

Oral Administration of *Garcinia dulcis* Flower Extract Lowers Arterial Blood Pressure of 2-kidneys-1-clip Renovascular Hypertensive Rat

(Pemberian Oral Ekstrak Bunga *Garcinia dulcis* Menurunkan Tekanan Darah Arteri daripada 2-buah pinggang-1-klip Tikus Hipertensi Renovaskular)

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Received: 4 November 2020/Accepted: 12 August 2021

ABSTRACT

The hexane insoluble fraction of the *Garcinia dulcis* (GD) flower extract comprises mainly camboginol and morelloflavone which possess potent *in vivo* and *in vitro* antioxidant properties. This study aimed to evaluate the effects of 4-week oral administration of GD flower extract on the arterial blood pressure (ABP) and the excretory function of the kidney in the 2-kidneys-1-clip (2K1C) renovascular hypertensive rats (total=12) compared to sham operated (SO) normotensive Wistar rats (total=12). Four weeks after hypertensive-induced surgery, either 50 mg/kg BW GD flower extract or vehicle was orally administered to the 2K1C or SO groups (n=6/group) daily for four weeks. ABP and the renal excretory function were studied in anesthetized rats, and expression of endothelial nitric oxide synthase (eNOS) mRNA in the isolated thoracic aorta were measured. In the 2K1C rats, GD flower extract significantly decreased ABP while increased significantly eNOS mRNA levels. GD flower extract did not exert a diuretic effect in either SO and 2K1C rats since there was no change in observed urine excretion, but it did tend to attenuated the renal tubular damage caused by renovascular hypertension. GD flower extract was anti-hypertensive in this model of renovascular hypertension and probably acts via the endothelial nitric oxide signaling pathway.

Keywords: Anti-hypertension; camboginol; *Garcinia dulcis*; morelloflavone; renovascular hypertension

ABSTRAK

Pecahan tidak larut heksana daripada ekstrak bunga *Garcinia dulcis* (GD) mengandungi kamboginol dan morelloflavon yang mempunyai sifat antioksidan *in vivo* dan *in vitro* yang kuat. Kajian ini bertujuan untuk menilai kesan pemberian secara oral ekstrak bunga GD selama 4 minggu pada tekanan darah arteri (ABP) dan fungsi perkumuhan buah pinggang pada tikus hipertensi renovaskular 2-buah pinggang-1-klip (2K1C) (jumlah= 12) berbanding tikus Wistar normotensif kendalian palsu (SO) (jumlah=12). Empat minggu selepas pembedahan akibat hipertensi, sama ada 50 mg/kg BW ekstrak bunga GD atau wahana diberikan secara oral kepada kumpulan 2K1C atau SO (n=6/kumpulan) setiap hari selama empat minggu. ABP dan fungsi perkumuhan buah pinggang telah dikaji pada tikus yang telah dibius dan pengekspresan mRNA nitrik oksida sintase (eNOS) endotelium dalam aorta toraks terpencil diukur. Pada tikus 2K1C, ekstrak bunga GD menurunkan ABP dengan ketara manakala paras mRNA eNOS meningkat dengan ketara. Ekstrak bunga GD tidak memberikan kesan diuretik sama ada pada tikus SO dan 2K1C kerana tiada perubahan dalam perkumuhan air kencing yang diperhatikan, tetapi ia cenderung untuk melemahkan kerosakan tiub buah pinggang yang disebabkan oleh hipertensi renovaskular. Ekstrak bunga GD adalah anti-hipertensi dalam model hipertensi renovaskular ini dan berkemungkinan bertindak melalui laluan isyarat nitrik oksida endotelium.

Kata kunci: Anti-hipertensi; *Garcinia dulcis*; hipertensi renovaskular; kamboginol; morelloflavon

INTRODUCTION

Renovascular hypertension (RVH) is common cause of secondary hypertension characterized by a reduction of renal blood flow (RBF). The animal model of 2-kidneys-1-clip (2K1C) is the optimal model for comparing to human RVH and involves restricting renal blood flow by placing a U-shape clip on one side of the renal artery leading to a permanent reduction in RBF in one kidney (Goldblatt et al. 1934). The induced hypertension is dependent upon activation of the renin-angiotensin-aldosterone system (RAAS), which plays a crucial role in the regulation of cardiovascular and renal fluid and electrolyte homeostasis. The reduced RBF stimulates an increase in renin production and release from the juxtaglomerular cells of the clipped kidney leading to increased renin-induced cleaving of angiotensin I from angiotensinogen and increased conversion to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II is a potent vasoconstrictor that increases arterial blood pressure (ABP) and stimulates the production and release of aldosterone from the zona glomerulosa of the adrenal cortex.

Intensive efforts have been conducted on medical plants with anti-hypertensive potential because conventional anti-hypertensive drugs are usually associated with a wide range of side effects. *Garcinia dulcis* (GD) is a plant in the Guttiferae family that is widely distributed in Thailand and other Southeast Asian countries. It has been reported that the GD contains at least four groups of chemical compounds including flavonoids, benzophenone, xanthenes, and benzophenone-xanthone (Daechathai et al. 2008, 2006, 2005; Mahabusarakam et al. 2016). Those compounds can be found in all parts of the GD including flower, leave, fruit, root, stem, and bark; however, the type and concentration of the chemical constituents depend on which part of the plant for extraction and purification. A previous study reports that the major chemical constituents in the hexane insoluble fraction of the acetone extract of GD flowers were camboginol and morelloflavone (Daechathai et al. 2006).

Camboginol (also called garcinol) is a plant benzophenone which exerts a wide range of physiological activities including free-radical scavenging (Yamaguchi et al. 2000a; 2000b), anti-inflammation (Hong et al. 2006; Kim et al. 2008; Liao et al. 2004), anti-cancer (Tanaka et al. 2000), anti-ulcer (Das et al. 1997), and anti-HIV (Balasubramanyam et al. 2004). Morelloflavone is the biflavonoids comprising two covalently-linked flavones,

apigenin and luteolin. Morelloflavone has anti-oxidant (Hutadilok-Towatana et al. 2007), anti-inflammatory (Gil et al. 1997), anti-atherosclerotic (Pinkaw et al. 2012), anti-cancer (Pang et al. 2009), anti-HIV (Lin et al. 1997), and hypo-cholesterolemic activity (Tuansulong et al. 2011). Studies in our laboratory have shown that the intravenous administration of either camboginol or morelloflavone extracted from the fruits of GD exerted diuretic and hypotensive effects in anesthetized normotensive and hypertensive rats (Thongsepee et al. 2018, 2017a, 2017b). Work on isolated thoracic aortas showed the vasorelaxant action of camboginol and morelloflavone in both normotensive and hypertensive rats; the proposed mechanism is increased activation of endothelial nitric oxide synthetase (eNOS) leading to increased production of the vasodilator, nitric oxide (NO) (Lamai et al. 2013; Thongsepee et al. 2017a, 2017b).

Our study aimed to evaluate the effects of orally administered GD flower extract on ABP, renal excretory function, and expression of vascular eNOS mRNA in 2K1C hypertensive compared to sham operated (SO) normotensive rats.

MATERIALS AND METHODS

THE GD FLOWER EXTRACT PREPARATION

The extraction and purification of the GD flower followed the method of Daechathai et al. (2006). This study used the hexane insoluble fraction composes mainly of camboginol and morelloflavone; their chemical structures and molecular weights are shown in Figure 1. The GD fraction was first dissolved in a small amount of dimethyl sulfoxide (0.3% DMSO; Sigma-Aldrich, Darmstadt, Germany) and then dissolved in corn oil (Mazola, Bangkok, Thailand) for oral gavage to the rats at the dose of 50 mg/kg BW, daily.

EXPERIMENTAL ANIMALS

Twenty-four male, six weeks old Wistar rats were purchased from the Siam Nomura International Co. Ltd. They were housed at the Animal Laboratory Center of Thammasat University and maintained under standard conditions; room temperature $22\pm 1^\circ\text{C}$, relative humidity 30-70%, light intensity 130-325 Lux, 12/12 dark-light cycle and were fed with a commercial pellet food and reverse osmosis water *ad libitum*. Rats were acclimatized for a week before experimentation. All experimental protocols adhered to NIH Guiding Principles in the Care

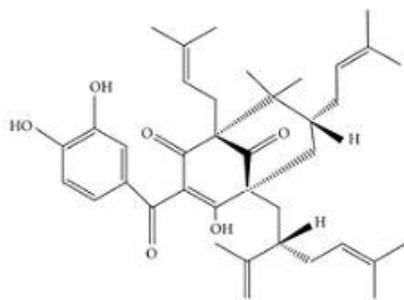
and Use of Animals and were approved by the Thammasat University Animal Care and Use Committee under Protocol No. 031/2018.

INDUCTIVE OPERATION OF 2K1C AND SO RATS

Rats were randomly assigned into four groups; two SO and two 2K1C groups (n=6 each). Each rat was

anesthetized with 5% isoflurane supplied with oxygen (O₂) at 4 liters per minute (L/min) for one minute. Then, the anesthetized rat was placed on an electrical temperature control pad and anesthesia maintained with 0.9-2% isoflurane and 0.9 L/min of O₂ using a facemask throughout the operation. The left kidney was exposed through a 1 cm retroperitoneal incision, the left renal

A) Camboginol



B) Morelloflavone

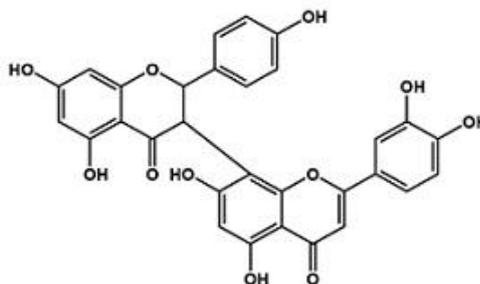


FIGURE 1. The chemical structure of camboginol (C₃₈H₅₀O₆, MW 602, A) and morelloflavone (C₃₀H₂₀O₁₁, MW 556, B) from the *Garcinia dulcis* flower extract as determined by the thin layer chromatography

artery was exposed and then cleared from the surrounding connective tissues. A U-shaped silver clip with a 0.20 mm gap was placed around the left renal artery close to the junction with the abdominal aorta. Then, the muscle and skin layer were sutured separately with 4/0 catgut. The SO included the entire surgery except for renal artery clipping. The rats recovered in separate cages for 30 minutes. Thereafter, rats were given subcutaneous carprofen (5 mg/kg BW) for pain relief once a day for 3 days and convalesced over one month to allow the development of hypertension. The body weights of all rats were recorded every week.

MEASUREMENT OF SERUM ANGIOTENSIN II LEVELS

Blood samples were collected before and 4 weeks after the inductive operation from the ventral artery and then centrifuged at 4,000 RPM for 10 minutes to obtain serum. Serum concentrations of angiotensin II were measured by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Sigma Aldrich, MO, USA) to ascertain whether hypertension had developed.

ORAL ADMINISTRATION OF GD FLOWER EXTRACT

Four weeks after the operation, the 2K1C or SO rats were further divided into 2 groups based on the orally gavage treatment; corn oil (as vehicle) or GD flower extract. Consequently, there are 4 groups of rats: I) SO + vehicle (SO as a control group), II) SO + GD, III) 2K1C + vehicle (2K1C), and IV) 2K1C + GD, n=6 per group. Each rat was orally administered with either vehicle or GD flower extract once a day for 4 weeks. The dose of oral gavage was 50 mg/kg BW given in a volume of 2.5 mL/kg BW. This dose of GD flower extract was applied based on our previous study which found that the oral administration of the GD flower extract at the doses of 50 and 100 mg/kg BW daily for two weeks significantly decreased the ABP in the normotensive rats (Thongsepee et al. 2020). The BW was recorded weekly.

MEASURING ARTERIAL BLOOD PRESSURE (ABP) AND RENAL CLEARANCE

Four weeks after oral administration, rats were anesthetized again, as described earlier. The left carotid

artery was cannulated for blood sampling and record continuously ABP using the PowerLab system (model 26T, ADInstruments, New South Wales, Australia). The urinary bladder was cannulated for urine sampling. The right jugular vein was cannulated to infuse the renal clearance markers, 1% PAH and 1% inulin (Sigma-Aldrich, Darmstadt, Germany) in 0.9% sodium chloride (NaCl) solution at a rate of 16 mL/min/kg BW.

The total experimental time was 150 min, 30 min of incubation and 120 min of renal clearance study during which urine was collected in four samples of 30 min (U_1 to U_4). 1 mL of arterial blood was collected (heparinized tube) at the end of U_1 and U_3 ; this blood loss was replaced with 0.9% NaCl. A small amount of this blood was used to measure the hematocrit and the remaining sample centrifuged at 4,000 RPM for 10 min for plasma collection.

Each urine sample was collected in pre-weighed tubes and the urine flow rate (V) was determined gravimetrically by assuming a density of 1 g/mL. The plasma and urine samples were kept at -20 °C for analysis of the PAH and the inulin levels using spectrophotometry following the methods of Smith et al. (1945) and Davidson and Sackner (1963), respectively. The plasma and urine osmolality (P_{Osm} and U_{Osm}) were also measured by using a Micro-Osmometer (model Osmomat 030D, Genetec, Berlin, Germany). The sodium (Na^+) and potassium (K^+) concentration in the plasma and urine samples were measured using the FUJI DRY-CHEM analyzer (Fujifilm FDC NX500, Tokyo, Japan). At the end of experimentation, the weights of both kidneys, heart, and liver were recorded.

SEMIQUANTITATIVE RT-PCR FOR EVALUATION OF eNOS EXPRESSION

The expression of eNOS in the thoracic aorta was evaluated by semiquantitative reverse-transcription PCR (RT-PCR). The total RNA of each excised thoracic aorta was extracted by TRIzol™ reagent (Invitrogen™, ThermoFisher Scientific, Carlsbad, CA, USA) according to the instruction manual. Total RNA concentration was measured by NanoDrop™-2000 spectrophotometer (ThermoFisher Scientific, Wilmington, DE, USA), and contaminated genomic DNA was removed by treating with DNase-I, RNase-free (ThermoFisher Scientific, Lithuania) at 37 °C for 30 min. One hundred nanograms of each DNase-I-treated RNA sample was then subjected to perform semiquantitative RT-PCR by using SensiFAST™ SYBR® No-ROX One-Step Kit

(Bioline, UK) following the recommendations but with some modifications in the Applied Biosystems StepOne Real-Time PCR System (Step-one, Applied Biosystems, Massachusetts, USA). GAPDH was used as an internal control and baseline of mRNA expression for foldchange comparison. The specific primers were included eNOS (F) 5'-ATTGCACCCTTCCGGGGATT-3', eNOS (R) 5'-ACGGTTTGCAGGACGCTGGTT-3', GAPDH (F) 5'-TGAAGGTCGGTGTCAACGGATTGGC-3', and GAPDH (R) 5'-CATGTAGGCCATGAGGTCCACCAC-3'. All samples were reverse transcribed at 45 °C for 10 min, polymerase activation at 95 °C for 10 min, and 40 cycles of denaturation at 95 °C for 5 s, annealing at 55 °C for 10 s, and extension at 72 °C for 5 s. Amplifications were performed in duplicate, three-independence. Gene expression fold change of the gene expression levels was performed using the $2^{-\Delta\Delta CT}$ method (Schmittgen & Livak 2008).

CALCULATION

Systolic blood pressure (SBP, mm Hg) and diastolic blood pressure (DBP, mm Hg) were determined at the mid-point of U_1 to U_4 periods. Mean arterial pressure (MAP, mm Hg), pulse pressure (PP, mm Hg), and renal vascular resistance [RVR, resistance unit (RU)] were computed using the following equations: $MAP = DBP + 1/3 PP$, $PP = SBP - DBP$, and $RVR = MAP \div ERPF$. The values during U_1 to U_4 periods were averaged.

The excretory functions of the kidneys were calculated using the following equations: $CX = (\dot{V} \times UX) \div PX$, $TCH_2O = C_{Osm} - \dot{V}$, Excretion rate of X = $UX \times \dot{V}$, $FE_x = (UX \div Px) \div$ total load of X, where, CX = clearance of X (mL/min/g KW), TCH_2O = negative free water clearance (mL/min/g KW), U_x = concentration of X in urine (mg/mL or mmol/L or mOsm/kg H_2O), P_x = concentration of X in plasma (mg/mL or mmol/L or mOsm/kg H_2O), FE_x = fractional excretion of X (%), X = inulin, PAH, Na^+ , K^+ or osmolality. The values were normalized by kidney weight (KW) and the values from U_1 to U_4 periods were averaged.

STATISTICAL ANALYSIS

Data were presented as mean \pm standard error of the mean (S.E.M.). Comparisons between the means values within-group and among groups of the SO and the 2K1C were performed with one-way analysis of variance followed by multiple t-test using GraphPad Prism 8 (San Diego, CA, USA). A p-value of less than 0.05 was considered a significant difference.

RESULTS

THE LEVELS OF SERUM ANGIOTENSIN II

The serum angiotensin II concentrations in the 2K1C rats at 4 weeks after the inductive operation increased by 4.75-fold compared with the level before the operation. While the levels of plasma angiotensin II of SO rats were unaltered (Figure 2).

EFFECTS OF THE GD FLOWER EXTRACT ON ABP AND RVR

The SBP, DBP, PP, MAP, and RVR in the group of 2K1C were significantly higher in comparison to the respective values in the SO group: SBP: 142±18 vs. 98±2, DBP: 77±13 vs. 49±3, PP: 65±5 vs. 49±2, MAP: 99±15 vs. 65±2 mm Hg, and RVR: 27±5 vs. 16±0.5 RU, $p < 0.05$.

HR in the 2K1C group did not differ significantly from the SO group.

The GD flower extract group significantly decreased the SBP, DBP, PP, and MAP in the group of 2K1C+GD compared to the 2K1C group: SBP: 99±6 vs. 142±18, DBP: 51±4 vs. 77±13, PP: 49±3 vs. 65±5, and MAP: 67±4 vs. 99±15 mm Hg, $p < 0.05$. The ABP and the RVR in the group of SO+GD did not change in comparison to the group of SO (Figure 3).

EFFECT OF GD FLOWER EXTRACT ON THE RENAL FUNCTIONS

The mean \dot{V} , C_{Osm} , and TCH_2O in the 2K1C group were significantly higher than those of the SO group: \dot{V} : 19.42±3.77 vs. 10.85±1.02, C_{Osm} : 63.36±5.75 vs. 37.82±2.10, and TCH_2O : 40.58±6.48 vs. 26.97±1.93 $\mu\text{L}/$

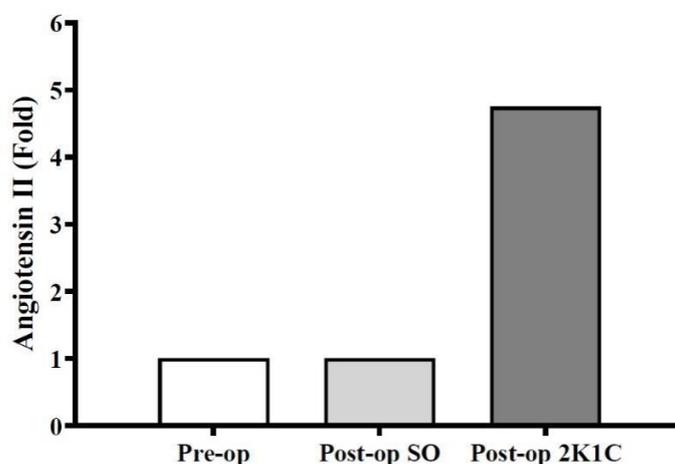


FIGURE 2. The fold change of the mean serum angiotensin II levels at 4 weeks after either the sham (Post-op SO, n=10) or the 2-kidneys-1-clip (post-op 2K1C, n=10) operation compared to the mean preoperative angiotensin II level (Pre-op, n=20)

min/g KW, $p < 0.05$. The mean P_{Osm} in the 2K1C was significantly lower than that of the SO group: 265±2 vs. 285±5 mOsm/kg H_2O ($p < 0.05$, Figure 4). The mean P_{Na} and P_K levels were significantly decreased in the group of 2K1C compared to those of the SO group: Na^+ : 120.3±4.4 vs. 137.9±1.1 and K^+ : 2.62±0.17 vs. 3.05±0.10 mmol/L, $p < 0.05$. The mean U_{Na} , FE_{Na} and FE_K levels in the 2K1C group were significantly higher than those of the SO group: U_{Na} : 2.85±0.19 vs. 1.79±0.26 mmol/min/g KW, FE_{Na} : 2.37±0.26 vs. 1.40±0.22%, and FE_K : 61.82±6.06 vs. 41.21 ± 3.03%, $p < 0.05$ (Figure 5).

GD flower extract did not affect the levels of C_{PAH} , C_{In} , \dot{V} , TCH_2O , P_{Osm} , U_{Osm} , and hematocrit in the 2K1C+GD group in comparison with the 2K1C group but decreased significantly ($p < 0.05$) the mean C_{Osm} levels vs. the 2K1C group: 48.08±2.35 vs. 63.36±5.75 $\mu\text{L}/\text{min}/\text{g}$ KW. Similarly, GD flower extract did not alter the levels of C_{PAH} , C_{In} , \dot{V} , C_{Osm} , TCH_2O , P_{Osm} , U_{Osm} , and hematocrit in the SO+GD vs. SO group (Figure 4). The P_{Na} levels in the 2K1C+GD group remained in the normal range and were significantly higher compared to the 2K1C group: 139.8±0.5 vs. 120.3±4.4 mmol/L ($p < 0.05$, Figure 5).

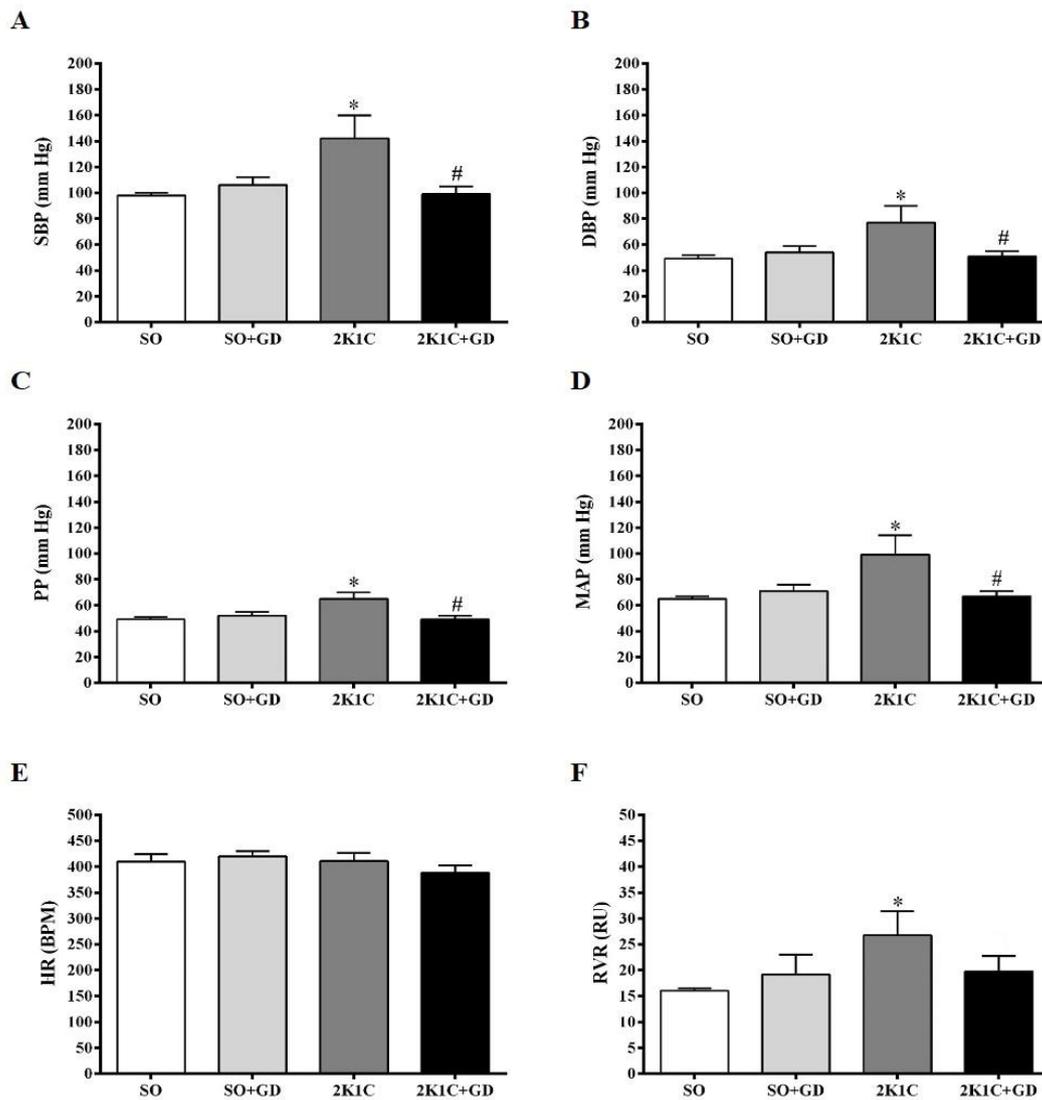


FIGURE 3. The effect of 50 mg/kg BW *Garcinia dulcis* (GD) flower extract on systolic blood pressure (SBP, A), diastolic blood pressure (DBP, B), pulse pressure (PP, C), mean arterial pressure (MAP, D), heart rate (HR, E), and renal vascular resistance (RVR, F) in the sham operation (SO) and the 2-kidneys-1-clip (2K1C) hypertensive rats. *,# $p < 0.05$ in comparison to SO and 2K1C, respectively

CHANGES IN BODY AND ORGAN WEIGHTS

The body weights at the beginning of the experiment, 4 weeks after the inductive operation, and 4 weeks after oral administration of the GD flower extract of both two SO and two 2K1C groups were not significantly different (Table 1). The mean weight of left clipped kidneys was significantly decreased while the right non-clipped

kidneys weight was significantly increased in both 2K1C groups compared to the SO group. The cardiac mass of the 2K1C group was significantly increased in comparison to the SO group. The cardiac mass in the group of 2K1C+GD was significantly decreased compared with the group of 2K1C. There was no change in liver weight among the four groups.

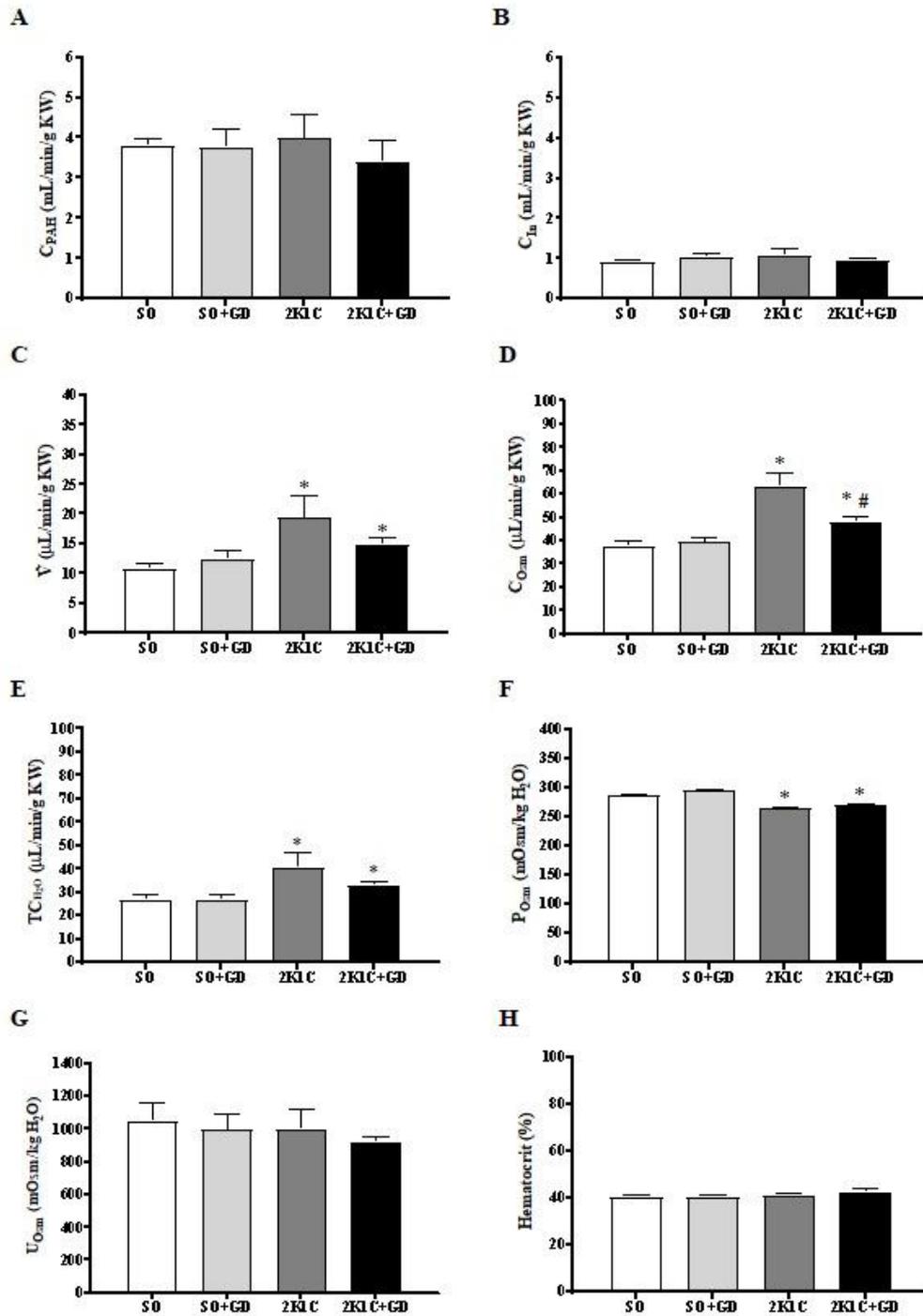


FIGURE 4. The effect of 50 mg/kg BW *Garcinia dulcis* (GD) flower extract on clearances of para-amino hippuric acid (C_{PAH} , A) and inulin (C_{In} , B), urine flow rate (V, C), osmolar clearance (C_{Osm} , D), negative free water clearance (TC_{H_2O} , E), plasma osmolarity (P_{Osm} , F), urine osmolarity (U_{Osm} , G), and hematocrit (H) in the sham operation (SO) and the 2-kidneys-1-clip (2K1C) hypertensive rats. *,# $p < 0.05$ in comparison to SO and 2K1C, respectively

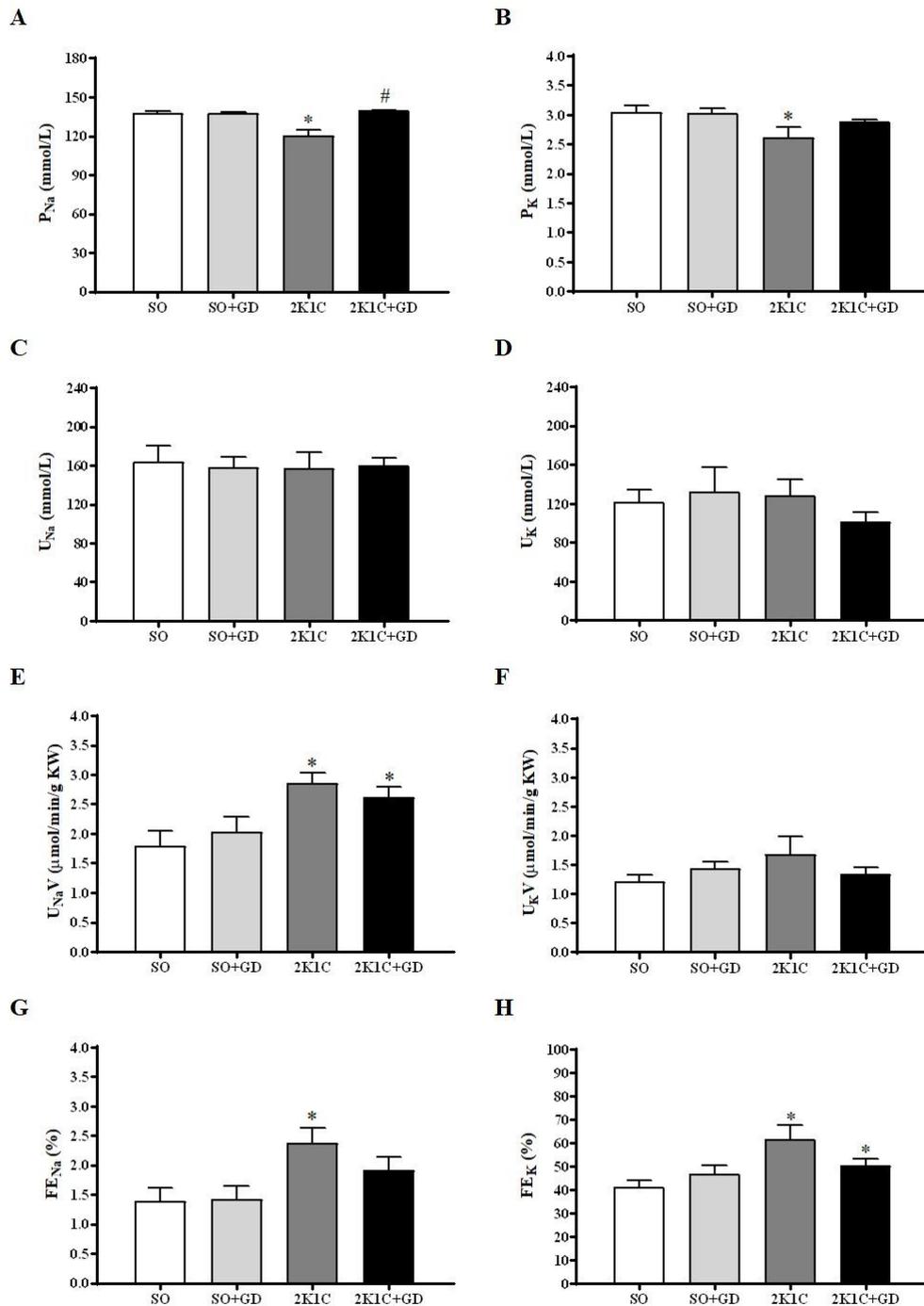


FIGURE 5. The effect of 50 mg/kg BW *Garcinia dulcis* (GD) flower extract on the levels of plasma sodium (P_{Na} , A), plasma potassium (P_K , B), urinary sodium (U_{Na} , C), urinary potassium (U_K , D), sodium excretion rate ($U_{Na}V$, E), potassium excretion rate (U_KV , F), fractional excretion of sodium (FE_{Na} , G), and fractional excretion of potassium (FE_K , H) in the sham operation (SO) and the 2-kidneys-1-clip (2K1C) hypertensive rats. * $p < 0.05$ in comparison to SO

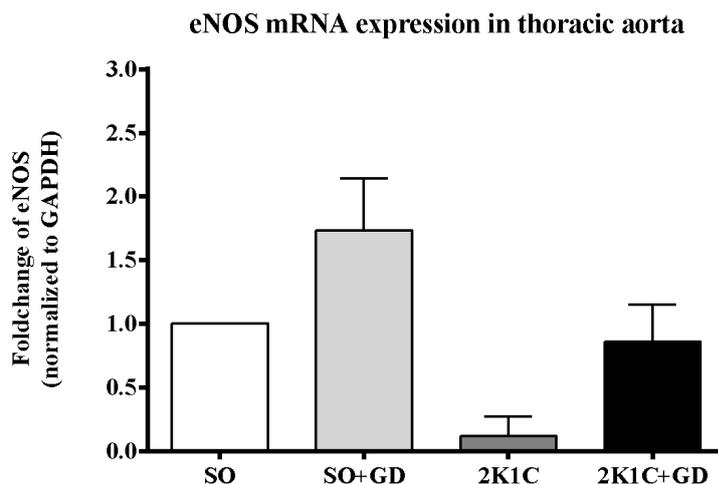


FIGURE 6. The mRNA expression levels of the endothelial nitric oxide synthase (eNOS) normalized to GAPDH in the thoracic aortas of the sham operation (SO) and the 2-kidneys-1-clip (2K1C) hypertensive rats after administration of either vehicle or of 50 mg/kg BW of *Garcinia dulcis* (GD) flower extract. GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

EFFECT OF GD FLOWER EXTRACT ON THE LEVELS OF eNOS

The expression of eNOS mRNA in the thoracic aorta of the 2K1C group was 8.3-fold lower vs. the SO group.

After treatment with the GD flower extract, the levels of eNOS mRNA in the group of SO+GD and 2K1C+GD were 1.73-fold higher and 1.16-fold lower vs. SO group, respectively.

TABLE 1. Body weight and organ weights of the Wistar rats

Descriptive data	SO	SO + GD	2K1C	2K1C + GD
Body weight (BW)				
Before inductive surgery (g)	189±4	192±5	193±6	188±4
4 weeks after inductive surgery (g)	347±12	359±16	364±15	358±8
4 weeks after treatment (g)	401±7	417±12	430±21	407±15
Left kidney weight (g% BW)	0.29±0.01	0.29±0.01	0.25±0.01*	0.26±0.01*
Right kidney weight (g% BW)	0.29±0.01	0.30±0.01	0.33±0.01*	0.36±0.02*
Cardiac mass (g% BW)	0.25±0.01	0.24±0.01	0.31±0.02*	0.26±0.03#
Liver (g% BW)	3.17±0.05	3.25±0.12	3.20±0.15	3.08±0.10

Data expressed as mean ± S.E.M. *p < 0.05 in comparison to SO. #p < 0.05 in comparison to 2K1C

DISCUSSION

The induction of RVH in the rat by unilateral renal artery stenosis was successful because the SBP, DBP, PP, MAP, and RVR in the group of 2K1C were all significantly higher than those of the SO group and serum levels of angiotensin II in the 2K1C rats increased 4.75-folds compared with preoperative values, suggesting increased activation of the RAAS during this period. An increase of circulating angiotensin II levels during the 4 weeks of the renal artery stenosis has also been reported by Lu et al. (2010). Moreover, the left clipped kidney weights of 2K1C rats decreased significantly while those of the right non-clipped kidney weights significantly increased compared with their respective ipsilateral kidneys of SO rats. Changes in the kidney weights were due to a substantial in RBF to the left side and compensatory response to maintain the renal function of the right side. The cardiac mass of the 2K1C groups also increased significantly (vs. SO groups), suggesting increased cardiac afterload in response to the raised total peripheral resistance (TPR) by angiotensin II-induced peripheral vasoconstriction. Moreover, high level of angiotensin II also promoted cardiac hypertrophy via its trophic effect (Mazzolai et al. 2000). All group of animals did not show significant changes in their body weight as previously reported (Thongsepee et al. 2018, 2017a, 2017b).

The anti-hypertensive effect of the GD flower extract was observed after 4 weeks of oral administration in the 2K1C but not SO groups. SBP, DBP, PP, and MAP were all significantly lower in the 2K1C+GD group compared to the 2K1C group and the cardiac mass in the 2K1C+GD group was significantly less vs. the 2K1C group, indicating a decrease in TPR. Endothelial dysfunction probably occurred in the 2K1C rats since the eNOS mRNA expression decreased markedly (8.3-fold) compared to the SO group, consistent with our previous immunohistological study which showed the lower eNOS expression in the thoracic aorta of the 2K1C rats (Thongsepee et al. 2018). Previous studies in hypertensive rat showed significantly reduced eNOS expression in the dysfunctional arteries (Sánchez et al. 2006; Ulker et al. 2003). Moreover, we found that the GD flower extract (2K1C+GD group) could restore the eNOS mRNA levels, resulting in a more modest fold expression of ~10% reduction in vs. SO group and increases in eNOS expression vs. 2K1C group and SO group vs. SO+GD group, suggesting strongly that GD's anti-hypertensive effect is mediated via endothelial NO signaling pathway.

The renal function studies in the 2K1C rat showed the ERPF and the GRF were preserved, levels of V, C_{Osm} ,

TC_{H_2O} , U_{Na} , FE_{Na} , and FE_K increased while the P_{Osm} , P_{Na} , and P_K decreased significantly compared to those SO group. These results agree with those previously studied (Anderson et al. 1985; Martinez-Maldonado 1991; Ploth et al. 1981). The higher V in the 2K1C group may be due to abnormal tubular electrolyte and water reabsorption in response to the high circulating level of angiotensin II. Angiotensin II had biphasic effects on tubular reabsorption; low concentrations stimulate whilst high levels inhibit tubular reabsorption (Harris & Young 1997). GD flower extract did not exert a diuretic effect in either SO or 2K1C rats, since there was no change in observed urine excretion, but it did tend to attenuate the renal tubular damage caused by renovascular hypertension.

The bioactive compounds of the GD flower extract are the antioxidants camboginol and morelloflavone that could have a role in reversing the pathophysiology involved in renal hypertension. Our previous studies in 2K1C rats have found that the plasma MDA levels were significantly increased, suggesting the induction of oxidative stress (Thongsepee et al. 2017b), sensitivity of the oxidative stress-induced baroreceptor reflex (BRS) is impaired (Thongsepee et al. 2017a), and vascular eNOS expression is reduced vs. control rats (Thongsepee et al. 2018). Treatment with camboginol or morelloflavone could counter these changes. Moreover, *in vitro* work found that the camboginol and morelloflavone vasodilate the isolated thoracic aorta from the normotensive and 2K1C rats and their mechanism of action involved the endothelial NO signaling pathway (Lamai et al. 2013; Sumalee et al. 2017; Thongsepee et al. 2018, 2017a, 2017b). These data suggest that the anti-oxidant properties of camboginol and morelloflavone acting on the endothelial NO signaling pathway reverse the effects of renal hypertension and protect renal function.

CONCLUSION

Orally administered GD flower extract at 50 mg/kg BW daily for 4 weeks demonstrated anti-hypertensive properties and was renoprotection. The proposed mechanisms of action were antagonizing the reduced eNOS expression. The possible clinical role of GD flower extract as an anti-hypertensive agent should be studied further.

ACKNOWLEDGEMENTS

This research was supported by a research grant from the Faculty of Medicine, Thammasat University, Thailand (Grant No. 2-11/2562) and Research Unit in Nutraceuticals and Food Safety, Faculty of Medicine, Thammasat University, Pathumthani, Thailand.

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