SESAME OIL THERAPEUTICALLY MITIGATES CARDIAC HYPERTROPHY IN CHRONIC KIDNEY DISEASE BY ATTENUATING OXIDATIVE STRESS AND HYPERTENSION

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Left ventricular hypertrophy (LVH), highly prevalent in patients with chronic kidney disease (CKD), represents one of the major hazards for mortality. Oxidative stress is involved in the progression of CKD, and triggers the pathogenesis of hypertension in kidney and arterial tissues. Sustained hypertension in CKD leads to the development of LVH. Sesame oil is a potent antioxidative agent, and protects multiple organ injury by attenuating oxidative stress in various animal models. We investigated the therapeutic effects of sesame oil on LVH in CKD rats. CKD rat model is established by subcutaneously injecting deoxycorticosterone acetate (DOCA, 15 mg/ml/kg; twice weekly for 5 weeks) and supplementing with 1% sodium chloride drinking water (DOCA/salt) to uninephrectomized rats. Sesame oil (0.5, 1 ml/kg; p.o.,) is administered for 7 days after 4 weeks of DOCA/salt treatment. Sesame oil decreased renal hydroxyl radical, peroxynitrite, lipid peroxidation, blood urea nitrogen, creatinine, urine volume; increased CCR level and renal nuclear Nrf2 levels in CKD rats. Furthermore, sesame oil ameliorated systolic/diastolic blood pressure, heart weight, and the size of cardiomyocyte. In conclusion, sesame oil therapeutically mitigates LVH by inhibiting renal oxidative stress, modulating Nrf2 expression and maintaining blood pressure. Therefore attenuating hypertension mitigates LVH in CKD rats. The finding of this study may be beneficial for treatment of LVH in patients with CKD.

Key words: left ventricular hypertrophy, chronic kidney disease, hypertension, oxidative stress, sesame oil
Background

Chronic kidney disease (CKD), a progressive loss of renal function, often leads to end-stage renal failure (ESRF) or death because it is frequently asymptomatic (Hsu, 2007). The prevalence of CKD in the United States rose from 10% in 1988-1994 to 13.1% in 1999-2004 (Coresh et al., 2007). The prevalence and mortality rates of CKD increased in Taiwan by about 500% between 1996 and 2003 (Kuo et al., 2007). More than 1 million people worldwide die of untreated kidney failure each year because kidney replacement therapy (dialysis or kidney transplantation) is unaffordable in most countries. This represents a huge economic burden, with global costs from 2000-2010 surpassing $1 trillion (Couser et al., 2011). Worsening kidney function is associated with a marked increased prevalence of cardiovascular disease (CVD), which is the leading cause of mortality in industrialized countries (Go et al., 2004). Careful attention to reducing traditional CVD risk factors in CKD is of great importance. Nevertheless, delay of ESRD remains a primary goal of CKD therapy simply because specific treatments to avoid CVD in this population do not currently exist.

Elevated oxidative stress and fibrosis in kidney and arterial tissues plays a central role in the pathogenesis of CKD. Oxidative stress is a condition in which the amount of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, nitric oxide, hydroxyl radical, and peroxynitrite exceed the capacity of the antioxidant defense system (ADS) (Rosenthal and Nocera, 2007; Bartz RR and Piantadosi, 2010). Accumulated ROS cause glomerular or tubulointerstitial cellular injury and necrosis via several mechanisms: peroxidation of membrane lipids, protein denaturation, and DNA damage (Dincer et al., 2008). One of potent ROS scavenger involved in ADS is mediated by activation of the nuclear factor-erythroid-2-related factor 2 (Nrf2) which regulates the coordinated induction of numerous genes that encode various antioxidants (Li et al., 2008; Wakabayashi et al., 2010). Nrf2 is held as an inactive complex bound to a repressor molecule known as Kelch-like ECH-associated protein1 (Keap1) in the cytoplasm. Under oxidative stress, Nrf2-Keap1-mediated repression unbounds Nrf2 and Keap1 in cytoplasm. Nrf2 translocates into nucleus and binds to antioxidant response element site. Therefore, activating Nrf2 has been recognized as one of the most important and promising molecular targets for protecting cells from oxidative stress and inflammatory insult (Rahman et al., 2006; Kim et al., 2010). It has been demonstrated that activation of Nrf2 decreased lipid peroxidation (LPO), down-regulated NAD(P)H oxidase, and 12-lipoxygenase in rats with CKD (Kim and Vaziri, 2010). Osteopontin (OPN) is an
extracellular phosphoprotein involved in cell adhesion and wound healing (Wang and Denhardt, 2008) of which related to aldosterone-induced oxidative stress, and interstitial fibrosis in the kidney (Irita et al., 2011). OPN expression leads to an accumulation of collagen associated with the progression of fibrosis in various organs, including the heart, liver, and kidney (Klingel et al., 2010; Syn et al., 2012). Serum OPN levels were found to be higher in patients with glomerular proteinuria condition (Wasilewska et al., 2011) and end-stage renal disease (Nakamura et al., 2006).

Sustained hypertension in CKD contribute to the development of LVH, which characterized by structural changes in the myocardium, such as collagen accumulation, increased ventricular wall thickness and enlarged cardiomyocytes (Mensah et al., 1993, Barrick et al., 2007). The incidence of hypertension in ESRF is in average about 20 times higher compared to normal population (Tesa, 2006).

Left ventricular hypertrophy (LVH), a strong predictor of cardiovascular morbidity and mortality, is highly prevalent in patients with CKD and ESRF (Nardi et al., 2007; Nardi et al., 2009). Complications of LVH include atria fibrillation, heart failure, and sudden death, of which is one of the major causes of death in the United States and around the world (Go et al., 2013). In the general population, sudden cardiac death accounts for one death per 1000 person-years and for 6–13% of all deaths, whereas among individuals with kidney failure, the rates are 59 deaths per 1000 person-years and 26% of total mortality. Among patients with ESRF, nearly 15% have systolic dysfunction, nearly 40% have heart failure and more than 70% have LVH (Foley et al., 1995; Levin et al., 1999). Hence, reducing LVH in CKD is of great importance.

Sesame oil, a potent natural antioxidant (Chandrasekaran et al., 2010; Periasamy et al., 2011; Periasamy et al., 2012), is a source of polyunsaturated fatty acids, potassium (400 mg/100 g sesame seed), magnesium (370 mg/100 g sesame seed), calcium (1200 mg/100 g sesame seed), phosphorus (540 mg/100 g sesame seed), vitamin B (1.2 mg/100 g sesame seed), and vitamin E (40 mg/100 g oil) (Namiki, 2007). Polyunsaturated fatty acids and vitamin E have been reported to lower blood pressure and prevent the development of hypertension in patients (Boshtam et al., 2002; Mori et al., 1999; Prisco et al., 1998). It has been suggested that sesame oil protects kidney against ROS damage by producing antioxidants to reduce excess free radicals (Abdou et al., 2012; Hsu et al., 2011). Sesame oil accelerates kidney recovery by decreasing OPN production and collagen
deposition in gentamicin-induced acute renal injury (Periasamy et al., 2010). The phenolic lignans from sesame oil, such as sesamin, sesamol, and sesamolin, contribute to its antioxidative activities (Kang et al., 1998; Nakano et al., 2002; Sharma et al., 2012). Sesamol (3,4-methylenedioxyphenol), one of the most active compound of sesame oil, attenuates oxidative stress and protects against liver and kidney injury (Hsu et al., 2007; Hsu et al., 2008; Chandrasekaran et al., 2009). Moreover, it has been demonstrated that sesamol attenuated myocardial infarction (Periasamy et al., 2011) and hypertension (Sharma et al., 2012) in animal models.

**Materials and Methods**

*Chemicals*

Deoxycorticosterone acetate (DOCA), sesame oil, and sesamol were purchased from Sigma-Aldrich (St. Louis, Mo., USA).

*Animals*

Forty-nine male specific-pathogen-free Sprague-Dawley rats weighing 250-300 g were purchased from our institution’s laboratory animal center and housed individually in a room with a 12-h light/dark cycle and central air conditioning (25°C, 70% humidity). They were allowed free access to tap water and pelleted rodent food (Richmond Standard; PMI Feeds, St. Louis, Mo., USA). The animal care and experimental protocols were in accord with nationally approved guidelines.

*Uni-nephrectomy*

All the rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). After a left lateral abdominal incision is made, the left renal vessels and ureter were ligated and then the left kidney was removed.

*Inducing CKD*

DOCA, a synthetic mineralocorticoid derivative, is used to establish an animal model of CKD. CKD was induced by injecting DOCA in mineral oil (15 mg/ml/kg, s.c., twice weekly) and supplemented with 1% NaCl for 5 weeks in uninephrectomized rats (Nakano et al., 2002).

*Experimental Design*

*Experiment 1*
After the uni-nephrectomy, 14 rats were divided into 2 groups of 7. Sham group rats were treated with vehicle (twice a week) without DOCA and normal drinking water. DOCA/salt group rats were treated with DOCA (15 mg/kg, s.c. twice a week) in vehicle with 1% salt in drinking water to induce LVH. Serum samples of blood urea nitrogen (BUN) and creatinine (CRE), blood systolic pressure (SP) and diastolic pressure (DP) were analyzed once a week.

**Experiment 2**

Thirty-five uninephrectomized rats were divided into 5 groups (n = 7 each). Group I rats receive mineral oil (s.c.) without injecting DOCA and unsalted drinking water. Group II rats were treated twice every 7 days with DOCA in mineral oil (15 mg/ml/kg, s.c.) and with salted drinking water to induce LVH. On day 29, the rats were orally gavaged with saline solution (1 ml/kg/day) for 7 days. Group III rats were treated to induce CKD. On day 29, the rats were orally gavaged with sesame oil (0.5 ml/kg/day) for 7 days. Group IV rats were treated to induce CKD. On day 29, the rats were orally gavaged with sesame oil (1 ml/kg/day) for 7 days. Group V rats were given mineral oil (s.c.) without DOCA and unsalted drinking water. On day 29, the rats were orally gavaged with sesame oil (1 ml/kg/day) for 7 days. On day 36, kidney hydroxyl radical, peroxynitrite, MDA, nuclear Nrf2, and OPN were estimated 24 h after the last dose of saline solution or sesame oil. Serum BUN, CRE, creatinine clearance rate (CCR), urine volume, serum and urinary, and blood pressure were measured. Heart and kidney tissues were collected for histological analysis.

**Measuring Hydroxyl Radical and Peroxynitrite in Renal Tissue**

The ongoing production of hydroxyl radical and peroxynitrite in kidney tissue from DOCA/salt treated rats were measured using a high-performance chemiluminescence (CL) analyzer (model CLA-2100; Tohoku Electronic Industrial Co. Ltd., Rifu, Japan). Briefly, 400 µL of a tissue homogenate was mixed with 200 µL of phosphate-buffered saline in a stainless dish, and then the background CL count was read for 60 s. One hundred microliters of indoxyl [beta]-d-glucuronide (17 mM dissolved in phosphate-buffered saline for determining hydroxyl radical counts) or luminol (17 mM dissolved in phosphate-buffered saline for determining peroxynitrite counts) was injected into the machine, and CL was counted for another 1,200 s at 10-s intervals. The data were analyzed using chemiluminescence analyzer data acquisition software (Tohoku Electronic Industrial Co., Sendai, Japan) (Hsu et al., 2006).
**Measuring Renal Lipid Peroxidation (LPO)**

Kidney tissue was homogenized in Tris-HCl (20 mM, pH 7.4). Tissue homogenate (500 µL) was centrifuged at 2,500 × g for 10 min at 4°C. Supernatant (200 µL) was taken for LPO measurement using a kit (lipid peroxidase assay kit; Calbiochem-Novabiochem, Darmstadt, Germany), and the results were read spectrophotometrically (DU 640B, Beckman, Fullerton, Calif., USA) at 586 nm (Hsu et al., 2005). Data are presented as nanomoles MDA per milligram of protein.

**Western Blotting: Quantifying Nrf2 Expression**

Cytoplasmic or nuclear proteins from kidney were prepared using NE-PER nuclear and cytoplasmic extraction reagents based on the manufacturer’s instruction (Pierce Biotechnology, Rockford, IL, USA). I loaded 60 µg of protein on 10% sodium dodecyl sulfate-polyacrylamide gel for electrophoresis and then transferred it to nitrocellulose sheets (NEN Life Science Products, Inc., Boston, MA, USA) in a transfer apparatus (Bio-Rad) run at 1.2 A for 3 h. After the blots had been blocked in 5% nonfat skim milk in Tris-buffered saline Tween-20, I incubated the blots with primary Nrf2 antibody (dilution, 1 : 500; Santa Cruz Biotechnology, Palo Alto, Calif., USA) against the target protein in 5% nonfat skim milk and then with anti-rabbit immunoglobulin G conjugated with alkaline phosphatase (dilution, 1 : 1,500; Jackson ImmunoResearch Laboratories, Inc., Philadelphia, PA, USA). Immunoblots were developed using alkaline phosphatase substrate solution (5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium; Kirkegaard and Perry Laboratories, Inc., Baltimore, MD, USA).

**Determining OPN Expression**

Renal tissue was homogenized in 0.1 M sodium bicarbonate (pH 9.6) and centrifuged at 2500 g for 10 min at 4°C. OPN expression levels in tissue supernatant were measured using antihuman OPN antibody (rabbit; 1 : 20,000) (Rockland Immunochemicals, Gilbertsville, Penn., USA) and secondary antibody; developing reagents were bought from R&D Systems (Minneapolis, Minn., USA). The OPN content was assessed by measuring absorbance at 450 nm. Protein concentrations in renal tissue were determined using a protein assay (Bio-Rad).

**Evaluating Renal Fibrosis**
Renal tissue samples (4 µm thick) were stained using Sirius red (Direct Red 80; Sigma-Aldrich). The stained slides were scored using photomicrographs taken randomly at 10 different locations under a light microscope.

Assessing Renal Function

Renal injury was assessed by measuring rises in BUN and CRE levels in serum using a blood biochemical analyzer (Dri-Chem 3500s; Fujifilm, Kanagawa, Japan). Urinary albumin concentration was measured using an ELISA kit (E90-134; Bethyl Laboratories, Montgomery, Tex., USA). For CCR assessment, the rats were placed in metabolic cages after treatment. Their serum and urine were collected separately 24 h post-treatment. CCR was calculated using the following formula:

\[
\text{CCR (ml/min)} = \frac{\text{Urine CRE (mg/dl)} \times \text{Urine amount (ml/24 h)}}{\text{Serum CRE (mg/dl)} \times 1440 \text{ min/24 h}}
\]

Histological Evaluation of Renal Injury

Histopathological examination was also used to assess renal injury. Briefly, organ tissue was fixed in 4% formaldehyde buffered with a phosphate solution (0.1 M [pH 7.4]) at room temperature. Renal tissue samples were washed in phosphate buffer, dehydrated in graded concentrations of ethanol, and then embedded in paraffin. From each tissue sample, 4-µm-thin sections were obtained and stained with hematoxylin and eosin to evaluate renal morphology, and then mounted using Depex Polystyrene dissolved in xylene mountant. The permanently mounted sections of kidney tissue were examined under a microscope (Eclipse E600; Nikon Instech, Kawasaki, Japan) (100 magnification) to assess renal injury and scored. The histopathological score was evaluated using the following criteria: loss of brush border, hyaline deposits, cellular infiltration, swelling, and congestion (Periasamy et al., 2010).

Hemodynamic and functional measurements

Measurement of Blood Pressure

Rats are fixed in thermostatic chamber of SINGA Xction View II with its tail pass clipped in pressurized-sensor. The rats are acclimatized for 10 min, the systolic and diastolic pressures of each rat are measured once per min for 10 min and the numbers were averaged. All data are analyzed using Xction View 2.0 software.

Estimation of Heart Weight/100 g Body Weight Ratio
Hearts are collected, and their wet weights are measured. The ratio of heart weight (g) to body weight (g) is assessed and used as a measure of cardiac hypertrophy.

Histological Evaluation of Cardiac Hypertrophy

A histological examination is also used to assess cardiac hypertrophy. Briefly, heart tissue is fixed in buffered 4% formaldehyde and then embedded in paraffin. From each tissue sample, 4-µm-thin sections are stained with hematoxylin and eosin (H&E) using standard procedure (Parlakpinar et al., 2005) and are examined under a microscope ((Eclipse E600; Nikon Instech, Kawasaki, Japan) 400× magnification).

For consistent determination of the cell size, the cardiomyocytes positioned perpendicularly to the plane of the section with a visible nucleus and cell membrane clearly outlined and unbroken are selected for the cross-sectional area measurements. For the determination of cell density, cardiomyocytes in 6-8 high power fields (HPF) of LV tissue sections from each group are counted.

Protein Assay

Protein concentration in kidney tissue is determined using protein assay dye (Bio-Rad Laboratories, Hercules, Calif), and bovine serum albumin is used as a standard.

Statistical Analysis

Data are means ± standard deviation (SD). Significant differences between measurements traits are analyzed using one-way ANOVA and then Student’s t-test. Significance was set at $p < 0.05$. 
Results

Experiment 1: The time course of renal dysfunction and hypertension in DOCA/salt-treated rats

To evaluate the optimal time for sesame oil administration on CKD rats, serum CRE, BUN, systemic DP and SP were measured weekly. Serum CRE, BUN, DP and SP became significantly higher in the DOCA/salt group than in the Sham group after 4 weeks of treatment (Fig. 1). Based on the finding here, I chose day 29 to initiate therapeutic sesame oil dosing.

Experiment 2: Therapeutic effect of sesame oil on hydroxyl radical, peroxynitrite, and MDA in CKD rats.

To examine the effect of sesame oil on oxidative stress in CKD rats, hydroxyl radical, peroxynitrite, and MDA were measured. Hydroxyl radical, peroxynitrite, and MDA significantly increased in Group II compared to Group I or Group V. Therapeutic sesame oil significantly reduced hydroxyl radical (Fig. 2A), peroxynitrite (Fig. 2B), and MDA (Fig. 2C) in Group IV compared to Group II.

Therapeutic effect of sesame oil on renal nuclear and cytoplasmic Nrf2 expression in CKD rats.

To investigate the effects of sesame oil on the nuclear translocation of Nrf2, the nuclear and cytoplasmic fractions were analyzed, and the protein levels of Nrf2 were measured by western blot analysis. Group II (CKD rats) showed significantly decreased nuclear Nrf2 level and increased cytoplasmic Nrf2 level compared to Group I or Group V. Therapeutic sesame oil significantly increased nuclear Nrf2 level (Fig. 3A) and decreased cytoplasmic Nrf2 level (Fig. 3B) in Group IV compared to Group II.

Therapeutic effect of sesame oil on OPN expression in CKD rats.

To evaluate the therapeutic effect of sesame oil on healing and interstitial fibrosis of kidney, I quantified OPN. CKD rats (Group II) showed significantly increased renal OPN compared to control rats (Group I) or sesame oil alone treated rats (Group V). Therapeutic sesame oil (Group IV) significantly decreased renal OPN compared to Group II (Fig. 4).

Therapeutic effect of sesame oil on renal fibrosis in CKD rats.
Sirius red staining was used to assess the therapeutic effect of sesame oil on renal fibrosis in CKD rats. Group II rats showed significantly increased collagen deposition in renal tubules than Group I or V (Fig. 5A). However, Group III and IV had significantly lower collagen deposition than Group II (Fig. 5B).

**Therapeutic effect of sesame oil on renal dysfunction in CKD rats.**

To assess the therapeutic effect of sesame oil on DOCA/salt induced renal dysfunction, serum BUN and CRE, urine volume, and CCR levels were determined. Serum BUN and CRE, and urine volume were significantly higher in Group II than in Group I or Group V (Fig. 6A-C). The CCR level was significantly lower in Group II than in Group I or Group V, and significantly higher in Group IV than in Group II (Fig. 6D). Therapeutic sesame oil significantly decreased serum BUN and CRE, and urine volume, but increased CCR in Group IV rats. Furthermore, no significant differences between Group V and I for all the parameters studied (Fig. 6A-D).

**Therapeutic effect of sesame oil on histological change in CKD rats.**

Histopathological examination was used to confirm the therapeutic effect of sesame oil on DOCA/salt induced CKD. Renal tissue from Group I and V showed normal architecture of glomeruli in the cortex and tubules in the medulla. Group II showed extensive hyaline deposits and cellular infiltration in the cortex, and a loss of brush border in the tubules. Group III and IV showed decreased inflammatory cell infiltration and congestion than Group II (Fig. 7).

**Therapeutic effects of sesame oil on cardiac function in CKD rats**

To evaluate the effect of sesame oil on the cardiac function in CKD rats, I measured blood pressure (SP and DP). DP/SP were significantly higher (p < 0.05) in Group II than in Group I, and these increases of blood pressure were markedly reduced in Group IV to the levels of Group I. No significant changes in blood pressure were detected in Group V (Fig. 8). The results here indicated that sesame oil ameliorated hypertension in CKD rats.

**Therapeutic effects of sesame oil on cardiac hypertrophy in CKD rats**

To assess the role of sesame oil on cardiac hypertrophy in CKD rats, I measured the thickness of the LV, and the wet weights of the heart. The thickness of LV (Fig. 9A) and the heart
weight (Fig. 9B) were significantly higher in Group II than in Group I, and both increases were reversed in Group IV. The protection by sesame oil against cardiac hypertrophy was further examined by histological analysis. The H&E staining of LV tissue sections demonstrated enlarged cell diameter and reduced cell density of cardiomyocytes in Group II than that in Group I and V (Fig. 10, 11). The numbers of cardiomyocytes per area of microscopic view were significantly higher in Group III and IV than in Group II, and the diameter of cardiomyocytes significantly lower in Group IV than in Group II.
Discussion

Sesame oil therapeutically instigated the regression of LVH in DOCA/salt-induced CKD rats. Sesame oil inhibited hydroxyl radical, peroxynitrite, MDA, cytoplasmic Nrf2, OPN, and collagen, but elevated nuclear Nrf2 expression in CKD rats. Sesame oil significantly decreased serum CRE, BUN, urine volume, but increased CCR in CKD rats. In addition, sesame oil effectively reversed systolic/diastolic pressure in CKD rats. Furthermore, sesame oil reduced the thickness of LV, heart weight, and the size of cardiomyocytes in CKD rats.

Sesame oil attenuated oxidative stress in CKD rats. Among oxygen free radicals, hydroxyl radical, and peroxynitrite have been suggested as the crucial radicals in the development of LPO during the progression of kidney damage (Lahera et al., 2006; Seija et al., 2012). Sesame oil blocked hydroxyl radical and peroxynitrite production, indicating that inhibiting both oxygen free radicals were involved in the sesame oil-associated reduction of LPO in CKD rats. Sesame oil efficiently repairs the acute renal injury by decreasing renal hydroxyl radical and peroxynitrite (Hsu et al., 2011; Nakano et al., 2002). Decreasing ROS in CKD rats may contribute to the depletion of OPN (Urtasun et al., 2012), thereby attenuation of renal fibrosis. Therapeutic sesame oil attenuated free radical-induced oxidative stress and may contribute to reduced OPN level and renal fibrosis in CKD rats. In addition, inhibited oxidative stress decreases systemic vascular resistance, renal sodium retention, and hence arterial pressure (Rodriguez-Iturbe et al., 2004; Wilcox, 2005; Vaziri and Rodriguez-Iturbe, 2006). Therefore, inhibiting renal oxidative stress may be the crucial step in sesame oil's protection in CKD rats.

Activation of Nrf2 may be involved in the amelioration of sesame oil on renal oxidative stress in CKD rats. Expression of Nrf2 is associated with detoxification in many organs such as liver and kidney (Copple et al., 2008). Dissociation of Nrf2 from Keap1, a cytoplasmic repressor, follow by the translocation of Nrf2 to the nucleus, then binds to ARE and promotes upregulation of Nrf2 target genes are thought to be responsible for the decreases of ROS (Zhang et al., 2014). Sesamin, a potent antioxidant of sesame oil, notably activate Nrf2 and ameliorate oxidative stress-related neurodegenerative diseases in in vitro study (Hamada et al., 2011). Therapeutic sesame oil significantly increased the nuclear Nrf2 protein, while decreasing cytoplasmic Nrf2; indicating...
translocation of Nrf2 to the nucleus and subsequent binding to ARE that might promote upregulation of antioxidant enzymes and decrease ROS in CKD rats.

Inhibiting renal OPN expression in CKD may be important for sesame oil’s anti-fibrotic effect. The accumulation of OPN in the kidney leads to renal fibrosis (Denhardt et al., 2001). Renal OPN is increased in animal models of DOCA/salt induced glomerulosclerosis (Hartner et al., 2001). In OPN-knockdown mice, depletion of OPN expression inhibited the renal interstitial fibrosis (Ophascharoensuk et al., 1999). Renal fibrosis in DOCA/salt induced CKD is related to interstitial collagen accumulation (Iwazu et al., 2011), which is the consequence of interstitial renal cell mediated inflammatory infiltration (Zheng et al., 2011). Sesame oil potently attenuated gentamicin-induced acute kidney damage by attenuating collagen accumulation (Li et al., 2012), subsequently renal fibrosis (Palm et al., 2011; Rodriguez-Iturbe and Garcia Garcia, 2010). Thus, sesame oil decreased renal OPN expression, inflammatory cell infiltration, and collagen accumulation thereby it decreased fibrosis in CKD rats.

Seven doses of sesame oil showed potent therapeutic effect against renal dysfunction in CKD rats. The absorption, bioavailability, and antioxidant activity of sesame oil and its components proved to be excellent. Sesame oil is absorbed immediately by the gastrointestinal system, starting from the oral cavity, which is from evident oil pulling therapy (Asokan et al., 2011). Studies from our laboratory (Chandrasekaran et al., 2008; Akita et al., 1998; Asmar et al., 2012) have consistently shown that sesame oil is both prophylactically as well as therapeutically effective within 3-12 h after a sesame oil gavage. Although ingesting too much sesame oil at one time may cause a bowel movement or mild diarrhea, and chronic over-ingestion may cause weight gain, there is no known health risks associated with sesame oil. We previously reported that prophylactic and therapeutic sesame oil were effective for kidney damage (Nakano et al., 2002; Li et al., 2012; Hsu et al., 2010). In the present study, one-week treatment of sesame oil significantly attenuated CKD in rats.

Therapeutic effect of sesame oil on LVH in CKD may be attributable to its components, such as phenolic lignans (sesamin and sesamol) and vitamin E. Sesamol effectively decreases blood pressure in diet-induced cardiometabolic syndrome in rats (Sharma et al., 2012). Sesamin attenuated the development of hypertension and cardiovascular impairment in DOCA/salt hypertensive rat
Vitamin E alone lowers blood pressure in spontaneously hypertensive rats (Newaz and Nawal, 1998), and synergizes with sesamin against hypertension and stroke by alleviating blood pressure, oxidative stress, and thrombotic tendency (Noguchi et al., 2001). Together, sesame oil may exert multiple functional properties against LVH in CKD rats.

The clinical implication of the current study is that sesame oil may be a potential candidate to mitigate LVH in CKD patients. Treatment of hypertension in patients with any stage of CKD is of paramount importance to slow or prevent CKD progression, and is the mainstay of cardiovascular protection. The use of combination treatment to achieve target blood pressure is the most important factor in hypertension control in patients with CKD (Nakao et al., 2003). Angiotensin-converting-enzyme inhibitor (ACEI) and angiotensin II receptor blockers (ARBs) combination treatment, the standard drugs for primary hypertension, is more effective than either drug alone in reducing BP and proteinuria in CKD (Kunz et al., 2008). If first-line therapy with an ACE inhibitor or ARB fails to achieve the target blood pressure, the addition of a loop diuretic is recommended by controlling fluid retention (Vogt et al., 2008). However, ACEI and ARB combination therapy might worsen renal function by doubling serum creatinine, and cause hyperkalemia in later stages of CKD (Mann et al., 2008). Adverse effect of a loop diuretic, such as ototoxicity, hyperkalemia and hypomagnesaemia has been reported (Leto et al., 2014). There is still no effective side-effect-free treatment against hypertension in CKD patients. Sesame oil is non-toxic nutritional oil, used as diet in most countries and effective against various diseases models, and it protects against multi-organ failure (Hsu and Liu, 2002) which could act as both disease prevention and symptom managing agent. Therefore, sesame oil is advantageous over chemical clinical management of LVH in CKD patients. However, further studies are required to test its clinical effectiveness.
Conclusion

Sesame oil therapeutically mitigates LVH by inhibiting oxidative stress-associated hypertension in CKD rats.
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Fig. 1. Time courses of renal dysfunction and hypertension in DOCA/salt-treated rats. The rats were divided into two groups of seven. Sham group: uninephrectomized, and subcutaneously (s.c.) injected with mineral oil (1 ml/kg; s.c.) twice weekly for 35 days; DOCA/salt group: uninephrectomized and then given DOCA (15 mg/kg suspended in mineral oil; s.c.) twice weekly and given drinking water with 1% NaCl for 35 days. Serum blood urea nitrogen (BUN) and creatinine (CRE) levels, systolic pressure and diastolic pressure were determined weekly. Data are means ± standard deviation (SD) (n = 7). * p < 0.05 compared with the Control group.
Fig. 2. Therapeutic effect of sesame oil on oxidative stress in CKD rats. Group I: uni-nephrectomized injected vehicle (mineral oil 1 ml/kg; s.c., twice a week) alone and normal drinking water for 35 days and strating from 29th day saline (1 ml/kg/day; p.o.,) for 7 days; Group II: uni-nephrectomized injected DOCA in vehicle (15 mg/kg; s.c., twice a week) with 1% salt in drinking water for 35 days and strating from 29th day saline (1 ml/kg/day; p.o.,) for 7 days; Group III: uni-nephrectomized injected DOCA in vehicle (15 mg/kg; s.c., twice a week) with 1% salt in drinking water for 35 days and strating from 29th day sesame oil (0.5 ml/kg/day; p.o.,) for 7 days; Group IV: uni-nephrectomized injected DOCA in vehicle (15 mg/kg; s.c., twice a week) with 1% salt in drinking water for 35 days and strating from 29th day sesame oil (1 ml/kg/day; p.o.,) for 7 days; Group V: uni-nephrectomized injected vehicle alone (1 ml/kg; s.c., twice a week) and normal drinking water for 35 days and strating from 29th day sesame oil (1 ml/kg/day; p.o.,) for 7 days. (A) Hydroxyl radical counts, (B) Peroxynitrite counts, (C) lipid peroxidation. Data are means ± SD. *<i>p</i> < 0.05 compared with Group I or Group V; #<i>p</i> < 0.05 compared with Group II.
Fig. 3. Therapeutic effect of sesame oil on nuclear and cytoplasmic Nrf2 expression in CKD rats (for treatment details, see legend for Fig. 2). (A) Nuclear Nrf2 expression, (B) Cytoplasmic Nrf2 expression. Data are means ± SD. * $p < 0.05$ compared with Group I or Group V; # $p < 0.05$ compared with Group II.
Fig. 4. Therapeutic effect of sesame oil on osteopontin expression in CKD rats (for treatment details, see legend for Fig. 2). Osteopontin level. Data are means ± SD. * p < 0.05 compared with Group I or Group V; # p < 0.05 compared with Group II.
Fig. 5. Therapeutic effect of sesame oil on renal fibrosis in CKD rats (for treatment details, see legend for Fig. 2). Collagen grading. Arrows show collagen accumulation. Data are means ± SD. * $p < 0.05$ compared with Group I or Group V; # $p < 0.05$ compared with Group II.
Fig. 6. Therapeutic effects of sesame oil on renal dysfunction in CKD rats (for treatment details, see legend for Fig. 2). (A) BUN, (B) CRE, (C) Urine volume, (D) CCR levels. Data are means ± SD. * $p < 0.05$ compared with Group I or Group V; # $p < 0.05$ compared with Group II.
Fig. 7. Therapeutic effect of sesame oil on histopathology in CKD rats (for treatment details, see legend for Fig. 2). Histological scoring. Data are means ± SD. * $p < 0.05$ compared with Group I or Group V; # $p < 0.05$ compared with Group II.
Fig. 8. Therapeutic effects of sesame oil on hypertension in CKD rats (for treatment details, see legend for Fig. 2). (A) Systolic pressure, (B) Diastolic pressure. Data are means ± SD. * $p < 0.05$ compared with Group I or Group V; # $p < 0.05$ compared with Group II.
Fig. 9. Therapeutic effect of sesame oil on cardiac hypertrophy in CKD rats (for treatment details, see legend for Fig. 2). (A) The thickness of the left ventricle, (B) Wet weights of heart. Data are means ± SD. * $p < 0.05$ compared with Group I or Group V; # $p < 0.05$ compared with Group II.
Fig. 10. Therapeutic effect of sesame oil on cardiomyocyte numbers in CKD rats (for treatment details, see legend for Fig. 2). Numbers of cardiomyocyte. Data are means ± SD. * $p < 0.05$ compared with Group I or Group V; # $p < 0.05$ compared with Group II.
Fig. 11. Therapeutic effect of sesame oil on cardiomyocyte size in CKD rats (for treatment details, see legend for Fig. 2). Size of cardiomyocytes. Data are means ± SD. * $p < 0.05$ compared with Group I or Group V; # $p < 0.05$ compared with Group II.