Vasorelaxant mechanisms of camboginol from Garcinia dulcis in normotensive and 2-kidneys-1-clip hypertensive rat

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<th>Journal:</th>
<th>Songklanakarin Journal of Science and Technology</th>
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<tr>
<td>Manuscript ID</td>
<td>SJST-2017-0136.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Original Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>18-Jul-2017</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
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<tr>
<td>Keyword:</td>
<td>camboginol, Garcinia dulcis, 2-kidneys-1-clip, antioxidant, vasorelaxation</td>
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Vasorelaxant mechanisms of camboginol from *Garcinia dulcis* in normotensive and 2-kidneys-1-clip hypertensive rat

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Abstract

The endothelial dysfunction along with an increase in reactive oxygen species observed in 2-kidneys-1-clip (2K1C) hypertensive rat has strengthened hypertension. This study aimed to investigate the vasorelaxant effect of camboginol from *Garcinia dulcis* which is a robust antioxidant in normotensive and 2K1C hypertensive rat (n=6, each). Intravenous injection of camboginol showed a transient reduction in arterial blood pressure and restored both the impaired baroreflex sensitivity and the elevated plasma malondialdehyde observed in 2K1C rat. Experiments in isolated thoracic aorta revealed the vasorelaxant action of camboginol with pD2 of 9.67±0.19 and 8.01±0.66 in normotensive and 2K1C hypertensive rat. The mechanisms of its actions involved the different extent of endothelial nitric oxide and prostacyclin signaling pathway, and the opening of ATP-activated potassium channel. Camboginol also enhanced an endothelial nitric oxide synthase expression in the isolated vessel from 2K1C rat. It is concluded that vasorelaxant mechanisms of camboginol may involve its antioxidant activity.

Keywords: camboginol; *Garcinia dulcis*; 2-kidneys-1-clip; vasorelaxation, antioxidant
1. Introduction

Normal arterial blood pressure is regulated by the balance between vasodilative and vasoconstrictive mechanisms. The possible mechanisms responsible for the development of pathological hypertension are the impairment of endothelial-derived vasodilative production such as nitric oxide (NO) or prostacyclin (PGI₂) in addition to an increase in circulating vasoconstrictors such as angiotensin II (AII). In an animal model, 2-kidneys-1-clip (2K1C) renovascular hypertension (RVH), an impairment of endothelium-dependent vasorelaxation was reported (Choi et al., 2014). Moreover, an increase in plasma AII along with an overproduction of reactive oxygen species (ROS), especially superoxide anion (O₂⁻), were also observed (McIntyre, Bohr, & Dominiczak, 1999). AII has shown to stimulate O₂⁻ generation by increasing the activity of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in cultured rat vascular smooth muscle cell (Griendling, Minieri, Ollerenshaw, & Alexander, 1994) and in intact aortas of AII-infused hypertensive rat (Rajagopalan et al., 1996). The role of O₂⁻ is related to endothelial dysfunction by which NO can be scavenged by O₂⁻ to from peroxynitrite (ONOO⁻) resulting in reduced NO bioavailability (Rubanyi & Vanhoutte, 1986). In addition, O₂⁻ has shown to involve in a diminishing of baroreflex sensitivity (BRS) in 2K1C hypertension (Botelho-Ono et al., 2011; Queiroz et al., 2012).

The scavenging of excessive body free radical using plant compounds in treatment of hypertension has been widely researched (Gilani et al., 2000; Edwards et al., 2007; Lv et al., 2013; Mozafari, Nekoeian, Panjeshahin, & Zare, 2015). Camboginol, an isoprenylated benzophenone, can be isolated from most Garcinia spp., including the folk medical plant *Garcinia dulcis* Kurz (family Guttiferae). The chemical structure of camboginol (Figure 1) was first elucidated in 1980 by Rao, Venkatswamy, and Pendse (1986). Its wide range of biological activities included anti-ulcer (Vaananen, Meddings, & Wallace, 1991; Das,
Bandyopadhyay, Bhattacharjee, & Banerjee, 1997), antioxidant (Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000a, Yamaguchi, Saito, Ariga, Yoshimura, & Nakazawa, 2000b; Hutadilok-Towatana, Kongkachuay, & Mahabusarakam, 2007), anti-cancer (Tanaka et al., 2000; Yoshida et al., 2005), anti-inflammation (Liao, Sang, Liang, Ho, & Lin, 2004; Hong et al., 2006; Kim et al., 2008), and anti-HIV (Balasubramanyam et al., 2004; Mantelingu et al., 2007).

The potent antioxidant activity of camboginol has been previously reported. It could scavenge the free radical 1, 1-diphenyl-2-picrylhydrazyl with three times greater potency than α-tocopherol (vitamin E), a well-known lipid-soluble natural antioxidant, in aqueous ethanol solution (Yamaguchi et al., 2000a). It was also able to scavenge both hydrophilic and hydrophobic agents including ROS (Yamaguchi et al., 2000b). Camboginol also exhibited strong anti-oxidation effect in both Fe²⁺-mediated and non-metal induced human low-density lipoprotein oxidations (Hutadilok-Towatana et al., 2007).

According to the development of hypertension which may be strengthened by an increase in body reactive oxygen or nitrogen species, we aimed to investigate the vasorelaxant effects of camboginol by determining its acute action on arterial blood pressure (ABP) and heart rate (HR), and also its action on BRS, plasma malondialdehyde (MDA) level in anesthetized 2K1C hypertensive and sham operative (SO) normotensive rats. The endothelium-dependent vasorelaxant mechanisms of camboginol action which might occur via the activation of NO signaling pathway was studied in isolated thoracic aortic rings of both groups using nitric oxide synthase inhibitor (Nω-Nitro-L-arginine methyl ester, or L-NAME) and endothelial nitric oxide synthase (eNOS) expression using immunohistochemistry. Other vasorelaxant mechanisms including the involvement of PGI₂ signaling pathway, ATP-activated potassium (K_ATP) channel and Ca²⁺-activated potassium
(K_{Ca}) channel were also studied using their specific blockers indomethacin, glibenclamide and tetraethylammonium (TEA), respectively.

2. Materials and Methods

2.1 Camboginol and chemicals

Camboginol was extracted from the fresh ripe fruits of *Garcinia dulcis* collected from Songkhla province, Thailand. The voucher specimen has been deposited at the Herbarium of Prince of Songkla University, Thailand. The extraction procedure of camboginol was described previously (Deachathai, Mahabusarakam, Phongpaichit, & Taylor, 2005). Its structure was illustrated in Figure 1. Other chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA including phenylephrine (PE), sodium nitroprusside (SNP), acetylcholine (ACh), dimethylsulfoxide (DMSO), malondialdehyde (MDA), thiobarbituric acid (TBA), L-NAME, indomethacin, glibenclamide and TEA. Pentobarbital sodium was purchased from CEVA Santé Animal, Brussels, Belgium. For immunohistochemical study, Freezing Medium® was purchased from Leica, Nussloch, Germany. Rabbit anti-eNOS antibody (Lot # QJ214409), biotinylated goat anti-rabbit IgG (Lot # QE215187), ABC Peroxidase staining kit and DAB substrate kit were purchased from Thermo Fisher, Rockford, IL, USA.

2.2 Experimental animals

Male Wistar rats (body weight 150-200 g, n=84) were obtained from the Southern Laboratory Animal Facility (Prince of Songkla University, Thailand). Rats were housed under control condition (temperature 23-24°C; humidity 50-55%; lighting 0600-1800 h), fed
a laboratory diet containing 34.2 mmol sodium chloride/kg dry weight food and were allowed
free access to reverse osmosis water.

For the immunohistochemical study of the rat thoracic aorta, rats (n=7) were purchased from the National Laboratory Animal Center (Mahidol University, Thailand) and were housed at the Animal Facility of Department of Zoology, Kasetsart University under control conditions as mentioned above. All experiments were approved by the Prince of Songkla University Animal Ethics Committee (Reference No. 31/2014).

2.3 Establishment of 2K1C hypertensive rat and experimental design

Rats were anaesthetized with a single dose of pentobarbital sodium (50 mg/kg BW i.p.). Only the deep sedated animals were gone through the following surgical protocol; the left kidney was exposed through a retroperitoneal incision, the left renal artery was then exposed and cleared from surrounding connective tissues and a U-shaped silver clip with a 0.20 mm gap placed around it close to the junction with the abdominal aorta. The muscle and skin layer were sutured separately with catgut and silk No. 4/0, respectively. The SO group included the entire surgery with the exception of renal artery clipping. At the end of the surgery, all animals received a single dose of ampicillin (50 mg/kg BW, i.m.) injection and were allowed to recover in separate cages for 2-3 h under an angle poise lamp and remained untouched for 4 weeks afterward in order to develop hypertension.

There were two parts of experiments the in vivo and in vitro study. Each part consisted of 4 groups of rats (n=6, each), namely SO + Vehicle (SO), SO + Camboginol (SO+C), 2K1C + Vehicle (2K1C) and 2K1C + Camboginol (2K1C+C).
Four weeks after renal artery clipping, rats were anesthetized with pentobarbital sodium (60 mg/kg BW i.p.; an additional dose was given when necessary) and placed on a thermostatically-controlled heated table to maintain body temperature at 37°C. A tracheotomy was performed and the left carotid artery was cannulated, using polyethylene tube (PE-50) filled with heparinized 0.9% NaCl, and connected to a pressure transducer, coupled to a PowerLab system (ADInstruments, Colorado Springs, CO, USA), to monitor systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR). The right jugular vein was cannulated using PE-50 filled with heparinized 0.9% NaCl. Rat was given 0.9% NaCl solution via jugular vein at a rate of 1.6 mL/h/100g BW until basal ABP and HR were stabled. Then, camboginol dissolved in DMSO was given as a bolus injection (0.1 mg/kg BW; volume of injection was 0.05 mL/100g BW) and the same volume of DMSO was given as vehicle in both SO and 2K1C groups. The maximal response in SBP, DBP, PP (SBP-DBP), MABP (DBP+1/3 PP) and HR were then determined.

The effect of camboginol on BRS was performed during the continuous intravenous infusion at the dose of 5 µg/min/kg BW. The experimental dose of camboginol was chosen based on the minimal effective dose of a specific AII receptor antagonist, candesartan (Hiranyachattada, Saetew, & Harris, 2005). After one hour equilibration, the base line values of HR and ABP were recorded. Then the animals received an acute injection of two sets vasoactive drugs; PE, a specific α1 receptor agonist and SNP, a NO donor, at the similar doses of 1, 2, 4, 8, 16 and 32 µg/kg BW. The maximal response in MABP and HR were then determined. BRS was calculated from ΔHR/ΔMABP.

At the end of the experiment, blood sample was collected via carotid artery catheter in heparinized tube and was then centrifuged at 4,000 rpm for 10 min then plasma was stored at -20°C until analysis for plasma MDA level. Rats were sacrificed by intravenous injection of
saturated MgSO₄. Both kidneys were removed, decapsulated, dried on blotting paper and weighed. The heart was also removed and weighed after carefully clearing away blood vessels, fat and connective tissues. The organ weight to total body weight ratio was calculated.

2.5 Determination of plasma MDA

The MDA content was assayed in the form of TBA reacting substances (modified from Ohkawa, Ohishi, & Yagi, 1979). Briefly, plasma sample, distilled water and MDA standard were added into tubes followed by 8.1% sodium dodecyl sulfate and 20% acetic acid. The mixture was adjusted to pH 3.5 with NaOH, then 0.8% TBA was added. The mixture was heated in 95°C water bath for 60 min and was cooled down in ice-bath. Distilled water and n-butanol were added and then the mixture was centrifuged. The absorbance of the organic layer was measured using spectrophotometer. The amount of TBA reactive substances was determined from standard curve generation by MDA from acid hydrolysis of 1, 1, 3, 3-tetramethoxypropane. The values of plasma MDA were expressed as µmol/L.

2.6 In vitro study: Preparation of isolated thoracic aortic rings

Rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and sacrificed by decapitation. The thoracic aorta was dissected and cut into four ring segments approximately 5 mm in length each (two endothelium-intact and two -denuded rings). The denuded rings were performed by mechanical removal of endothelium. The rings were mounted in 20 mL organ baths containing 37°C Krebs Henseleit solution which was composed of (mM) 118.41 NaCl, 4.6 KCl, 1.12 MgSO₄.7H₂O, 1.18 KH₂PO₄, 1.9 CaCl₂, 25.0 NaHCO₃ and 11.66 D-glucose. The pH of solution was maintained at 7.4 by continuous aeration with 95% O₂ and 5% CO₂. The 1 g resting tension was set and the tension changes during the course of
experiment were recorded using force displacement transducer (Model FT03, Grass Instrument Co., Quincy, MA, USA) connected to PowerLab system (ADInstruments, USA).

The endothelial function of aortic rings was tested by the addition of $10^{-5}$ M ACh into the $10^{-7}$ M PE precontracted rings. The 80% relaxation was accepted and considered as an intact endothelium and the disappearance of relaxation was considered as denuded endothelium (Molina, Hidalgo, & García de Boto, 1992).

2.7 Concentration response curve of camboginol

After 45 min equilibration, both endothelium-intact and denuded aortic rings from 2K1C and SO rats were precontracted by addition of $10^{-7}$ M PE. When the maximal contraction response developed, the tension was recorded. Either camboginol or vehicle (DMSO) was added cumulatively, allowing the final concentration to be $10^{-13}$-$10^{-5}$ M and 0.1-0.9%, respectively. Subsequent concentrations were added after the maximal response by the previous concentration developed and recorded.

2.8 Effect of specific inhibitors on vasorelaxation response of camboginol

After 15 min equilibration, the endothelium-intact aortic rings from 2K1C and SO rats were incubated with each specific inhibitor for 30 min before precontraction with $10^{-7}$ M PE. The doses of four inhibitors were $10^{-4}$ M L-NAME, $10^{-6}$ M indomethacin, $10^{-5}$ M glibencamide and $10^{-3}$ M TEA. Then, either camboginol ($10^{-13}$-$10^{-5}$ M) or vehicle (0.1% DMSO) was added cumulatively after the maximal contraction was developed and sustained 5-7 min. Subsequent concentrations were added after the maximal response by the previous developed and recorded concentration.

2.9 Immunohistochemical study of eNOS expression in thoracic aorta endothelium
One hour after camboginol (0.1 mg/kg + 5 µg/min/kg) was given via jugular vein of the anesthetized 2K1C rat, it was then terminated by overdose injection of pentobarbital sodium and thoracic aorta was removed, dissected and cut into 5 mm segments. The segments were fixed in 4% buffered formaldehyde, embedded in Tissue Freezing Medium® and was cut on a cryostat. The eNOS expression in aortic section was evaluated using floating-technique immunohistochemistry. Following several standard pretreatment steps, the section was incubated with rabbit anti-eNOS antibody (dilute 1:500) at 4°C for 24 h on shaker. Next, the section was incubated with biotinylated goat anti-rabbit IgG (dilute 1:2000) for 1 h and finally, was stained using ABC Peroxidase staining kit and metal enhanced DAB substrate kit, respectively. The section was mounted onto slide and covered slip section and then digitized using an Olympus DP 73 microscope (Olympus Optical Co, Ltd, Tokyo, Japan).

For this immunohistochemical study, the animals were divided into 4 groups; control (n=2), negative control (n=2), 2K1C (n=2) and 2K1C+C (n=3).

2.10 Statistical analyses

All data were expressed as the mean ± S.E.M. The degree of vasorelaxation in each experiment was expressed as a percent relaxation from PE (10⁻⁷ M) precontraction tension. The negative logarithm (pD₂) value was calculated using GraphPad Prism 5 (San Diego, CA, USA). Significant differences between the group means were determined using ANOVA followed by Student-Newman Keuls post hoc test or Student t-test. A p value < 0.05 was considered significantly different.

3. Results
3.1 Changes in body weight, kidney weight and cardiac mass

As shown in Table 1, the body weight changes between SO and 2K1C rats after four weeks of experimental renal stenosis were not significantly different. The left clipped and the right non-clipped kidney weight of 2K1C rat decreased by 73% and increased by 24% when compared to the respective ipsilateral kidneys of SO rat. The atrophic kidney was due to the reduction in renal blood flow while the right hypertrophic kidney was due to the compensation. The cardiac mass of 2K1C groups significantly increased in comparison to those SO groups. This finding suggested the increased afterload in this rat model.

3.2 Acute effect of camboginol on ABP and HR

As shown in Figure 2, four weeks after experimental renal stenosis, the resting SBP, DBP, PP and MABP in 2K1C rats were significantly higher than in those of SO rats (SBP; 208±8 vs. 155±4, DBP; 143±4 vs. 123±4, PP; 64±7 vs. 32±3 and MABP; 165±5 vs. 134±4 mm Hg, respectively, p < 0.05). However, the resting HR of 2K1C rats was not significantly different from SO rats (212±5 vs. 210±5 bpm).

The bolus injection of 0.1 mg/kg BW camboginol in SO rats significantly decreased SBP, DBP, MABP and HR in comparison to the vehicle injection (SBP; 104±3 vs. 153±4, DBP; 51±4 vs. 120±3, MABP; 69±3 vs.131±3 mm Hg and HR 154± 9 vs. 210 ± 5 bpm, respectively, p < 0.05). In 2K1C rats, the injection of 0.1 mg/kg BW also decreased the SBP, DBP, MABP and HR significantly when compared to vehicle injection (SBP; 118±9 vs. 208±9, DBP; 62±5 vs. 144±5, MABP; 81±4 vs. 165±5 mm Hg and HR; 178± 3 vs. 212 ± 5 bpm, respectively, p < 0.05). These results suggested the hypotensive potential of camboginol.

3.3 Effect of camboginol on BRS
As shown in Figure 3, after intravenous injection of six consecutive doses of PE and SNP, the changes of HR in response to changed MABP and the calculated BRS of 2K1C were significantly lower than those of SO rat at all respective experimental doses (p < 0.05). This finding suggested an impaired BRS in 2K1C rats. However, camboginol treatment restored this impairment by increasing HR and hence BRS in response to both PE and SNP effects.

3.4 Effect of camboginol on plasma MDA level

As shown in Figure 4, levels of plasma MDA in 2K1C rats were significantly higher than SO rats (64.3±8.9 vs. 30.0±2.5 µmol/L, p < 0.05, respectively). Camboginol treatment significantly lowers the plasma MDA level in 2K1C rats to 31.3±11.4 µmol/L, p < 0.05. This finding suggested the *in vivo* free radical scavenging property of camboginol.

3.5 Vasorelaxant effect of camboginol in isolated thoracic aortic ring

As shown in Figure 5A, the addition of the cumulative doses of camboginol (10^{-13}-10^{-5} M) significantly relaxed the PE-precontracted endothelium-intact aortic rings of both 2K1C and SO group in a concentration-dependent manner (2K1C; pD_{2} = 8.01±0.66 and SO; pD_{2} = 9.67±0.19, p < 0.05). Denudation of the functional endothelium completely abolished camboginol-induced vasorelaxation in both groups (Figure 5B). This suggested the vasorelaxant effect of camboginol is endothelium-dependent.

3.6 Effect of specific inhibitors on camboginol-induced vasorelaxation

Pretreatment of the endothelium-intact aortic rings with 10^{-4} M L-NAME significantly and completely abolished camboginol-induced vasorelaxation in both SO and 2K1C rats (Figure 6A and B). *In the presence of 10^{-6} M indomethacin* and 10^{-5} M glibenclamide, the
relaxation of SO aortic rings by addition of camboginol were partial abolished (Figure 6C and E). However, in the presence of $10^{-6}$ M indomethacin, the percent relaxation of the aortic rings from 2K1C was not abolished (Figure 6D) when compared to those of SO suggesting the endothelial PGI$_2$ synthesis might be impaired in 2K1C. In contrast, the percent relaxation of the aortic rings from 2K1C rat in response to camboginol in presence of $10^{-5}$ M glibenclamide was similar to SO (Figure 6E and F) suggesting the partial involvement of vascular smooth muscle K$_{ATP}$ in this signaling vasorelaxation was present in both groups. Pretreatment of the endothelium-intact aortic rings from SO and 2K1C rats with $10^{-3}$ M TEA did not affect the percent relaxation response to camboginol (Figure 6G and H). These findings suggested the vasorelaxant mechanisms of camboginol action in SO thoracic aorta involves mainly NO-signaling pathways with partial involvement of PGI$_2$ and K$_{ATP}$. In 2K1C, the contribution of NO-signaling and partial K$_{ATP}$ and K$_{Ca}$-involved pathways were observed.

3.7 Effect of camboginol on eNOS expression

As shown in Figure 7, 2K1C rats exhibited a significant reduction of eNOS expression in aortic endothelium when compared with control rats. The treatment with camboginol was able to enhance protein expression of aortic eNOS in 2K1C rat. This finding further supported the mechanism of vasorelaxation by camboginol which involves the endothelial NO-signaling pathway.

4. Discussion and Conclusion

2K1C is the most relevant model characteristics to human RVH which involves unilateral stenosis of the renal artery. This leads to a permanent reduction in renal perfusion pressure in one kidney resulting in increased AII production (Goldblatt, Lynch, Hanzal, &
Summerville, 1934; Navar et al., 1998). In this study, four weeks after experimental renal artery stenosis, SBP, DBP, PP and MABP of 2K1C rats measured under pentobarbitone sodium anesthetization were significantly higher than those of SO rats suggesting the development of hypertension was successful. These results were also supported by the significant increase in the cardiac mass and the atrophy of the clipped left kidney with the hypertrophy of the non-clipped right kidney of 2K1C when compared to SO rat.

It is found that an acute intravenous administration of camboginol showed a significant transient reduction in SBP, DBP, MABP and HR in 2K1C and SO rats suggesting the hypotensive potential of this plant compound. This hypotensive effect did not sustain during the infusion of our experimental dose (0.1 mg/kg BW + 5 µg/min /kg BW) in both 2K1C and SO. One of the explanations of this may be due to the well regulation of baroreflex in SO and the improved BRS in 2K1C caused by camboginol. The selected dose of camboginol in this in vivo study may not be high enough to maintain its hypotensive action comparable to candesartan, a specific AII receptor antagonist previously reported (Hiranyachattada et al., 2005).

However, the acute bradycardic effect of camboginol may involve NO action on sinoatrial node firing rate since NO has been shown to exert biphasic effect depending on its concentration. NO could increase HR by activating hyperpolarization-activated pacemaker current ($I_h$) at low concentration but it could potentially decrease HR by inhibition of L-type calcium current at high concentration (Mani, Nahavandi, Moosavi, Safarinejad, & Dehpour, 2002). Moreover, it is likely that camboginol may act directly on the nucleus tractus solitarii (NTS), the cardiovascular regulating center in medulla oblongata, to cause bradycardia since the overexpression of eNOS was reported in the NTS which consequently reduces the sympathetic nerve activity, HR and ABP in conscious rats (Sakai et al., 2000).
2K1C rats also exhibited the impairment of BRS which may due to the inability to increase HR in response to a decreased ABP and vice versa. It has been proved that an impairment of BRS in RVH was related to an overproduction of \( \text{O}_2^- \) by NADPH oxidase enzyme (Botelho-Ono et al., 2011; Queiroz, Guimarães, Mendes-Junior, & Braga, 2012). Acute administration of Vitamin C or apocynin, a NADPH oxidase inhibitor prominently improved the blunt BRS in 2K1C rat (Botelho-One et al., 2011). Likewise, it is likely that the antioxidant property of camboginol may improve the impairment of 2K1C autonomic function.

The raising of plasma MDA level confirmed the oxidative stress status in 2K1C rats in this study. Camboginol treatment suppressed an elevated plasma MDA in 2K1C supporting the robust antioxidant property of camboginol which previously was reported by Yamaguchi et al. (2000a, 2000b) and Hutadilok-Towatana et al. (2007).

The percent relaxation of thoracic aorta from 2K1C by cumulative addition of camboginol showed the lower \( pD_2 \) when compared to SO rat suggesting the higher concentration of camboginol was required to induce the vasorelaxation to the similar degree as in SO. The mechanisms of camboginol action in SO aorta were found to be endothelial dependent involving mainly NO generation and partial both PGI\( _2 \) production and the operation of vascular \( K_{\text{ATP}} \) channel. However in 2K1C, the camboginol actions occurred mainly through NO generation and partial \( K_{\text{ATP}} \) channel with the lesser extent than in SO as shown in Figure 6. This may be due to an impairment of endothelial function in 2K1C rat. However, it is unlikely that the vasorelaxant effect of camboginol could occur via vascular smooth muscle \( K_{\text{ATP}} \) or \( K_{\text{Ca}} \) stimulation since the denudation of both SO and 2K1C aortic rings completely abolished the relaxation as shown in Figure 5.

Our immunohistological study indicated the obviously less eNOS expression in 2K1C when compared to control. This finding is similar to the previous reports by Sánchez et al.
(2006) and Ulker, McMaster, McKeown, and Bayraktutan (2003) that showed the eNOS expression significantly decreases in the dysfunctional arteries from hypertensive rat. An overproduction of $O_2^-$ reduced bioactive NO by promoting NO inactivation and by promoting eNOS uncoupling (Förstermann, 2010). Camboginol treatment augmented eNOS expression in the vascular endothelium of 2K1C. This finding further supported the mechanism of camboginol action in 2K1C that might occur via eNOS expression in the vascular endothelium.

It is concluded that the vasorelaxant mechanisms of camboginol action in 2K1C hypertensive rats are likely to be endothelial dependent involving NO production and the opening of vascular $K_{ATP}$ channel similar to normotensive rat but with lesser degree. The partial involvement of PGI$_2$ production in the vasorelaxant mechanisms by camboginol in normotensive disappeared in 2K1C. Regarding to the robust antioxidant activity and the hypotensive potential of camboginol, camboginol may be beneficial in long term treatment as anti-hypertensive supplement. However, an application of this plant benzophenone as an alternative treatment of hypertension requires further pharmacological studies regarding its effects in the resistant vessels and intensive cellular mechanisms of its action.

Acknowledgements

This research was financially supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission [SC75705685], the PSU-Ph.D. Scholarships, the Graduate School Research Support Funding for Thesis and Faculty of Science Research Fund, Academic Year 2015. Prince of Songkla University, Hat Yai, Songkhla, Thailand.

References


**Table 1** Comparisons of pre-body weight (BW) at the beginning of experiment and post-BW at the end of experiment, left and right kidney weight (KW) and cardiac mass in 2-kidneys-1-clip (2K1C) and sham operated (SO) rats which were treated with either vehicle (V) or camboginol (C) at 4 weeks after induction of hypertension.

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<td>Right KW (g)</td>
<td>1.18 ± 0.02</td>
<td>0.98 ± 0.03</td>
<td>1.46 ± 0.07*</td>
<td>1.30 ± 0.10*</td>
</tr>
<tr>
<td>Right KW/BW (%)</td>
<td>0.30 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.39 ± 0.02*</td>
<td>0.36 ± 0.02*</td>
</tr>
<tr>
<td>Cardiac mass (g)</td>
<td>1.06 ± 0.02</td>
<td>1.01 ± 0.01</td>
<td>1.23 ± 0.12*</td>
<td>1.17 ± 0.03*</td>
</tr>
<tr>
<td>Cardiac mass/BW (%)</td>
<td>0.27 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>0.32 ± 0.02*</td>
<td>0.34 ± 0.01*</td>
</tr>
</tbody>
</table>

Data are mean ± S.E.M.

*p < 0.05 compared with respective SO groups (Student t-test).
Figure 1. Chemical structure of camboginol which was firstly isolated from latex of *Garcinia cambogia* by Rao et al. in 1980 (Molecular formula C$_{38}$H$_{50}$O$_{6}$, Molecular weight 602).
Figure 2. Acute hypotensive effects of camboginol (C) 0.1 mg/kg BW in the 2-kidneys-1-clip (2K1C) and sham operation (SO) groups (n=6 each). Upper panels show the recorded tracing of arterial blood pressure (ABP) and heart rate (HR) and lower panels represent A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) pulse pressure (PP) and D) mean arterial blood pressure (MABP). Data are mean ± S.E.M. *, # p < 0.05 compared with SO and 2K1C group, respectively (one-way ANOVA with Newman-Keuls post hoc test).
**Figure 3.** Baroreflex sensitivity (BRS) in response to either phenylephrine (PE; left panel) or sodium nitroprusside (SNP; right panel) in 2-kidneys-1-clip (2K1C) and sham operation (SO) group (n=6 each), during treatment with camboginol (C; 0.1 mg/kg BW + 5 µg/min/kg BW). BRS = \( \frac{\Delta HR}{\Delta MABP} \). Data are mean ± S.E.M. *, #p < 0.05 compared with the SO and 2K1C group at the respective concentrations of PE or SNP (one-way ANOVA with Newman-Keuls post hoc test).
Figure 4. Effect of camboginol (0.1 mg/kg BW + 5 µg/min/kg BW) on plasma malondialdehyde (MDA) levels in 2-kidneys-1-clip (2K1C) hypertensive and sham operative (SO) normotensive rats (n=6 each). Data are mean ± S.E.M. *# p < 0.05 compared with SO and 2K1C group, respectively (one-way ANOVA with Newman-Keuls post hoc test).
Figure 5. Effects of camboginol ($10^{-13}$-$10^{-5}$ M) or vehicle (0.1-0.9% DMSO) on vasorelaxation of A) endothelium-intact and B) -denuded aortic rings from 2-kidneys-1-clip (2K1C) or sham operative (SO) groups (n=6 each). Values are mean ± S.E.M of percentage relaxation from $10^{-7}$ M phenylephrine (PE) pre-contraction. *, # p < 0.05 compared with respective vehicle groups and SO + camboginol group (one-way ANOVA with Newman-Keuls post hoc test).
Figure 6. Effects of camboginol (10^{-13}-10^{-5} M) on vasorelaxation of endothelium-intact aortic rings from sham operative (SO, left panels) and 2-kidneys-1-clip (2K1C, right panels) group (n=6 each) in the presence of specific inhibitors; A&B) 10^{-4} M L-NAME, C&D) 10^{-6} M indomethacin, E&F) 10^{-5} M glibenclamide and G&H) 10^{-3} M TEA. Values are mean ± S.E.M. *,# p < 0.05 compared with vehicle and camboginol group respectively (one-way ANOVA with Newman-Keuls post hoc test).
Figure 7. Endothelial nitric oxide synthase (eNOS) expression in aortic endothelium of control and 2-kidneys-1-clip (2K1C) rat; A) control (n=2), B) negative control (n=2), C) 2K1C (n=2) and D) after camboginol (0.1 mg/kg + 5 µg/min/kg BW) in 2K1C (n=3). Brown staining in aortic endothelium represents eNOS in the aortic wall. Black staining represents nucleus of vascular smooth muscle cell. Arrow indicates the eNOS location. 400X, Scale bar = 20 µm.