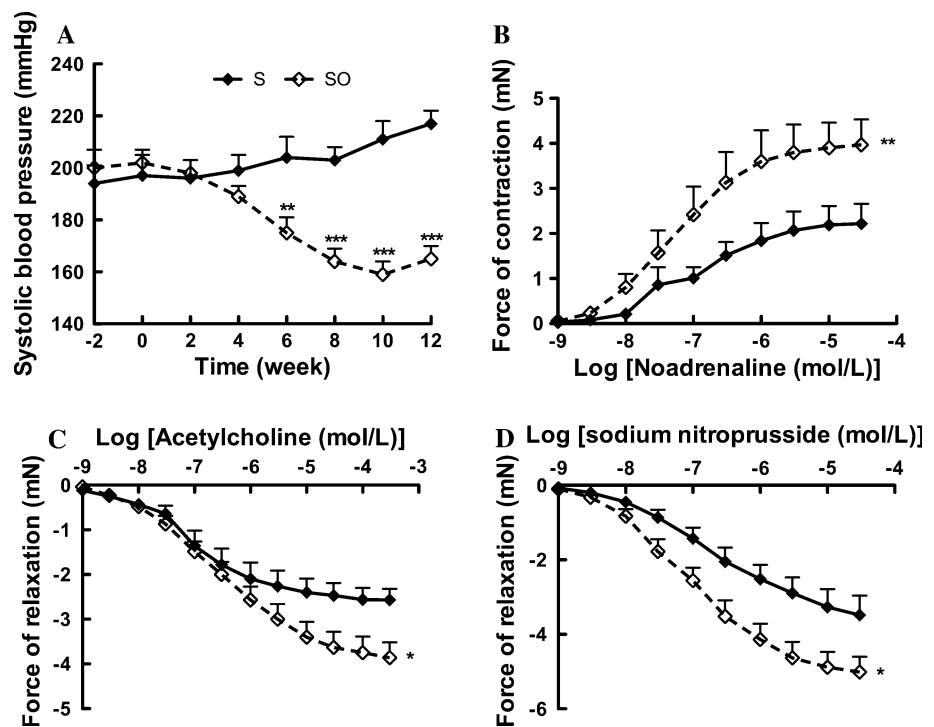
The improved cardiovascular parameters were also measured in adult SHR, the genetic model of choice to mimic human essential hypertension with extensive cardiovascular remodelling [19, 22]. OBE-treated rats had lower systolic blood pressure, ventricular collagen deposition and diastolic cardiac stiffness. Similar effects were previously reported with the ellagitannin-rich pomegranate juice in isoproterenol-induced cardiac necrosis model and

Table 4 Effects of OBE on cardiovascular structure and function in SHR

Variables	S	SO
LVIDd (mm)	8.01 ± 0.28	7.05 ± 0.22*
LVPWd (mm)	1.86 ± 0.08	1.72 ± 0.06
Systolic volume (μL)	138 ± 4	112 ± 4***
Relative wall thickness	0.54 ± 0.01	0.53 ± 0.01
Fractional shortening (%)	32.2 ± 1.2	38.5 ± 1.4**
Ejection fraction (%)	68.1 ± 1.0	74.6 ± 1.2***
Estimated left ventricular mass (g)	0.96 ± 0.04	0.82 ± 0.04*
Left ventricular + septum wet weight (mg/mm tibial length)	28.2 ± 0.8	25.3 ± 0.6**
Right ventricular weight (mg/mm tibial length)	4.5 ± 0.3	4.5 ± 0.2
Left ventricular diastolic stiffness constant, κ	32.4 ± 1.3	28.6 ± 1.1*

Values are mean ± SEM and $n = 8$ – 10 for each group. * versus S and $P < 0.05$, ** versus S and $P < 0.01$, *** versus S and $P < 0.001$. S SHR, SO SHR supplemented with OBE

Fig. 6 Effects of OBE on systolic blood pressure (a), vascular contraction by noradrenaline (b) and vascular relaxation by acetylcholine (c) and sodium nitroprusside (d) in SHR (S) and SHR supplemented with OBE (SO). Values are mean ± SEM and $n = 10$ for each group. * versus S ($P < 0.05$), ** versus S ($P < 0.01$) and *** versus S ($P < 0.001$)



in angiotensin II-induced hypertension in streptozotocin-induced diabetic rats [12, 13]. Improvement in vascular responses in OBE-treated SHR also suggests higher bioavailability of NO [36]. Hence, these results with SHR support the cardioprotective roles of ellagitannins derived from oak bark.

In conclusion, the health effects of red wine have been attributed to polyphenols such as resveratrol, rather than to oak-derived ellagitannins. This study has defined the protective effects of an oak bark extract containing vescalagin, castalagin, grandinin and roburin E on the heart and the liver together with an improved metabolic profile in two rat models of human metabolic syndrome and human hypertension. These results imply that the ellagitannins from oak may be important mediators of the benefits of red wine in

humans due to their antioxidant and anti-inflammatory responses. Clinical trials should be considered with these ellagitannin extracts.

Acknowledgments This study was supported by The Prince Charles Hospital Foundation, Brisbane, Australia. We thank Mr. Paul Addison, School of Biomedical Sciences, and Mr. Gary Wilson, School of Human Movement Studies, both at The University of Queensland, Australia, for their help with histopathological studies and plasma oxidant analyses, respectively. We also thank Dr. Fiona Campbell and Mr. Brian Bynon both at School of Veterinary Science, The University of Queensland, Australia, for their assistance with echocardiography and plasma analyses, respectively; Dr Thiruma Arumugam (The University of Queensland) for assistance with expression studies and Dr Kate Kauter (University of Southern Queensland) for assistance with C-reactive protein measurements. We also thank Prof. Stéphane Quideau, European Institute of Chemistry and Biology, University of Bordeaux I, France, and Dr. Daniel Jardine, Flinders

University, Australia, for providing ellagitannin standards and ellagitannin analyses in the extract, respectively.

Conflict of interest No conflicts of interest.

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