

Effects of oral administration of *Garcinia dulcis* flower extract on arterial blood pressure and renal excretory functions in rats

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Received 25 Mar 2020

Accepted 25 Oct 2020

ABSTRACT: Intravenous infusion of either camboginol or morelloflavone from *Garcinia dulcis* (GD) exerted diuretic, hypotensive, and vasorelaxant effects in either normotensive or hypertensive rats. This study aims to investigate the effects of GD flower extract on arterial blood pressure (ABP) and renal excretory functions. Male Wistar rats (8-week-old) were divided into 4 groups (group I–IV, $n = 6$ each) in both acute and sub-chronic protocols. The GD extract was orally administered to group II–IV at the dose of 50, 100, or 200 mg/kg, respectively, while group I served as vehicle control. The oral administration was performed before the experiment in acute protocol and daily for 2 weeks in the sub-chronic protocol. The ABP and renal excretory functions were measured in the anesthetized rats. The levels of fasting blood glucose (FBG), plasma lipid profiles, and liver enzymes were evaluated in the sub-chronic experiment along with liver histology. The results showed that acute administration of GD extract significantly decreased ABP but increased renal blood flow, glomerular filtration rate, urine flow rate, osmolar clearance, and negative free water clearance when compared with the control. In the sub-chronic protocol, the GD extract significantly decreased ABP but did not alter the renal excretory functions. The plasma levels of FBG, lipid profiles, liver enzymes, and the histology of liver were not changed. It is concluded that acute oral administration of GD extract possessed hypotensive and diuretic effects whereas the sub-chronic treatment of GD showed hypotensive effect and no alterations in liver function, FBG, and plasma lipid profiles.

KEYWORDS: *Garcinia dulcis*, camboginol, morelloflavone, diuretic effect, hypotensive effect

INTRODUCTION

Hypertension is a chronic cardiovascular disease in which the arterial blood pressure (ABP) is elevated leading to an increase in the risks of heart, brain, kidney, and other diseases and promotes premature death. In 2019, the World Health Organization estimated 1.13 billion people worldwide have hypertension. The pathophysiological mechanisms associated with hypertension are generally understood. One of the possible causes of hypertension is an overproduction of free radicals that damage cardiovascular and renal systems in which characterized by an overactivity of the sympathetic nervous

system [1] and renin-angiotensin-aldosterone system (RAAS) [2], the inability of kidneys to excrete sodium [3], and the endothelial dysfunction [4], resulting in increased vascular resistance and fluid overload and finally raised ABP. Recently, a large number of local plants with antihypertensive therapeutic potential have been studied because of their various bioactivities [5], more safety and efficacy, cost-effectiveness, and fewer side effects. However, more scientific researches are needed to verify the effectiveness and elucidate the safety profile of such herbal remedies for their antihypertensive potential.

The tropical plant *Garcinia dulcis* (GD) Kurz,

local Thai name Ma-Phud, belongs to the *Guttiferae* family and grows mainly in the Southeast Asia region. Various parts of this plant have been used as medicine; leaves and seeds were used for lymphatitis, parotitis, and struma treatment; stem bark was used as an antiseptic agent; fruit juice was used for anti-scurvy and expectorant for the relief of cough and sore throat; and its root extract was used as an antipyretic and antitoxin agent [6–9]. The extracted substances from GD contained at least 4 groups of phenolic compounds including flavonoids, benzophenone, xanthenes, and benzophenone-xanthone dimer [6–9]. The yield of chemical constituents extracted from GD depended on the part of the plant specimens and the procedure of purification. Its phenolic compounds which have been shown to possess antibacterial and antioxidative activity including cambogin, camboginol, dulcisflavone, epicatechin, and morelloflavone from fruits [6], dulcisxanthone C-F and dulcinone from flowers [7], and dulcisxanthone G from seeds [8]. Our previous studies found that the intravenous administration of either camboginol or morelloflavone extracted from the fruits of GD exerted diuretic and hypotensive effects in either anesthetized normotensive or hypertensive rats. An experiment in isolated thoracic aorta revealed the vasorelaxant action of camboginol and morelloflavone in both normotensive and hypertensive rats, and their mechanism of action involved an endothelial nitric oxide (NO)-dependent pathway [10–13].

This study aims to evaluate the effects of acute and sub-chronic oral administration of the GD flower extract on cardiovascular parameters including arterial blood pressure (ABP) and renal excretory functions comprising renal vascular resistance (RVR), clearance of para-amino hippuric acid (C_{PAH}) and inulin (C_{In}) as markers of effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), respectively, urine flow rate (\dot{V}), osmolar clearance (C_{Osm}), negative free water clearance (TC_{H_2O}), plasma osmolarity (P_{Osm}), and urine osmolarity (U_{Osm}) in anesthetized rats. The effects of GD flower extract on the levels of fasting blood glucose (FBG), plasma lipid profiles, liver enzymes, and histology of liver were also evaluated in the sub-chronic protocol to assess its toxicity.

MATERIALS AND METHODS

Preparation of *Garcinia dulcis* flower extract

The flowers of GD were collected from Songkhla province in the southern part of Thailand. The

voucher specimen has been deposited at Prince of Songkla University Herbarium (Collection No. 02 and Herbarium No. 0012625), Faculty of Science, Prince of Songkla University, Songkhla, Thailand. The GD flowers (488 g) were extracted at room temperature sequentially with acetone for 72 h. Removal of the solvents from the extract yielded the acetone extract (85.50 g). This acetone extract was fractionated by dissolving in hexane to give a soluble (13.45 g) and insoluble (70.06 g) fractions. The hexane insoluble fraction was used in this study. To identify the chemical components, 20 mg of the tested fraction was subjected to a silica gel column chromatography and eluted with the 10% MeOH in CH_2Cl_2 . The collected fractions were chromatographed on thin-layer chromatography (TLC) using silica gel as a stationary phase and 10% MeOH in CH_2Cl_2 as a mobile phase compared with the reference compounds including camboginol and morelloflavone.

The hexane insoluble fraction of the GD flower extract was dissolved in a small amount of dimethyl sulfoxide (0.3% DMSO; Sigma-Aldrich, Darmstadt, Germany), then further dissolved in corn oil (Mazola, Bangkok, Thailand) for oral gavage at the doses of 0 (corn oil as a vehicle), 50, 100, and 200 mg/kg body weight (BW). The volume of oral gavage is 2.5 mL/kg BW.

Animals and the experimental protocol

Male Wistar rats (7-week-old, $n = 48$) were purchased for the Siam Nomura International Co. Ltd. and were transported to the Animal Laboratory Center of Thammasat University. They were housed under standard conditions: room temperature 22 ± 1 °C, relative humidity 30–70% RH, light intensity 130–325 Lux, and 12/12 dark-light cycle and were fed with commercial pellet food and a reverse osmosis water *ad libitum*.

After acclimatization for a week, the rats were randomly divided into 4 groups: I–IV ($n = 6$ each), based on the dose of the GD flower extract administration: 0 (vehicle), 50, 100, and 200 mg/kg BW, respectively, in each acute and sub-chronic protocol. The oral administration was performed immediately before the experiment in the acute protocol and daily for 2 weeks in the sub-chronic protocol. The experimental protocols were 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. 007/2018.

Arterial blood pressure (ABP) and renal excretory function study

A similar protocol of ABP and renal excretory function study was used in both acute and sub-chronic groups. On the day of the experiment, either vehicle or GD flower extract was orally administered to each rat. Immediately, a rat was put in the chamber for induction to be anesthetized with 5% isoflurane (RWD, Shenzhen, China) supplied with oxygen 4 L/min for 1 min, and then the anesthetized rat was removed from the chamber, placed on an electrical temperature control pad, and maintained the anesthetized status with 0.9–2% isoflurane supplied with oxygen 0.9 L/min on face mask throughout the experiment. The right jugular vein was cannulated for infusion of the renal clearance markers containing 1% PAH (Sigma-Aldrich, Darmstadt, Germany) and 1% inulin (Sigma-Aldrich, Darmstadt, Germany) in 0.9% saline solution at the rate of 16 mL/min/kg BW. The left carotid artery was cannulated for blood sampling and continuous ABP recording using the PowerLab system (model 26T, ADInstruments, New South Wales, Australia). The urinary bladder was cannulated for urine sampling.

After infusion of clearance marker for 30 min, 4 consecutive 30-min urine collections (U_1 to U_4) were performed during the 120 min of the experimental period. Arterial blood samples (1 mL) were taken at the end of U_1 and U_3 periods and centrifuged at 3000 rpm for 10 min for plasma collection. The plasma and urine samples were kept at -20°C until analysis of the PAH and the inulin levels using spectrophotometry [14, 15]. The \dot{V} was determined gravimetrically by collecting urine into pre-weighed tubes and assuming a density of 1 g/mL. P_{Osm} and U_{Osm} were measured by using Micro-Osmometer (model Osmomat 030D, Gonotec, Berlin, Germany).

The C_{PAH} , C_{In} , and C_{Osm} were calculated according to the clearance equation; $C_x = (\dot{V} \times U_x) / P_x$, when \dot{V} is the urine flow rate, U_x is urine concentration of X, P_x is the plasma concentration of X, and X is either of PAH, inulin, or osmolality. $\text{TC}_{\text{H}_2\text{O}}$ was calculated using equation $\text{TC}_{\text{H}_2\text{O}} = C_{\text{Osm}} - \dot{V}$. The C_{PAH} and C_{In} were taken as the indices of ERPF and GFR, respectively. The C_{Osm} and $\text{TC}_{\text{H}_2\text{O}}$ were the indices of urinary electrolyte excretion and free water reabsorption, respectively. The values of C_{PAH} , C_{In} , C_{Osm} , $\text{TC}_{\text{H}_2\text{O}}$, and \dot{V} in each rat were normalized by kidney weight (KW). Values from U_1 to U_4 periods were averaged. Mean arterial pressure (MAP) was determined at the mid-point of U_1 to U_4 periods

and calculated from diastolic blood pressure (DBP) +1/3 pulse pressure (PP). The RVR was calculated using equation $\text{RVR} = \text{MAP} / \text{ERPF}$. The MAP and RVR during U_1 to U_4 periods were also averaged.

Test of toxicity of GD flower extract in the sub-chronic protocol

The animals in this group were orally administered with either vehicle, 50, 100, or 200 mg/kg BW GD flower extract daily for 2 weeks. One night before the ABP, renal, and excretory function study, the rat was deprived of food but still allowed to access the drinking water. On the day of the experiment, the rat was anesthetized with isoflurane supplied with oxygen. Before an infusion of renal clearance markers, a small amount of blood was collected from the carotid artery catheter for measuring a level of FBG using a digital glucometer (Accu-Check, Basel, Switzerland). Then, the renal and excretory function study was performed as previously described. At the end of the experiment, 5 mL of blood was collected from the carotid artery and centrifuged at 3000 rpm for 10 min for plasma collection, and then the plasma sample was kept at -20°C until analysis for the levels of plasma lipid profiles composed of cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) and liver enzyme levels including aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) using standard technique (Health Care Service Center, Faculty of Allied Health Science, Thammasat University, Pathum Thani, Thailand). Finally, the rat was perfused with 0.1 M phosphate buffer solution, and liver tissue samples were fixed in 10% formalin for histological study by hematoxylin and eosin (H&E) staining (Laboratory of Pathology, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand).

Statistics

Data were presented as mean \pm standard error of the mean (SEM). Comparisons between the means values within group and among groups of acute and sub-chronic treatment were performed with one-way analysis of variance followed by Tukey post hoc test using GraphPad Prism 6 (San Diego, CA, USA). A p -value of less than 0.05 was considered a significant difference.

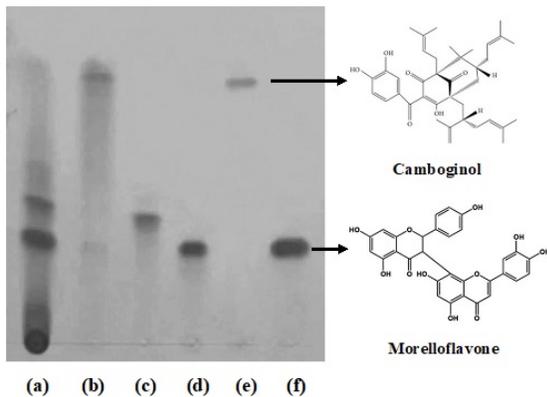


Fig. 1 The chromatogram of the *Garcinia dulcis* flower extract: (a) hexane insoluble fraction, (b) fraction 1, (c) fraction 2, (d) fraction 3, (e) camboginol ($C_{38}H_{50}O_6$, MW 602), and (f) morelloflavone ($C_{30}H_{20}O_{11}$, MW 556).

RESULTS

The chemical components of the GD flower extract

The chromatogram showed that the hexane insoluble fraction is composed of camboginol and morelloflavone as shown in Fig. 1.

Hypotensive effect of GD flower extract in acute and sub-chronic treatment groups

Acute oral administration of GD flower extract at the doses of 100 and 200 mg/kg BW significantly decreased SBP, DBP, and MAP but did not change PP and HR in comparison to the vehicle as shown in Fig. 2a (SBP; GD-100: 89 ± 3 , GD-200: 88 ± 3 , and vehicle: 102 ± 4 mm Hg, DBP; GD-100: 43 ± 2 , GD-200: 44 ± 2 , and vehicle: 57 ± 4 mm Hg, MAP; GD-100: 59 ± 2 , GD-200: 54 ± 3 , and vehicle: 72 ± 4 mm Hg, $p < 0.05$). Acute treatment of 50 mg/kg BW GD flower extract did not affect the SBP, DBP, PP, MAP, and HR.

In sub-chronic treatment, oral administration 50 and 100 mg/kg BW GD flower extract significantly decreased SBP, DBP, and MAP in comparison to the vehicle as shown in Fig. 2b (SBP; GD-50: 94 ± 2 , GD-100: 94 ± 2 , and vehicle: 104 ± 1 mm Hg, DBP; GD-50: 38 ± 1 , GD-100: 37 ± 4 , and vehicle: 48 ± 2 mm Hg, MAP; GD-50: 56 ± 0.5 , GD-100: 56 ± 3 , and vehicle: 67 ± 1 mm Hg, $p < 0.05$). Oral administration of 200 mg/kg BW GD flower extract significantly decreased SBP and PP but did not change DBP, MAP, and HR compared with vehicle (SBP; GD-200: 95 ± 3 and vehicle: 104 ± 1 , PP; GD-

Table 1 Effect of acute and sub-chronic treatment of either vehicle or *Garcinia dulcis* flower extract at the doses of 50, 100, and 200 mg/kg BW on heart rate (HR), hematocrit (Hct), and plasma osmolarity (P_{Osm}).

Group	HR (BPM)	Hct (%)	P_{Osm} (mOsm/kg H_2O)
Acute treatment			
Vehicle	402 ± 12	41.0 ± 0.9	291 ± 2
GD-50	426 ± 6	40.5 ± 0.9	289 ± 2
GD-100	414 ± 9	41.8 ± 0.3	289 ± 1
GD-200	422 ± 15	38.8 ± 1.3	292 ± 1
Sub-chronic treatment			
Vehicle	418 ± 11	38.9 ± 0.7	291 ± 3
GD-50	390 ± 9	$43.3 \pm 1.1^*$	$296 \pm 2^{*,**}$
GD-100	406 ± 20	$41.4 \pm 0.9^*$	$294 \pm 2^{*,**}$
GD-200	419 ± 12	$47.0 \pm 3.2^{*,**}$	$300 \pm 2^{*,**}$

Data were represented as mean \pm standard error of the mean (SEM). * $p < 0.05$ in comparison with the vehicle within either acute or sub-chronic group and ** $p < 0.05$ when compared between the acute and the sub-chronic treatment at the same dose of either vehicle or GD flower extract. BPM, beat per minute.

200: 42 ± 4 and vehicle: 56 ± 2 mm Hg, $p < 0.05$).

The diuretic effect of GD flower extract

All doses of acute oral administration of GD flower extract significantly decreased RVR compared with the vehicle as shown in Fig. 3 (GD-50: 15 ± 2 , GD-100: 13 ± 3 , GD-200: 13 ± 2 , and vehicle: 28 ± 12 RU, $p < 0.05$). The extract significantly raised C_{PAH} , C_{In} , \dot{V} , C_{Osm} , and TC_{H_2O} in comparison to vehicle (C_{PAH} ; GD-50: 4.92 ± 0.96 , GD-100: 4.96 ± 0.63 , GD-200: 4.98 ± 0.99 , and vehicle: 2.72 ± 0.06 mL/min/g KW, C_{In} ; GD-50: 1.69 ± 0.14 , GD-100: 1.74 ± 0.15 , GD-200: 1.67 ± 0.36 , and vehicle: 0.79 ± 0.07 mL/min/g KW, \dot{V} ; GD-50: 11.36 ± 0.28 , GD-100: 11.65 ± 0.32 , GD-200: 12.17 ± 1.01 , and vehicle: 8.87 ± 0.94 mL/min/g KW, C_{Osm} ; GD-50: 48 ± 3 , GD-100: 53 ± 3 , GD-200: 49 ± 6 , and vehicle: 33 ± 3 μ L/min/g KW, TC_{H_2O} ; GD-50: 34 ± 3 , GD-100: 41 ± 3 , GD-200: 37 ± 6 , and vehicle: 24 ± 3 μ L/min/g KW, $p < 0.05$). All doses of acute treatment of GD flower extract did not change U_{Osm} and P_{Osm} .

The C_{PAH} , \dot{V} , C_{Osm} , TC_{H_2O} , and U_{Osm} did not change after treatment of GD flower extract sub-chronically as shown in Fig. 4. However, the RVR significantly decreased in the 100 mg/kg BW GD treatment group but significantly increased in the 200 mg/kg BW GD treatment group in comparison to the vehicle (GD-100: 14.90 ± 1.13 , GD-

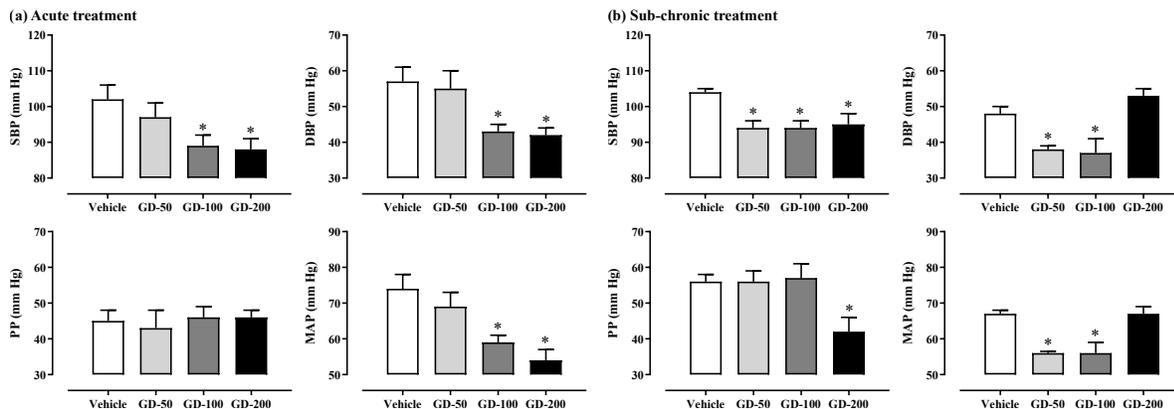


Fig. 2 The hypotensive effect of the *Garcinia dulcis* flower extract at the doses of 0 (vehicle), 50, 100, and 200 mg/kg BW: (a) acute and (b) sub-chronic treatment protocol. * $p < 0.05$ in comparison to vehicle. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; and MAP, mean arterial pressure.

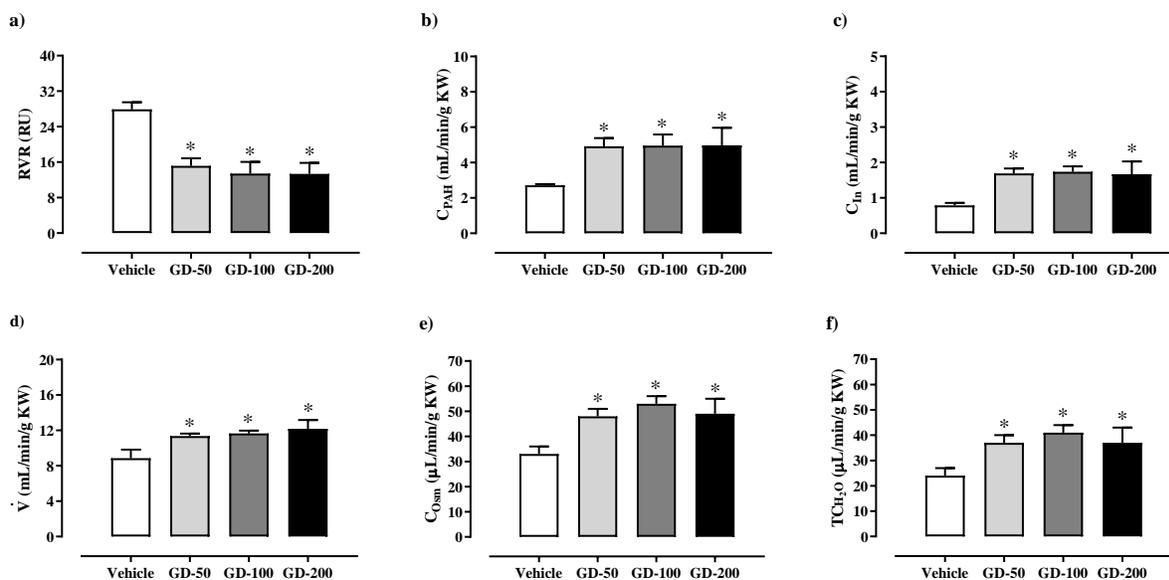


Fig. 3 The acute diuretic effect of the *Garcinia dulcis* flower extract at the doses of 0 (vehicle), 50, 100, and 200 mg/kg BW: a) renal vascular resistance (RVR), b) clearance of para-amino hippuric acid (C_{PAH}), c) clearance of inulin (C_{In}), d) urine flow rate (\dot{V}), e) osmolar clearance (C_{Osm}), and f) negative free water clearance (TC_{H_2O}). * $p < 0.05$ in comparison to vehicle. KW, kidney weight.

200: 25.26 ± 1.52 , and vehicle: 18.73 ± 1.15 RU, $p < 0.05$). It was also found that C_{In} significantly increased in the group of 200 mg/kg BW GD flower extract treatment in comparison to the vehicle (GD-200: 2.69 ± 0.22 and vehicle: 1.05 ± 0.05 , $p < 0.05$). Moreover, it was found that treatment of GD flower extract significantly increased hematocrit (Hct) and P_{Osm} in comparison to the vehicle as represented in Table 1 (Hct; GD-50: 43.3 ± 1.1 , GD-100: 41.4 ± 0.9 , GD-200: 47.0 ± 3.2 ,

and vehicle: $38.9 \pm 0.7\%$, P_{Osm} ; GD-50: 296 ± 2 , GD-100: 294 ± 2 , GD-200: 300 ± 2 , and vehicle: 291 ± 3 mOsm/kg H_2O , $p < 0.05$).

BW, FBG, plasma lipid profile, liver enzymes, and histology of liver

In the sub-chronic protocol, all doses of GD flower extract did not change the BW, FBG levels, the plasma levels of cholesterol, triglyceride, HDL, LDL, AST, ALT, and ALP in comparison to vehicle

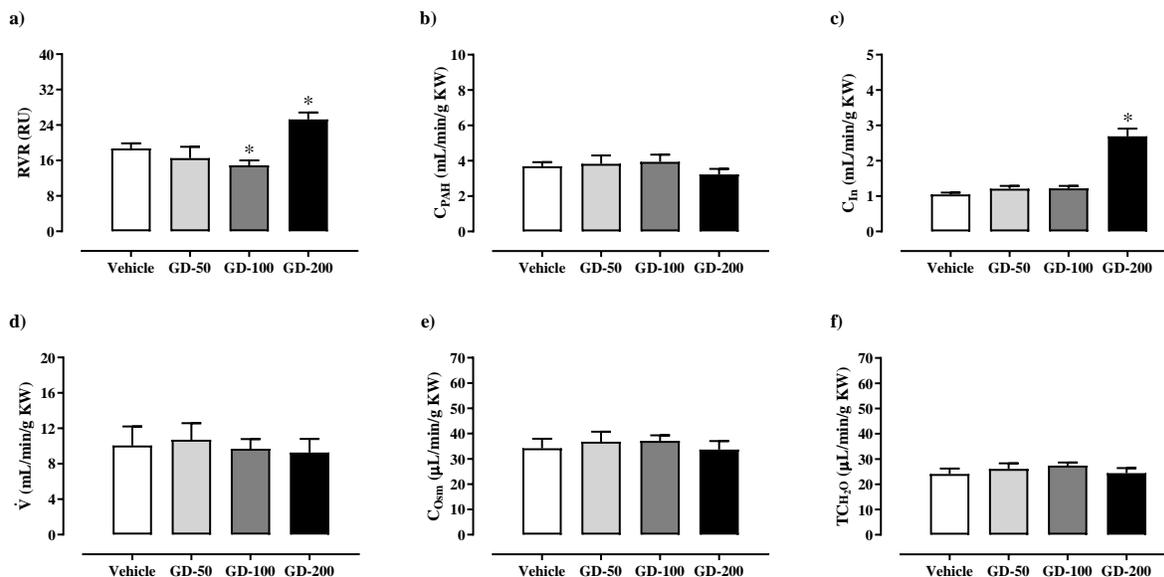


Fig. 4 Effect of sub-chronic treatment of the *Garcinia dulcis* flower extract at the doses of 0 (vehicle), 50, 100, and 200 mg/kg BW on renal excretory functions: a) renal vascular resistance (RVR), b) clearance of para-amino hippuric acid (C_{PAH}), c) clearance of inulin (C_{In}), d) urine flow rate (\dot{V}), e) osmolar clearance (C_{Osm}), and f) negative free water clearance (TC_{H_2O}). * $p < 0.05$ in comparison to vehicle. KW, kidney weight.

(Table S1). The H&E staining of liver tissues did not observe any pathology change (Fig. S1).

DISCUSSION

The TLC showed that the hexane insoluble fraction of the GD flower extract is composed of camboginol and morelloflavone (Fig. 1). Camboginol, also called garcinol, is a benzophenone which exerted a wide range of physiological activities including antioxidation, vasodilation, anti-inflammation, anti-cancer, anti-HIV, and anti-ulcer [10–12, 16–23]. Another active compound in this fraction is morelloflavone biflavonoid comprising 2 flavones: apigenin and luteolin occurring in most *Garcinia* species [24]. The previous study reported that morelloflavone inhibits vascular smooth muscle cell migration, invasion, and lamellipodium formation through activation of multiple migration-related kinases including focal adhesion kinase, Src, extracellular signal-regulated kinase, and RhoA [25]. Oral morelloflavone therapy for 8 months significantly reduced the atherosclerotic areas of the mouse aortae [26]. The possible mechanism of those actions of camboginol and morelloflavone may be related to its antioxidant property.

The findings showed that the hypotensive effect of GD flower extract can be observed at the doses of

100 and 200 mg/kg BW in the acute treatment and 50 and 100 mg/kg BW in the sub-chronic treatment (Fig. 2). It was also found that the RVR decreased in all groups of acute treatment and the group of 100 mg/kg BW in the sub-chronic treatment (Fig. 3a and Fig. 4a). The decreased RVR in those groups was due to a decreased MAP with an increased ERPF since the RVR was calculated using the equation; $RVR = MAP/ERPF$. Our previous studies found that both camboginol and morelloflavone induced vasorelaxation of the isolated thoracic aorta from either normotensive or hypertensive rat, and its mechanism of action involved endothelial NO-dependent signaling pathway [10, 11, 13]. The data suggested that the possible mechanism of hypotensive action may be due to vascular relaxation and reduction of the vascular resistance which involved endothelial NO-dependent pathway.

The hypotensive effect was absent in the group of acute 50 mg/kg BW treatment which may be due to less concentration of the GD flower extract (Fig. 2a). Interestingly, the DBP in the sub-chronic treatment groups at the doses of 50 and 100 mg/kg BW were greatly decreased, thus reflecting the strong vasorelaxation (Fig. 2b). While the DBP and MAP turned to the basal level, the SBP was still significantly lower than the control group in the sub-

chronic treatment of 200 mg/kg BW. It was also found that the RVR and the C_{In} (as GFR) were significantly higher than the other groups because (1) the MAP turned to a normal level while the ERPF was slightly lower than the control and (2) the increased GFR suggested the vasoconstriction of the efferent arteriole. The absence of the hypotensive effect of the GD flower extract at the high dose may be due to the compensatory mechanisms of the body including baroreceptor reflex to increase sympathetic activity and the RAAS to increase angiotensin II level and then increased basal vascular tone [27].

The diuretic effect of GD flower extract was observed in the acute treatment of the GD flower extract (Fig. 3). The C_{PAH} , C_{In} , \dot{V} , C_{Osm} , and TC_{H_2O} were significantly increased in comparison to the vehicle at all doses of GD flower extract. The findings corresponded with our previous studies which found that intravenous infusion of either cambojinol or morelloflavone exerted diuretic action in both normotensive and hypertensive rats [10, 12]. The mechanism of diuretic action may involve both glomerular and tubular functions. The GD flower extract relaxed the afferent arteriole and induced an increase of blood flow (C_{PAH}) leading to an increase of intravenous hydrostatic pressure in the glomerulus, resulting in increased GFR (C_{In}), \dot{V} , and urinary electrolyte excretion (C_{Osm}). The water reabsorption (TC_{H_2O}) was significantly increased in the GD extract treatment groups which may be due to the action of anti-diuretic hormone (ADH) in response to hypotension and hypovolemia [28].

The diuresis of the GD flower extract was not observed in the sub-chronic treatment groups (Fig. 4). The water contraction was observed in those sub-chronic treated groups since the level of hematocrit and P_{Osm} were significantly increased compared to vehicle control (Table 1). The mechanism of the water retraction may be because of the inhibitory effect of the extract on the thirst center. The extract may diminish action of ADH on the renal distal and collecting duct or diminish ADH synthesis and release itself. Physiologically, the compensatory responses to the water contraction include increased vascular tone by activation of the sympathetic nervous system and secretion of angiotensin II which induced vasoconstriction of both afferent and efferent arterioles. Moreover, the plasma levels of ADH and aldosterone were also increased in response to water contraction, and increasing of P_{Osm} resulted in increased water and sodium reabsorption at late distal tubule and collecting duct and finally decreased

the urine output [27].

Oral administration of GD flower extract for 2 weeks did not change the BW, FBG levels, and the plasma levels of cholesterol, triglyceride, HDL, and LDL. These findings corresponded with the previous study which reported that oral morelloflavone therapy for 8 months did not change the plasma lipid profiles in *Ldlr*^{-/-}*Apobec1*^{-/-} mice [26]. Moreover, sub-chronic treatment of GD flower extract did not change the levels of AST, ALT, and ALP in comparison to the vehicle and did not cause the histological change of the liver suggesting oral administration of flower GD extract was non-toxic (Table S1 and Fig. S1).

It is concluded that acute oral administration of GD flower extract possessed hypotensive and diuretic effects. Sub-chronic treatment of GD flower extract showed a hypotensive effect and the absence of liver toxicity. The hypotensive and diuretic action of GD flower extract may relate to its antioxidant activity. The application of GD flower extract as an antihypertensive agent should be further pharmacologically studied in the hypertensive model.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at <http://dx.doi.org/10.2306/scienceasia1513-1874.2020.095>.

Acknowledgements: This research was financially supported by the Thammasat University Research Fund under the TU Research Scholar, Contract No. 2/41/2561. It also was supported by the Laboratory of Pathology, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand for histological study.

REFERENCES

1. Julius S, Nesebitt S (1996) Sympathetic overactivity in hypertension. A moving target. *Am J Hypertens* **9**, 113S–120S.
2. Manrique C, Lastra G, Gardner M, Sowers JR (2009) The renin-angiotensin-aldosterone system in hypertension: roles of insulin resistance and oxidative stress. *Med Clin North Am* **93**, 569–582.
3. Blaustein MP, Zhang J, Chen L, Hamilton BP (2006) How does salt retention raise blood pressure? *Am J Physiol Regul Integr Comp Physiol* **290**, R514–R523.
4. Brandes RP (2014) Endothelial dysfunction and hypertension. *Hypertension* **64**, 924–928.
5. Yan W, Liu K, Zheng Y, Cao J, Zhang A, Wei G, Zeng Q (2018) Antihypertensive effect of a Chinese herbal formula (*Salvia miltiorrhiza* compound recipe) in hypertensive rats induced by high-fructose, high-salt, and high-fat diet. *ScienceAsia* **44**, 187–196.

6. Deachathai S, Mahabusarakam W, Phongpaichit S, Taylor WC (2005) Phenolic compounds from the fruit of *Garcinia dulcis*. *Phytochemistry* **66**, 2368–2375.
7. Dechathai S, Mahabusarakam W, Phongpaichit S, Taylor WC, Zhang YJ, Yang CR (2006) Phenolic compounds from the flowers of *Garcinia dulcis*. *Phytochemistry* **67**, 464–469.
8. Deachathai S, Phongpaichit S, Mahabusarakam W (2008) Phenolic compounds from the seeds of *Garcinia dulcis*. *Nat Prod Res* **22**, 1327–1332.
9. Mahabusarakam W, Mecawun P, Phongpaichit S (2016) Xanthones from the green branch of *Garcinia dulcis*. *Nat Prod Res* **30**, 2323–2328.
10. Thongsepee N, Mahabusarakam W, Hiranyachattada S (2017) Diuretic and hypotensive effect of morelloflavone from *Garcinia dulcis* in two-kidneys-one-clip (2K1C) hypertensive rat. *Sains Malaysiana* **46**, 1479–1490.
11. Thongsepee N, Mahabusarakam W, Hiranyachattada S (2018) Vasorelaxant mechanisms of camboginol from *Garcinia dulcis* in normotensive and 2-kidneys-1-clip hypertensive rat. *Songklanakarin J Sci Technol* **40**, 1248–1258.
12. Thongsepee N, Mahabusarakam W, Hiranyachattada S (2017) Diuretic effect of camboginol from *Garcinia dulcis* in 2-kidneys-1-clip hypertensive rat. In: *Proc of the Burapha University International Conference 2017*, Chonburi, Thailand, pp 613–622.
13. Lamai J, Mahabusarakam W, Ratithammatorn T, Hiranyachattada S (2013) Effects of morelloflavone from *Garcinia dulcis* on vasorelaxation of isolated rat thoracic aorta. *J Physiol Biomed Sci* **26**, 13–17.
14. Smith HW, Finkelstein N, Aliminoso L, Crawford B, Graber M (1945) The renal clearances of substituted hippuric and derivatives and other aromatic acids in dogs and man. *J Clin Invest* **24**, 388–404.
15. Davidson WD, Sackner MA (1963) Simplification of anthrone method of the determination of inulin in clearance studies. *J Lab Clin Med* **62**, 351–356.
16. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H (2000) Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. *J Agr Food Chem* **48**, 180–185.
17. Yamaguchi F, Saito M, Ariga T, Yoshimura Y, Nakazawa H (2000) Free radical scavenging activity and antiulcer activity of garcinol from *Garcinia indica* fruit rind. *J Agr Food Chem* **48**, 2320–2325.
18. Liao CH, Sang S, Liang YC, Ho CT, Lin JK (2004) Suppression of inducible nitric oxide synthase and cyclooxygenase-2 in downregulating nuclear factor-kappa B pathway by Garcinol. *Mol Carcinog* **41**, 140–149.
19. Hong J, Sang S, Park HJ, Park HJ, Kwon SJ, Suh N, Yang CS (2006) Modulation of arachidonic acid metabolism and nitric oxide synthesis by garcinol and its derivatives. *Carcinogenesis* **27**, 278–286.
20. Kim SY, Kim TB, Moon KA, Kim TJ, Shin D, Cho YS, Lee KY (2008) Regulation of pro-inflammatory responses by lipoxygenases via intracellular reactive oxygen species *in vitro* and *in vivo*. *Exp Mol Med* **40**, 461–476.
21. Tanaka T, Kohno H, Shimada R, Kagami S, Yamaguchi F, Kataoka S, Ohigashi H (2000) Prevention of colonic aberrant crypt foci by dietary feeding of garcinol in male F344 rats. *Carcinogenesis* **21**, 1183–1189.
22. Balasubramanyam K, Altaf M, Varier RA, Swaminathan V, Ravindran A, Sadhale PP, Kundu TK (2004) Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression. *J Bio Chem* **279**, 33716–33726.
23. Das D, Bandyopadhyay D, Bhattacharjee M, Banerjee RK (1997). Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. *Free Radical Bio Med* **23**, 8–18.
24. Verbeek R, Plomp AC, van Tol EA, van Noort JM (2004) The flavones luteolin and apigenin inhibit *in vitro* antigen-specific proliferation and interferon-gamma production by murine and human autoimmune T cells. *Biochem Pharmacol* **68**, 621–629.
25. Pinkaew D, Cho SG, Hui DY, Wiktorowicz JE, Hutadilok-Towatana N, Mahabusarakam W, Tongnunt M, Stafford LJ, et al (2009) Morelloflavone blocks injury-induced neointimal formation by inhibiting vascular smooth muscle cell migration. *Biochim Biophys Acta* **1790**, 31–39.
26. Pinkaew D, Hutadilok-Towatana N, Teng BB, Mahabusarakam W, Fujise K (2012) Morelloflavone, a biflavonoid inhibitor of migration-related kinases, ameliorates atherosclerosis in mice. *Am J Physiol Heart Circ Physiol* **302**, H451–H458.
27. Guyton AC, Hall JE (2006) *Textbook of Medical Physiology*, Philadelphia, Elsevier Inc., Pennsylvania, USA.
28. Boone M, Deen PM (2008) Physiology and pathophysiology of the vasopressin-regulated renal water reabsorption. *Pflugers Arch* **456**, 1005–1024.

Appendix A. Supplementary data

Table S1 Effect of Sub-chronic treatment of either vehicle or *Garcinia dulcis* flower extract at the doses of 50, 100, and 200 mg/kg BW on body weight change, fasting blood glucose, plasma lipid profile, and liver enzymes.

Parameter	Vehicle (n = 6)	GD-50 (n = 6)	GD-100 (n = 6)	GD-200 (n = 6)	p
BW at day 0, g	284 ± 14	323 ± 10	310 ± 10	318 ± 7	ns
ΔBW at day 14, g	+47 ± 5	+50 ± 10	+55 ± 8	+53 ± 3	ns
FBG, mg/dL	126 ± 13	118 ± 15	124 ± 10	124 ± 18	ns
Cholesterol, mg/dL	52 ± 4	51 ± 7	53 ± 2	49 ± 4	ns
Triglyceride, mg/dL	54 ± 8	48 ± 4	72 ± 9	65 ± 13	ns
HDL, mg/dL	33 ± 3	32 ± 2	34 ± 1	29 ± 2	ns
LDL, mg/dL	5 ± 1	3 ± 1	5 ± 1	4 ± 1	ns
AST, U/L	57 ± 2	49 ± 3	53 ± 3	64 ± 3	ns
ALT, U/L	18 ± 2	18 ± 3	16 ± 4	14 ± 1	ns
ALP, U/L	64 ± 5	55 ± 7	61 ± 8	52 ± 5	ns

Data were represented as mean ± standard error of the mean (SEM). ΔBW, body weight change (+increase); FBG, fasting blood glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; and ns, non-significant when compared with vehicle.

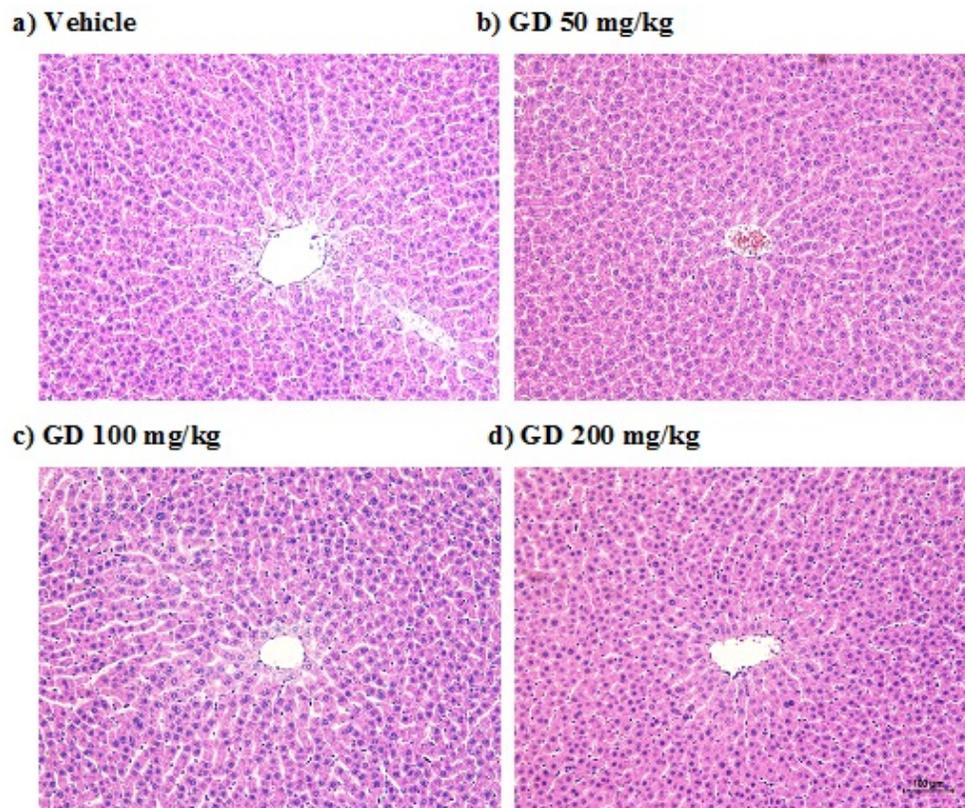


Fig. S1 Histological examination (hematoxylin and eosin staining) of sub-chronic treatment of either a) vehicle or *Garcinia dulcis* flower extract at the dose of b) 50, c) 100, and d) 200 mg/kg BW. Magnification power = 200 × .