Journal of Basic and Applied Pharmacology
Vol. 3 No. 2 July - December 2023

Page: [O54-67]

Original research article

Sacha inchi (*Plukenetia volubilis* L.) oil ameliorates hypertensive and vascular remodeling in nitric oxide synthase inhibitor-induced hypertensive rats

Pakaporn Sa-nguanpong[1], Anatcha Thongsit[1], Jhantanipa Boonsang[1], Anjaree Inchan[1], Worasak Kaewkong[2], Wanthanee Hanchang[1], Krongkarn Chootip[1], Tippaporn Bualeong[1*]

[1]*Department of Physiology, Faculty of Medical Science, Naresuan University, Phitsanulok, 65000, Thailand*
[2]*Department of Biochemistry, Faculty of Medical Science, Naresuan University, Phitsanulok, 65000, Thailand*

Received 13 June 2023; Received in revised form 22 July 2023
Accepted 15 August 2023; Available online 26 August 2023

ABSTRACT

Sacha inchi (SI) oil possesses a wealth of vital polyunsaturated fatty acids (PUFA), vitamin E (including γ- and δ-tocopherols), and beneficial antioxidants like phenolic compounds, making it highly recommended for promoting heart health. Therefore, our ambition was to analyze the potential anti-hypertensive effect of SI oil on cardiovascular remodeling in nitric oxide synthase inhibitor-induced hypertensive rats. Hypertension was provoked in male Sprague-Dawley rats by daily handling of L-NAME through their drinking water at a dose of 40 mg/kg for three weeks. The rats were then administrated with L-NAME in drinking water plus SI oil 0.5, 1.0, 2.0 ml/kg/day or captopril 5 mg/kg/day which was delivered via oral gavage for 5 weeks. All rats had their tail cuff blood pressure measured weekly. Upon completion of the experiment, the measurement of direct blood pressure was conducted by cannulating the common carotid artery. Plasma nitric oxide metabolites (NOx), levels of the adhesion molecule VCAM-I, cardiac histopathology, fibrosis in cardiac muscle, and aorta were also investigated. SI oil at 2.0 ml/kg administered once daily for 5 weeks significantly reduced SBP, DBP, MAP, and decreased VCAM-I and fibrosis in hypertensive rats. Furthermore, SI oil also mitigated endothelial dysfunction by enhancing the vascular response to acetylcholine (ACh) in aortic rings. Taken together, these results indicated that SI oil attenuated blood pressure and improved vascular dysfunction. However, the protective effect of SI oil will be further studied because the damage to organs from hypertension is difficult to restore after pathological damage has occurred.

Keywords: Sacha inchi oil, L-NAME, hypertension

***Corresponding author**: tippapornb@nu.ac.th

1. Introduction

Hypertension, classified as a chronic non-communicable disease (NCD), is considered when the systolic blood pressure (SBP) gets to or goes over 140 mmHg, or when the diastolic blood pressure (DBP) gets to or goes over 90 mmHg.[1] Hypertension is the most typical cause of cardiovascular, cerebrovascular, and kidney diseases[2-4] and affects about 1.28 billion people worldwide. However, it is estimated that only 23% of females and 18% of males are aware of having the disease and are being treated for it.[5,6] Hypertension is associated with endothelial dysfunction, where high arterial pressure damages endothelial cells, leading to atherosclerotic plaque formation and increased arteriolar resistance or total peripheral resistance (TPR). High TPR inserts additional stress on the heart by increasing afterload, causing left ventricle hypertrophy as the heart works harder against the high-resistance arterioles.[7,8] Hypertension also triggers oxidative stress, with reactive oxygen species (ROS) playing a significant role. ROS originate from various sources, for instance, endothelial cells, vascular smooth muscle cells, fibroblasts, and monocytes/macrophages.[7,9,10] High ROS levels can cause cellular damage, promote inflammation, and contribute to cardiac and vascular remodeling.[10,11]

Chronic blockage of nitric oxide synthase (NOS) by the potent nitric oxide synthase inhibitor Nω-nitro-L-arginine-methyl ester hydrochloride (L-NAME) has been demonstrated to induce hypertension together with cardiovascular remodeling such as left ventricular hypertrophy, myocardial fibrosis, endothelial dysfunction, perivascular thickening and perivascular fibrosis.[12-15] L-NAME-inhibited NOS activity leads to a decrease in NO production, which in turn results in vasoconstriction, increased TPR, and elevation of blood pressure. In addition, L-NAME-induced hypertension is related to the renin-angiotensin-aldosterone system (RAAS). The RAAS assumes a crucial function in handling blood pressure and responds to changes in vascular resistance or blood volume. L-NAME activates the RAAS by increasing the production of renin and angiotensin II, thereby contributing to the progress of hypertension. Moreover, the inhibition of NO synthases also causes oxidative stress and inflammation through an increase in ROS. Reduction of NO bioavailability and increase of ROS generate the stimulation of nuclear factor kappa-B (NF-kB) transcription, subsequently bringing about an increase in proinflammatory cytokines such as tumor necrosis factor (TNF-alpha) and interleukin-1beta (IL-1β). Additionally, this process is responsible for the enhancement of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1).[16-18]

Sacha inchi (*Plukenetia volubilis* L.) (SI) is likewise referred to as Daao Inca or Inca peanut. SI seeds contain numerous unsaturated fatty, proteins, and a variety of health-promoting compounds such as phenolic compounds, tocopherol, and phytosterol.[19] SI seeds are a valuable source of oil (35-60%) and protein (24-33%).[20] SI oil is notably abundant in polyunsaturated fatty acids (PUFAs), accounting for approximately 77.5-84.4% of its composition, comparable to flax and chia seed oils. It also contains 8.4-13.1% monounsaturated fatty acids (MUFAs) and 6.8-9.1% saturated fatty acids (SFAs). The PUFA content primarily comprises alpha-linolenic acid (C18:3 n-3, ω-3, ALA) and linolenic acid (C18:2 n-6, ω-6, LA), which are necessary fatty acids that cannot be synthesized by the body.[20] However, consuming excessive amounts of ω-6 fatty acid-rich seed oil including corn, soybean, and safflower oil and a high ratio of ω-6/ω-3 fatty acid promotes coronary heart disease, cancer, allergic diseases, inflammatory and autoimmune diseases.[21,22] In contrast, a low ratio of ω-6/ω-3 fatty acid decreases the likelihood of cardiovascular disease and chronic diseases.[23] The ideal recommendation

for promoting human health is to maintain a proportion of ω-6 to ω-3 fatty acids at 1:1.[21]

Interestingly, the ratio of ω-6/ω-3 fatty acids in the SI oil ranges from 0.81 to 1.12 and has been reported in several studies.[24,25] Similarly, Chirinos et al. (2013) mentioned that the ratio ω-6/ω-3 fatty acid of the SI oil was within the 0.89-1.09 range.[19] SI oil has a high amount of total phenolic content (TPC: 63.89-120.32 mg GAE/100 g oil), total tocopherols (161.87-282.20 mg/100 g oil), total phytosterols (162.3-289.9 mg/100 g oil), and flavonoids (approximately 617.0 mg /100 g oil).[26-32] These substances have a role in antioxidation and inflammation and several studies have demonstrated their prevention of hypercholesterolemia and cardiovascular diseases.

In this study, captopril was employed as a reference treatment for hypertension. Captopril functions as an antagonist of angiotensin-converting enzyme (ACE), hindering the transition from angiotensin I (Ang I) to angiotensin II (Ang II). Ang II, a powerful vascular tone enhancer, has the potential to raise blood pressure. Ang II plays a vital role as an essential part within the renin-angiotensin-aldosterone system (RAAS). The effect of captopril is to decrease plasma concentrations of Ang II and aldosterone, resulting in reduced mean arterial blood pressure (MAP) through decreased blood volume, which in turn leads to decreased cardiac output (CO) and decreased total peripheral resistance (TPR).[33]

Currently, there are no studies investigating the alleviating of high blood pressure by SI oil in hypertensive rats. Therefore, the ongoing investigation examines the impact of SI oil on blood pressure and cardiovascular remodelling, specifically hypertrophy and fibrosis, in NO-deficient rats.

2. Materials and Methods

2.1 Medications and chemical substances

Sacha Inchi pure cold pressed 100% extra virgin oil was purchased from Omega 3.6.9 and Lycopene Co. Ltd. (Kamphaeng Phet, Thailand). Its composition is shown in Table 1. Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) and captopril were purchased from Sigma-Aldrich Pte Ltd (USA).

2.2 Animal study and research protocol

Male Sprague-Dawley rats weighing between 200 and 250 grams were acquired from Nomura Siam International Co., Ltd. (Bangkok, Thailand). The rats were accommodated at the Center for Animal Research, Naresuan University, Phitsanulok, Thailand, in clear plastic cages with autoclaved corncob bedding, the under-regulated environment of a 12-hour dark-light cycle and a temperature of 22.0 ± 1.0°C. Throughout the experiment, the rats were provided with pasteurized pellets and reverse osmosis (RO) filtered water. All trial protocols were followed in compliance with the protocol's instructions for the treatment and handling of experimental animals. The Animal Ethics Committee of Naresuan University, Phitsanulok, Thailand, approved all the experimental protocols (Approval No. NU-AE620617).

After one week of acclimatization, the rats were subjected to hypertension induction by administering 40 mg/kg body weight nitric oxide synthase inhibitor in their drinking water, while the control rats were given reverse osmosis (RO) water. (Fig.1). Random allocation was used to distribute the rats into six groups (n=8) each as follows:

Group 1: Control (Control; RO 10 ml/100 kg BW.)
Group 2: Hypertensive (LN; L-NAME 40 mg/kg BW.)
Group 3: L-NAME + Sacha inchi oil 0.5 ml (LN + SI 0.5; L-NAME 40 mg/kg BW + SI 0.5 ml/kg BW.)
Group 4: L-NAME + Sacha inchi oil 1.0 ml (LN + SI 1.0; L-NAME 40 mg/kg BW + SI 1.0 ml/kg BW.)
Group 5: L-NAME + Sacha inchi oil 2.0 ml (LN + SI 2.0; L-NAME 40 mg/kg BW.+ SI 2.0 ml/kg BW.)
Group 6: L-NAME + captopril (LN+Cap; L-NAME 40 mg/kg BW.+ captopril 5 mg/kg BW.)

2.3 Assessment of blood pressure

2.3.1 Indirect blood pressure measurement

The acclimatized rats were trained to become accustomed to being in a rat restrainer for 5 days and initial blood pressure readings, including systolic blood pressure (SBP), were then obtained as baseline measurements at week 0. Throughout the 8-week study, we utilized an indirect tail-cuff blood pressure measurement system (ML 125 NIBP system, AD Instruments, Sydney, Australia) in conjunction with a PowerLab measurement recording system (AD Instruments, Sydney, Australia) to measure the SBP of conscious rats on a weekly basis. This approach enabled continuous monitoring and recording of any changes or fluctuations in blood pressure throughout the study period. Subsequently, the collected data were assessed offline operating with the LabChart physiological data analysis software (AD Instruments; Software: LabChart for Windows v. 7 PowerLab).

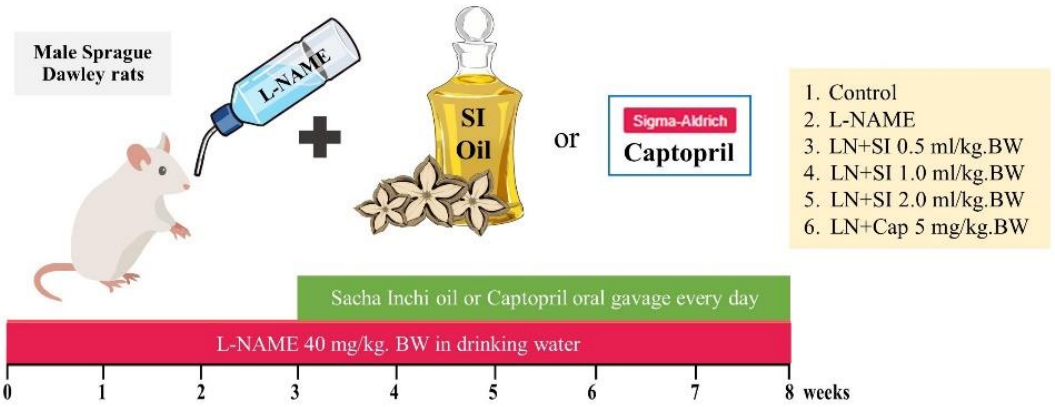

Fig. 1. Conceptual framework.

2.3.2 Direct blood pressure measurement

Upon completion of the study, invasive blood pressure measurements were performed on the rats under inhalation anesthesia using 2% isoflurane. Cannulation of the right common carotid artery was conducted for each rat, enabling the connection of a pressure transducer (iWorx, Dover, NH, USA) to the PowerLab system. This setup facilitated the monitoring of baseline values for SBP, diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR) using the Lab Chart 7 software (AD Instruments, Sydney, Australia). The Lab Chart 7 software was utilized for both data recording and analysis purposes.

2.4 Histological and morphometric analysis

The weighing was conducted to determine the ratios of heart weight to body weight (HW/BW) and heart weight to tibia length (HW/TL). The harvested heart and thoracic aortas were preserved in a solution of 10% neutral buffered formalin, subsequently embedded in paraffin mold, and sliced into sections with a thickness of 3 µm. Afterward, hematoxylin and eosin (H&E) staining was employed to evaluate left ventricular hypertrophy and the medial thickness of the thoracic aortas, while Masson-Goldner staining was used to evaluate fibrosis in the left ventricle and thoracic aortas. The sections were detected and captured employing a light microscope (BX53F2, Olympus). The analysis of left ventricular and thoracic aorta thickness was conducted using the Image J software from the National Institute of Mental Health in Bethesda, Maryland, USA.

Table 1. The composition of SI oil.

Fatty acids	Percentage (g/100 g)
Myristic acid (C14:0)	0.02
Palmitic acid (C16:0)	4.41
Heptadecanoic acid (C17:0)	0.11
Stearic acid (C18:0)	3.62
Arachidic acid (C20:0)	0.11
Behenic acid (C22:0)	0.03
Tricosanoic acid (C23:0)	0.01
Lignoceric acid (C24:0)	0.01
Saturated fat	8.32
Palmitoleic acid (C16:1n7)	0.04
Cis-10-heptadecenoic acid (C17:1n10)	0.05
Trans-9-elaidic acid (C18:1n9t)	0.05
Cis-9-oleic acid (C18:1n9c)	9.16
Cis-11-eicosenoic acid (C20:1n11)	0.27
Monounsaturated fatty acid	9.58
Cis-9,12-linoleic acid (C18:2n6)	40.54
γ-Linolenic acid (C18:3n6)	0.2
α-Linolenic acid (C18:3n3)	41.29
Cis-11,14-eicosadienoic acid (C20:2)	0.05
Cis-11,14,17-eicosatrienoic acid (C20:3n3)	0.01
Polyunsaturated fatty acid	82.1
Unsaturated fat	91.68
Omega-3	41.30
Omega-6	40.75
Omega-9	9.16

2.5 Quantification of plasma nitric oxide metabolites (nitrite/nitrate, NOx) and plasma TNF-α levels

A sample of blood was obtained from the common carotid artery of each rat, and these samples were subjected to centrifugation at 3,500 rpm and 4°C for 15 minutes to distinguish the plasma. Eventually, it was preserved at a temperature of -80°C. The concentration of plasma NOx was evaluated using a nitric oxide assay kit (ELISA) (ab65328, Abcam). Similarly, the plasma concentration of TNF-α was determined utilizing an enzyme-immunoassay (ELISA) kits (ab100784, Abcam)

2.6 Western Blot analysis of VCAM-I protein expressions in plasma

Plasma protein expression of VCAM-I was assessed using the following procedure: Samples were subjected to electrophoresis on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) system. Subsequently, the proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane and blocked with 5% skimmed milk in 1x Tris-buffered saline, 0.1% Tween 20 detergent (TBST), at room temperature for 1 hour. Following one wash, the membrane was exposed to primary antibodies during the incubation process (Rabbit anti-VCAM-I, diluted 1:5,000) at 4°C throughout the night. After another wash, a secondary antibody (Goat anti-Rabbit, diluted 1:5,000) was applied to the membrane during the incubation process at room temperature in the dark for 1 hour. Following a final wash, detection was performed using ImageQuant™ LAS 500. The band intensity was standardized relative to GADPH, and the results were displayed as a percentage of the value established in the control group from the same gel.

2.7 Vascular function study

To assess the potential improvement in aortic vascular function in rats with hypertension caused by nitric oxide deficiency, the vascular reactivity of the aorta was evaluated. The aorta was isolated and immediately transferred to a Krebs solution containing the following components (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 12, with a pH of 7.3. The connective tissue and fat were delicately extracted, then the aorta was divided into segments, each measuring 2-3 mm in length, to form rings. Subsequently, these rings were submerged in an organ bath consisting of Krebs solution, which was continuously oxygenated with 95% O_2 and kept at a consistent temperature of 37°C.[34] The rings underwent a 60-minute equilibration period, during which they were maintained and subjected to a resting tension of 1 g. Throughout the equilibration period, the Krebs solution was replaced every 15 minutes to ensure its freshness and stability. Once the rings reached a plateau of

contraction elicited by the administration of phenylephrine (10 μM), acetylcholine (ACh, 10^{-9}–10^{-4} M) or sodium nitroprusside (SNP, 10^{-9}–10^{-4} M) was added cumulatively to the organ bath. The alterations in tension were captured using force transducers (CB Sciences Inc., Milford, USA) linked to a PowerLab Data Acquisition System equipped with Lab Chart software version 7.0 (A.D. Instrument, Castle Hill, Australia). The findings were presented as a percentage of relaxation relative to the contraction induced by phenylephrine, with 100% indicating complete relaxation.

2.8 Data analysis

Statistical analysis was rendered using one-way ANOVA sequentially the Newman-Keuls and Turkey's post-hoc test to assess the significance between the groups. GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA) was utilized for the statistical calculations. Differences between experimental groups were considered significant when $p < 0.05$. The data were expressed as the mean ± standard error of the mean (SEM).

3. Results

3.1 The influence of SI oil and captopril on SBP in conscious rats

The tail-cuff plethysmography method is an indirect blood pressure measurement technique used to measure SBP in awake rats. Initially, at week 0, there were no relevant differences in SBP between all the groups. However, after administering L-NAME for 3 weeks, there was a progressive and significant rise in SBP in the L-NAME group compared to the normotensive group. Furthermore, throughout the entire experimental period, both the L-NAME group and the LN+SI 0.5 group exhibited a significant elevation in SBP. Alternatively, the simultaneous application of L-NAME and SI oil at doses of 1.0 and 2.0 ml/kg caused a significant reduction in SBP in the L-NAME group. Additionally, the SBP in the captopril group, as well as the groups receiving SI oil at doses of 1.0 and 2.0, exhibited a significant reduction compared to the hypertensive group. (Fig. 2)

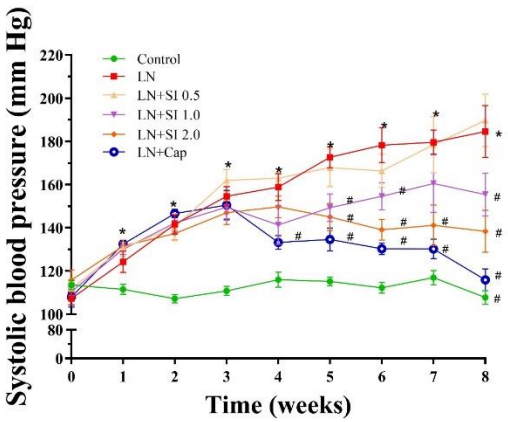

Fig. 2. Effect of L-NAME or L-NAME and SI oil on weekly SBP during treatment in all experimental groups. Control; normotensive rats, LN (L-NAME); hypertensive rats, LN+SI 0.5; hypertensive rats treated with SI oil 0.5 ml/kg, LN+SI 1.0; hypertensive rats treated with SI oil 1.0 ml/kg, LN+SI 2.0; hypertensive rats treated with SI oil 2.0 ml/kg, and LN+Cap; hypertensive rats treated with captopril 5 mg/kg. The data is presented in the form of mean ± SEM (n=5-8/group), $*p < 0.05$ vs. control, $^{\#}p < 0.05$ vs. L-NAME.

3.2 The impact of SI oil and captopril on hemodynamic parameters in rats under anesthesia

Direct blood pressure measurements were conducted on the anesthetized rats at the end of week 8 using the common carotid artery. The L-NAME-induced hypertensive rats exhibited significant elevations in SBP, DBP, and MAP in comparison to the control group (163.3 ± 13.3 vs. 106.8 ± 5.6, 123.9 ± 9.9 vs. 76.6 ± 6.9, 144.3 ± 10.8 vs. 91.1 ± 6.3, mmHg respectively). The concurrent regimen of L-NAME and SI oil given at 2.0 ml/kg or captopril 5 mg/kg revealed significantly lower SBP, DBP, and MAP than the L-NAME group (141.5 ± 7.5 and 115.9 ± 5.0 vs. 163.3 ± 13.3, 100.6 ± 6.7 and 85.5 ± 3.4 vs. 123.9 ± 9.9, 118.6 ± 7.6 and 100.9 ± 4.0 vs. 144.3 ± 10.8 mmHg, respectively). Nevertheless, no notable distinctions were detected in HR among the groups. (Table 2.)

Table 2. The impact of SI oil and captopril on hemodynamic parameters in rats under anesthesia.

Parameter	Control	LN	LN+SI 0.5	LN+SI 1.0	LN+SI 2.0	LN+Cap
SBP (mmHg)	106.8 ± 5.6	163.3 ± 13.3*	178.4 ± 12.7	152.6 ± 9.6	141.5 ± 7.5$^{\#}$	115.9 ± 5.0$^{\#}$
DBP (mmHg)	76.6 ± 6.9	123.9 ± 9.9*	127.6 ± 7.8	115.7 ± 6.5	100.6 ± 6.7$^{\#}$	85.5 ± 3.4$^{\#}$
MAP (mmHg)	91.1 ± 6.3	144.3 ± 10.8*	150.4 ± 9.4	133.3 ± 7.6	118.6 ± 7.6$^{\#}$	100.9 ± 4.0$^{\#}$
HR (mmHg)	314.6 ± 5.8	346.2 ± 11.2	327.8 ± 14.3	345.7 ± 19.5	315.6 ± 17.2	331.1 ± 19.8

The data is presented in the form of mean ± SEM (n=7-8/group), *$p < 0.05$ vs. control, $^{\#}p < 0.05$ vs. L-NAME

Table 3. Effect of L-NAME or L-NAME and SI oil on body weight (BW), heart weight (HW), and tibia length (TL).

Parameter	Control	LN	LN+SI 0.5	LN+SI 1.0	LN+SI 2.0	LN+Cap
BW (g)	565.7 ± 12.6	544.2 ± 24.0	570.7 ± 19.6	551.7 ± 20.0	551.7 ± 16.1	541.5 ± 11.6
HW (mg)	1,363.8 ± 26.5	1,421.4 ± 82.3	1,481.7 ± 31.2	1,425.0 ± 41.8	1,470.0 ± 40.7	1,352.5 ± 23.1
HW/BW (mg/g)	2.42 ± 0.05	2.51 ± 0.12	2.61 ± 0.10	2.38 ± 0.06	2.68 ± 0.09	2.51 ± 0.07
HW/TL (mg/mm)	27.1 ± 0.55	28.1 ± 1.58	29.9 ± 0.70	28.7 ± 0.88	29.7 ± 0.99	27.3 ± 0.56

The data is presented in the form of mean ± SEM (n=7-8/group), *$p < 0.05$ vs. control, $^{\#}p < 0.05$ vs. L-NAME

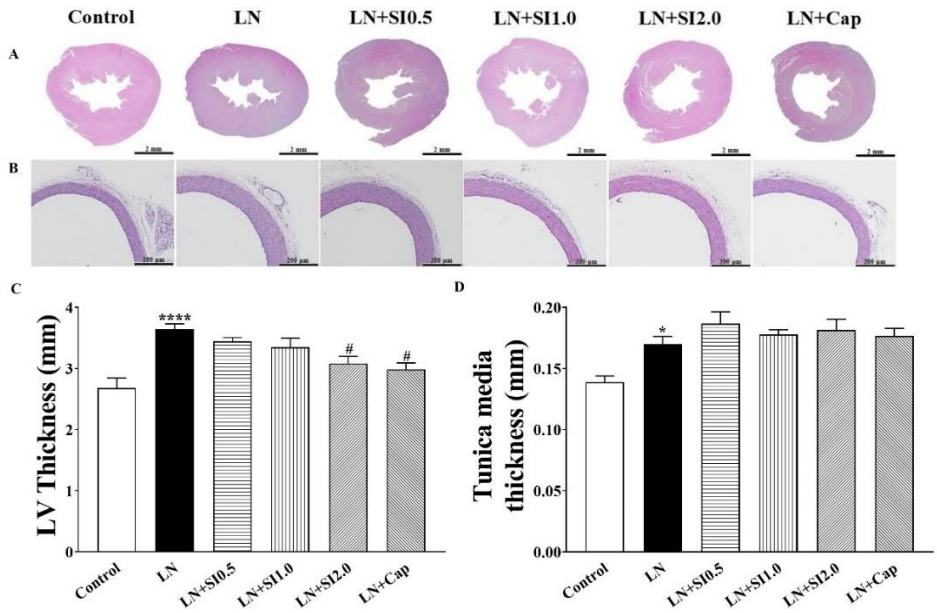

Fig. 3. Effect of SI oil and captopril on histomorphology of the LV and thoracic aorta in L-NAME-induced hypertensive rats. (A) Illustrative images of LV sections stained with hematoxylin and eosin under a light microscope using 1.25x magnification, scale bar = 1 mm. (B) Illustrative images of thoracic aorta sections stained with hematoxylin and eosin under a light microscope using 10x magnification, scale bar = 200 µm. (C) LV thickness (D) Tunica media thickness in Control, LN, LN+SI 0.5, LN+SI 1.0 /kg, LN+SI 2.0, and LN+Cap. The data is presented in the form of mean ± SEM *$p < 0.05$,****$p <$ 0.0001 vs. Control, $^{\#}p < 0.05$ vs. L-NAME, (n=7-8/group).

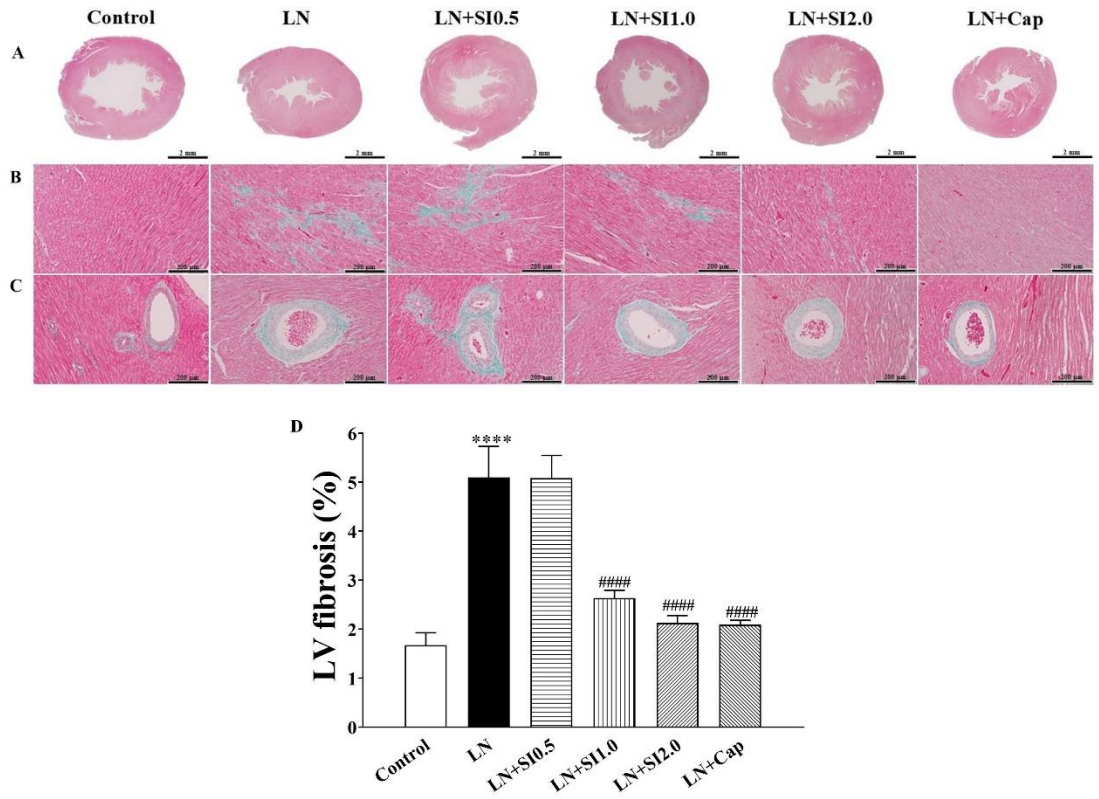

Fig. 4. Effect of SI and captopril on left ventricular and perivascular fibrosis. (A) Illustrative images of LV sections stained with Masson-Goldner under a light microscope using 1.25x magnification, scale bar = 1 mm. (B) Illustrative images of left ventricular sections stained with Masson-Goldner under a light microscope using 20x magnification, scale bar = 100 µm. (C) Illustrative images of perivascular fibrosis of the coronary artery in the left ventricular cardiac tissue sections stained with Masson-Goldner under a light microscope using 20x magnification, scale bar = 200 µm. (D) Left ventricular fibrosis in Control, LN, LN+SI 0.5, LN+SI 1.0 /kg, LN+SI 2.0, and LN+Cap. The data is presented in the form of mean ± SEM ,$^{****}p < 0.0001$ vs. Control, $^{\#\#\#\#}p < 0.0001$ vs. L-NAME, (n=7-8/group).

3.3 Effect of SI oil or captopril on body weight, heart weight, and the ratio of heart weight body weight, and heart weight tibia length

After administration of L-NAME or treatment with SI oil or captopril, there were no statistically considerable variations observed in body weight (BW), heart weight (HW), heart weight/body weight (HW/BW) ratio, and heart weight/tibia length (HW/TL) ratio among all the studied groups. (Table 3.)

3.4 Effect of SI oil or captopril on the left ventricular and thoracic aorta histo-morphology

Histomorphometry analysis utilizing hematoxylin and eosin (H&E) staining revealed that the chronic application of L-NAME generated a significant escalation in LV wall thickness and thoracic aorta thickness when compared to the normotensive group (3.65 ± 0.08 vs. 2.68 ± 0.16, and 0.17 ± 0.01 vs. 0.14 ± 0.01 mm, respectively) (Fig. 3A, 3C). This result confirms that the application of L-NAME elicited LV hypertrophy and vascular wall hypertrophy in rats.

Interestingly, treatment with SI oil at a dose of 2. 0 ml/ kg, or captopril, significantly decreased LV wall thickness compared to the group of hypertensive rats. However, concomitant SI oil and L-NAME could not restore thoracic aorta thickness. (Fig. 3B, 3D).

3. 5 Effect of SI oil or captopril on left ventricular and perivascular fibrosis

The left ventricular and perivascular fibrosis were evaluated from the Masson-Goldner stain section. Chronic administration of L-NAME significantly exhibited fibrosis in the cardiac section of the left ventricle to a greater extent than observed in the normotensive group (5.10 ± 0.64 vs. 1.68 ± 0. 25, respectively) . The treatment of hypertensive rats with SI oil at doses of 1.0 and 2. 0, as well as captopril, led to significantly diminished fibrosis in the cardiac tissue when compared to the L-NAME group (2.64 ± 0.15, 2.13 ± 0.15, 2.09 ± 0.09 vs. 5.10 ± 0.64, respectively) (Fig. 4). Furthermore, a higher presence of fibrosis surrounding the coronary artery of the left ventricle was noticed in the hypertensive group in comparison with the remaining groups.

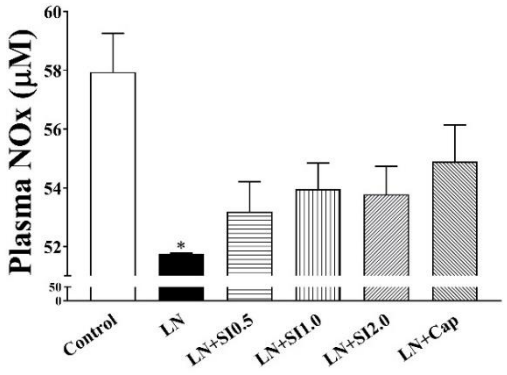

Fig. 5. Effect of L-NAME or L-NAME and Sacha Inchi or captopril on the concentration of plasma NOx. Control; normotensive rats, L- NAME; hypertensive rats, LN+SI 0.5; hypertensive rats received SI 0.5 ml/kg, LN+SI 1.0; hypertensive rats received SI 1. 0 ml/ kg, LN+ SI 2. 0; hypertensive rats received SI 2. 0 ml/ kg, and LN+Cap; hypertensive rats received captopril 5 mg/kg. Data is presented in the form of mean ± SEM (n=7-8/group). *$p < 0.05$ vs. Control.

3.6 Effect of SI oil on plasma NOx concentration

The administration of L- NAME caused a rise in blood pressure and significantly reduced plasma NOx concentration compared to the normotensive group. However, treatment with SI oil did not cause any changes in plasma NOx levels when compared to the L-NAME group (53.2 ± 1.0, 54.0 ± 0.89, 53.8 ± 0.86, 54.9 ± 1.24 vs. 51.8 ± 0.04 μM, respectively). (Fig. 5)

3. 7 Effect of SI oil or captopril on plasma TNF- α concentration and cardiac muscle VCAM-1 expression

The expression of TNF-α concentration in plasma from the various experimental groups is depicted in Figure 5. A. There were no notable distinctions observed among the groups. (Fig. 6A). Nevertheless, there was a significant expression of VCAM-1 protein in the hypertensive group relative to the normotensive group (1.76 ± 0.10 vs. 1.00 ± 0.10, respectively). Treatment with SI oil at a dose of 1.0 or 2.0 ml/kg significantly decreased the level of VCAM-1 expression relative to the L-NAME group (1.16 ± 0.02 and 1.03 ± 0.11 vs. 1.76 ± 0.10, respectively) (Fig. 6B).

3.8 Effect of SI on vasorelaxation response of aortic rings to ACh and SNP

The vasorelaxation responses to ACh (10^{-9}– 10^{-4} M) in the aorta were significantly diminished in hypertensive rats induced by nitric oxide deficiency in comparison to the control group (Emax, 30.8 ± 1.46 vs. 82.5 ± 5.60%, $p < 0.01$; Fig. 7A). Treatment with SI oil at a dose of 1.0 and 2.0 mg/ kg or captopril demonstrated a significant increase in the percentage of vasorelaxation of aortic rings in hypertensive rats (Emax, 57.9 ± 2.69, 59.3 ± 4.33 and 63.1 ± 5. 32, respectively, $p < 0.01$; Fig. 7A), whereas after treatment with 0.5 mg/kg SI there were no notable discrepancies in L-NAME group. Moreover, no significant

variations were noticed in the relaxation of blood vessels in response to SNP (10^{-9}–10^{-4} M) in all groups (Fig. 7B).

4. Discussion

This study provides evidence that the chronic administering of L-NAME orally at a dosage of 40 mg/kg BW for 8 weeks results in NO deficiency, which promotes the progression of hypertension and ultimately leads to cardiac remodeling. Cardiac remodeling is revealed by LV hypertrophy, myocardial fibrosis, and thickening of the thoracic aorta wall. Treatment with SI oil diminished high blood pressure and mitigated endothelial dysfunction, as proven by an acceleration in the vascular response to ACh in aortic rings.

L-NAME-induced hypertension in this study is a well-established model used in numerous studies.[12,14,35-37] One of several precise mechanisms of L-NAME involved in cardiac remodeling is an imbalance of vascular tone from reducing NO production.[14,38,39] It is well-documented that L-NAME generates NO-deficient hypertension by inhibiting nitric oxide synthase activity, leading to decreased NO production and consequent attenuated vascular relaxation and finally elevated blood pressure.[40,41] Our study provides confirmation that treatment with SI oil after L-NAME-induced hypertension effectively lowers blood pressure and exhibits a trend toward increased plasma nitric oxide concentration levels. It is possible that the duration of the SI oil treatment for 5 weeks, which implies acute treatment, was not long enough to increase the concentration of nitric oxide in the plasma. This discovery aligns with the findings of a study conducted by Goya et al. in 2022, where it was observed that the consumption of wild olive (acebuche) oil for a duration of 12 weeks tends to significantly enhance nitric oxide bioavailability.[26] Nitric oxide synthase inhibitors induce a marked elevation of blood pressure as well as deteriorated cardiac muscle and blood vessels by increased levels of proinflammatory cytokines, oxidative stress markers, and ultimately a fibrotic scar.[42-48] The high resistance of blood vessels due to increased vascular constriction causes increasing afterload-induced cardiac workload adaptive response to compensate for the cardiac output resulting in LV hypertrophy, myocardial degeneration, and progression of cardiac fibrosis and perivascular fibrosis.[12,37,49] There are well-known studies which have reported that treatment with L-NAME causes marked fibrosis in the left ventricular wall, as well as perivascular fibrosis.[14,38,50,51]

Interestingly, the findings of this study indicate that the administration of SI oil provides relief from hypertension and mitigates cardiovascular remodeling induced by L-NAME. SI oil may have potential blood pressure lowering effects due to several reasons including 1) plentiful ω-3 fatty acids, notably alpha-linolenic acid (ALA) which improves endothelial function, reduces inflammation, and promotes vasodilation,[52,53] 2) antioxidant properties, as SI oil contains antioxidants including vitamin E and carotenoids, which have been shown to decrease blood pressure by reducing oxidative stress and inflammation,[54] 3) angiotensin-converting enzyme (ACE) inhibition, as SI oil has been found to inhibit ACE which promotes vasodilation and consequently decreases blood pressure,[55] 4) nitric oxide production, as SI oil has demonstrated the ability to enhance nitric oxide production and exhibit anti-inflammatory effects, which potentially contribute to its capacity to lower blood pressure.[56] These effects might be attributed to the presence of PUFAs in SI oil, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These essential fatty acids have a crucial role in promoting human health by improving blood pressure, enhancing blood flow and cardiovascular function, and regulating thrombosis and inflammation, thereby contributing to the prevention of cardiovascular disease.[52,56] Earlier, in a study conducted by Gonzales and Gonzales (2014), a non-biased allocation,

double- blind, placebo- controlled trial was implemented over a duration of 4 months to evaluate the toxicity of orally administered 10 or 15 ml/kg SI oil in healthy individuals. The results demonstrated that no statistically significant ($p > 0.05$) alterations were observed in biomarkers related to liver and kidney function. In addition, SBP and DBP decreased significantly ($p < 0.05$).[57] Although there have been no previous studies of SI oil on cardiovascular remodeling in nitric oxide deficiency hypertensive rats, SI oil may also have a cardioprotective effect. It is possible that omega- 3 fatty acids in SI oil might improve endothelial function, promote vasodilation, antioxidant, anti-inflammatory, and antithrombotic action, slow down the progress of plaques, and decrease vessel wall thickness.[19,26,52,56]

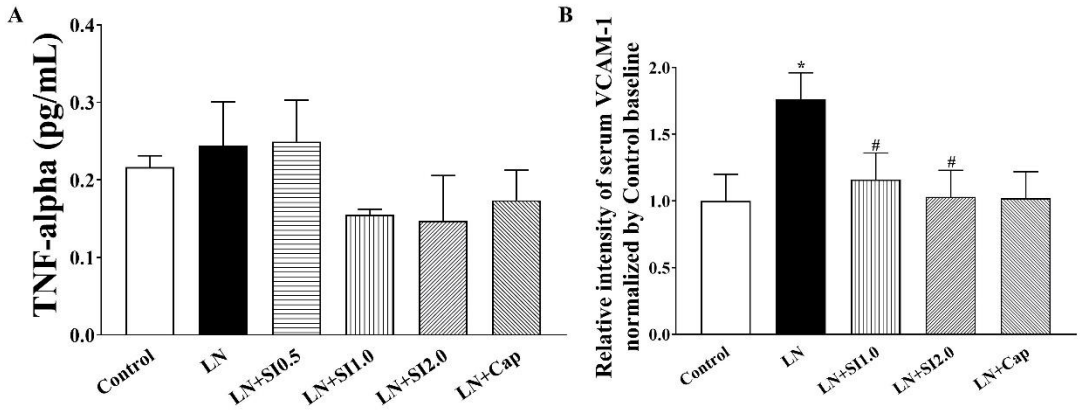

Fig. 6. Effect of SI oil or captopril on plasma TNF-α (A) and cardiac muscle VCAM-1 protein expressions (B). Control; normotensive rat, L-NAME; hypertensive rat, LN+SI 0.5; hypertensive rats received SI 0.5 ml/kg, LN+SI 1.0; hypertensive rats received SI 1.0 ml/kg, LN+SI 2.0; hypertensive rats received SI 2.0 ml/kg, and LN+Cap; hypertensive rats received captopril 5 mg/kg, The data is presented in the form of mean ± SEM (n=7-8/group). *$p < 0.05$ vs. Control and $^{\#}p < 0.05$ vs. L-NAME.

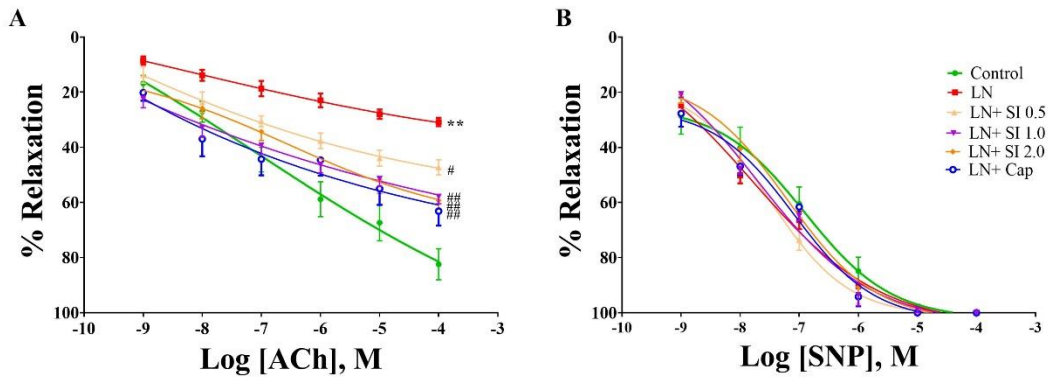

Fig. 7. Cumulative concentration-relaxation curves of (A) acetylcholine (ACh, 10^{-9}–10^{-4} M) and (B) sodium nitroprusside (SNP, 10^{-9}–10^{-4} M) in endothelial intact aortic rings pre-contracted with 10 µM phenylephrine (PE). SI, Sacha Inchi; L-NAME; LN, L-NAME. The data is presented in the form of mean ± SEM (n = 6). **$p < 0.01$ vs Control, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. L-NAME.

5. Conclusion

The present study has shown that a dose of 2.0 ml/kg of SI oil alleviates left ventricular hypertrophy, fibrosis, and VCAM-I protein expression. It also alleviates endothelial dysfunction, as verified by an enhancement in the vascular response to ACh in aortic rings. While these results suggest that SI oil can lower blood pressure and enhance vascular function, the protective effect of SI oil requires further investigation, given that organ damage caused by hypertension can be challenging to reverse once it has become pathological.

Acknowledgements

This work was supported by the Agricultural Research Development Agency of Thailand (Grant no. CPR6205031640).

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Organization WH. Guideline for the pharmacological treatment of hypertension in adults. World Health Organization; 2021.

[2] Collins R, Peto R, MacMahon S, Godwin J, Qizilbash N, Hebert P, et al. Blood pressure, stroke, and coronary heart disease: part 2, short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. Lancet. 1990; 335(8693):827-838.

[3] Wright Jr JT, Bakris G, Greene T, Agodoa LY, Appel LJ, Charleston J, et al. Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. JAMA. 2002;288(19):2421-2431.

[4] Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature. 2006;444(7121):875-880.

[5] World Health Organization (WHO). Guideline for the pharmacological treatment of hypertension in adults: summary. 2022 [cited 2023 May 10]. Available from: https://www.who.int/publications/i/item/9789240050969

[6] Kebadnew M, Tensaykahsay. Factors associated with hypertension among age groups of 18 years and above in Southwestern, Ethiopia, 2020: A community based cross-sectional study. Divers Equal Health Care. 2020;17(7):202-208.

[7] Laher I. Systems biology of free radicals and antioxidants. Springer; 2014.

[8] Copstead-Kirkhorn L-EC, Banasik JL. Pathophysiology-E-Book. Elsevier Health Sciences; 2014.

[9] Paravicini TM, Touyz RM. Redox signaling in hypertension. Cardiovasc Res. 2006; 71(2):247-258.

[10] Tousoulis D, Stefanadis C. Biomarkers in cardiovascular diseases. CRC Press; 2013.

[11] Schulman IH, Zhou MS, Raij L. Interaction between nitric oxide and angiotensin II in the endothelium: role in atherosclerosis and hypertension. J Hypertens Suppl. 2006; 24(1):S45-50.

[12] Maneesai P, Bunbupha S, Potue P, Berkban T, Kukongviriyapan U, Kukongviriyapan V, et al. Hesperidin prevents nitric oxide deficiency-induced cardiovascular remodeling in rats via suppressing TGF-β1 and MMPs protein expression. Nutrients. 2018;10(10):1549.

[13] Pechánová O, Bernátová I, Babál P, Martínez MC, Kyselá S, Stvrtina S, et al. Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension. J Hypertens. 2004;22(8):1551-1559.

[14] Bunbupha S, Prachaney P, Kukongviriyapan U, Kukongviriyapan V, Welbat JU, Pakdeechote P. Asiatic acid alleviates cardiovascular remodelling in rats with L-NAME-induced hypertension. Clin Exp Pharmacol. 2015; 42(11):1189-1197.

[15] Bernátová I, Pechánová O, Babál P, Kyselá S, Stvrtina S, Andriantsitohaina R. Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. Am J Physiol Heart. 2002;282(3):H942-H948.

[16] Pechanova O, Simko F. The role of nuclear factor kappa B and nitric oxide interaction in heart remodelling. J Hypertens. 2010;28: S39-S44.

[17] Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2(1):17023.

[18] Harrison DG, Gongora MC. Oxidative stress and hypertension. Med Clin. 2009; 93(3):621-635.

[19] Chirinos R, Zuloeta G, Pedreschi R, Mignolet E, Larondelle Y, Campos D. Sacha inchi (*Plukenetia volubilis*): A seed source of polyunsaturated fatty acids, tocopherols, phytosterols, phenolic compounds and antioxidant capacity. Food Chem. 2013;141(3):1732-1739.

[20] Mhd Rodzi NAR, Lee LK. Sacha Inchi (*Plukenetia volubilis* L.): recent insight on phytochemistry, pharmacology, organoleptic, safety and toxicity perspectives. Heliyon. 2022;8(9):e10572.

[21] Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002;56(8):365-379.

[22] DiNicolantonio JJ, O'Keefe J. The importance of maintaining a low omega-6/omega-3 ratio for reducing the risk of autoimmune diseases, asthma, and allergies. Mo Med. 2021;118(5):453-459.

[23] Simopoulos AP. The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. Asia Pac J Clin Nutr. 2008;17(Suppl 1):131-134.

[24] Gutiérrez LF, Rosada LM, Jiménez Á. Chemical composition of Sacha Inchi (*Plukenetia volubilis* L.) seeds and characteristics of their lipid fraction. Grasas Aceites. 2011;62(1):76-83.

[25] Maurer NE, Hatta-Sakoda B, Pascual-Chagman G, Rodriguez-Saona LE. Characterization and authentication of a novel vegetable source of omega-3 fatty acids, sacha inchi (*Plukenetia volubilis* L.) oil. Food Chem. 2012;134(2):1173-1180.

[26] Goyal A, Tanwar B, Kumar Sihag M, Sharma V. Sacha inchi (*Plukenetia volubilis* L.): An emerging source of nutrients, omega-3 fatty acid and phytochemicals. Food Chem. 2022;373:131459.

[27] Cisneros FH, Paredes D, Arana A, Cisneros-Zevallos L. Chemical composition, oxidative stability and antioxidant capacity of oil extracted from roasted seeds of Sacha-inchi (*Plukenetia volubilis* L.). J Agric Food Chem. 2014;62(22):5191-5197.

[28] Fanali C, Dugo L, Cacciola F, Beccaria M, Grasso S, Dacha M, et al. Chemical characterization of Sacha inchi (*Plukenetia volubilis* L.) oil. J Agric Food Chem. 2011;59(24):13043-13049.

[29] Liu Q, Xu Y-K, Zhang P, Na Z, Tang T, Shi Y. Chemical composition and oxidative evolution of Sacha Inchi (*Plukentia volubilis* L.) oil from Xishuangbanna (China). Grasas Aceites. 2014;65(1):e012.

[30] Chasquibol NA, del Aguila C, Yácono JC, Guinda Á, Moreda W, Gómez-Coca RB, et al. Characterization of glyceridic and unsaponifiable compounds of Sacha Inchi (*Plukenetia huayllabambana* L.) oils. J Agric Food Chem. 2014;62(41):10162-10169.

[31] Zanqui AB, da Silva CM, de Morais DR, Santos JM, Ribeiro SAO, Eberlin MN, et al. Sacha inchi (*Plukenetia volubilis* L.) oil composition varies with changes in temperature and pressure in subcritical extraction with n-propane. Ind Crops Prod. 2016;87:64-70.

[32] Xuan TD, Gangqiang G, Minh TN, Quy TN, Khanh TD. An overview of chemical profiles, antioxidant and antimicrobial activities of commercial vegetable edible oils marketed in Japan. Foods. 2018;7(2): 21.

[33] Brogden RN, Todd PA, Sorkin EM. Captopril. An update of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension and congestive heart failure. Drugs. 1988;36(5):540-600.

[34] Inchan A, Pathomwichaiwat T, Bualeong T, Tipratchadaporn S, Chootip K. Anti-hypotensive effect of "Yahom Navakot" in rats with orthostatic hypotension. J Tradit Complement Med. 2022;12(2):180-189.

[35] Ribeiro MO, Antunes E, de Nucci G, Lovisolo SM, Zatz R. Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. J Hypertens. 1992; 20(3):298-303.

[36] Bernátová I, Pechánová Og, Kristek F. Mechanism of structural remodelling of the rat aorta during long-term NG-nitro-L-arginine methyl ester treatment. J Pharmacol. 1999; 81(1):99-106.

[37] Maneesai P, Prasarttong P, Bunbupha S, Kukongviriyapan U, Kukongviriyapan V, Tangsucharit P, et al. Synergistic antihypertensive effect of *Carthamus tinctorius* L. extract and captopril in L-NAME-induced hypertensive rats via restoration of eNOS and AT1R expression. Nutrients. 2016;8(3):122.

[38] Paulis L, Zicha J, Kunes J, Hojna S, Behuliak M, Celec P, et al. Regression of L-NAME-induced hypertension: the role of nitric oxide and endothelium-derived constricting factor. Hypertens Res. 2008; 31(4):793-803.

[39] Sonoda K, Ohtake K, Uchida H, Ito J, Uchida M, Natsume H, et al. Dietary nitrite supplementation attenuates cardiac remodeling in l-NAME-induced hypertensive rats. Nitric Oxide. 2017;67:1-9.

[40] Holécyová A, Török J, Bernátová I, Pechánová O. Restriction of nitric oxide rather than elevated blood pressure is responsible for alterations of vascular responses in nitric oxide-deficient hypertension. Physiol Res. 1996;45(4):317-321.

[41] Aekthammarat D, Pannangpetch P, Tangsucharit P. Moringa oleifera leaf extract lowers high blood pressure by alleviating vascular dysfunction and decreasing oxidative stress in L-NAME hypertensive rats. Phytomedicine. 2019;54:9-16.

[42] Hale TM, Robertson SJ, Burns KD, deBlois D. Short-term ACE inhibition confers long-term protection against target organ damage. Hypertens Res. 2012;35(6):604-610.

[43] Ono Y, Ono H, Matsuoka H, Fujimori T, Frohlich ED. Apoptosis, coronary arterial remodeling, and myocardial infarction after nitric oxide inhibition in SHR. J Hypertens. 1999;34(4 Pt 1):609-616.

[44] Tomita H, Egashira K, Kubo-Inoue M, Usui M, Koyanagi M, Shimokawa H, et al. Inhibition of NO synthesis induces inflammatory changes and monocyte chemoattractant protein-1 expression in rat hearts and vessels. Arterioscler Thromb Vasc Biol. 1998;18(9): 1456-1464.

[45] Miguel-Carrasco JL, Mate A, Monserrat MT, Arias JL, Aramburu O, Vázquez CM. The role of inflammatory markers in the cardioprotective effect of L-carnitine in L-NAME-induced hypertension. Am J Hypertens. 2008;21(11):1231-1237.

[46] Koyanagi M, Egashira K, Kitamoto S, Ni W, Shimokawa H, Takeya M, et al. Role of monocyte chemoattractant protein-1 in cardiovascular remodeling induced by chronic blockade of nitric oxide synthesis. Circulation. 2000;102(18):2243-2248.

[47] Gross V, Obst M, Kiss E, Janke J, Mazak I, Shagdarsuren E, et al. Cardiac hypertrophy and fibrosis in chronic L-NAME-treated AT2 receptor-deficient mice. J Hypertens. 2004;22(5):997-1005.

[48] Pecháňová O, Bernatova I, Pelouch V, Babal P. L-NAME-induced protein remodeling and fibrosis. Physiol Res. 1999;48:353-362.

[49] Nemtsova V, Vischer AS, Burkard T. Hypertensive heart disease: A narrative review series—Part 1: Pathophysiology and microstructural changes. J Clin Med. 2023;12(7):2606.

[50] Akashiba A, Ono H, Ono Y, Ishimitsu T, Matsuoka H. Valsartan improves L-NAME-exacerbated cardiac fibrosis with TGF-ß inhibition and apoptosis induction in spontaneously hypertensive rats. J Cardiol. 2008;52(3):239-246.

[51] Boonprom P, Boonla O, Chayaburakul K, Welbat JU, Pannangpetch P, Kukongviriyapan U, et al. *Garcinia mangostana* pericarp extract protects against oxidative stress and cardiovascular remodeling via suppression of p47(phox) and iNOS in nitric oxide deficient rats. Ann Anat. 2017;212:27-36.

[52] Djuricic I, Calder PC. Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: An update for 2021. Nutrients. 2021;13(7):2421.

[53] Hajizadeh-Sharafabad F, Sharifi Zahabi E. Role of alpha-lipoic acid in vascular function: A systematic review of human intervention studies. Crit Rev Food Sci Nutr. 2022;62(11):2928-2941.

[54] Wang S, Zhu F, Kakuda Y. Sacha inchi (*Plukenetia volubilis* L.): Nutritional composition, biological activity, and uses. Food Chem. 2018;265:316-328.

[55] Chirinos R, Pedreschi R, Campos D. Enzyme-assisted hydrolysates from sacha inchi (*Plukenetia volubilis*) protein with in vitro antioxidant and antihypertensive properties. J Food Process Preserv. 2020; 44(12):e14969.

[56] Kodahl N, Sørensen M. Sacha Inchi (*Plukenetia volubilis* L.) is an underutilized crop with a great potential. Agron. 2021; 11(6):1066.

[57] Gonzales GF, Gonzales C. A randomized, double-blind placebo-controlled study on acceptability, safety and efficacy of oral administration of sacha inchi oil (*Plukenetia volubilis* L.) in adult human subjects. Food Chem Toxicol. 2014;65:168-176.