ฤทธิ์ของสารสกัดไพลต่อความตึงตัวของหลอดเลือดเอออร์ตาของหนูแรท

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บทคัดย่อ

ไพลถูกนำมาใช้อย่างแพร่หลายในการแพทย์แผนไทยโบราณเพื่อรักษาโรคต่างๆ การศึกษาเมื่อไม่นานมานี้รายงานว่าสารสกัดไพลสามารถลดความดันหลอดเลือดแดงได้ อย่างไรก็ตาม ยังไม่มีหลักฐานเกี่ยวกับฤทธิ์ของสารสกัดไพลต่อความตึงตัวของหลอดเลือดและกลไกที่เกี่ยวข้อง การศึกษานี้มีจุดประสงค์เพื่อศึกษาฤทธิ์ของสารสกัดไพลต่อความตึงตัวของหลอดเลือดและการตอบสนองของหลอดเลือดต่อสารที่มีผลต่อหลอดเลือด โดยใช้หลอดเลือดเอออร์ตาของหนูแรท ผลการศึกษาพบว่าสารสกัดไพลที่ความเข้มข้น 0.1 ถึง 10 ไมโครกรัมต่อมิลลิลิตร มีฤทธิ์ทำให้หลอดเลือดเอออร์ตาของหนูคลายตัว นอกจากนี้ สารสกัดไพลยังมีฤทธิ์ลดการหดตัวของหลอดเลือดโดย methoxamine และ $CaCl_2$ อย่างไรก็ตาม สารสกัดไพลไม่มีผลต่อการคลายตัวของหลอดเลือดโดย carbachol และ sodium nitroprusside ผลจากการศึกษานี้โดยใช้หลอดเลือดเอออร์ตาของหนูแรทแสดงให้เห็นว่าฤทธิ์ของสารสกัดไพลที่ทำให้เกิดการคลายตัวของหลอดเลือดอย่างน้อยที่สุดมีกลไกที่เกี่ยวข้องกับการยับยั้งการผ่านของ Ca^{2+} จากภายนอกเซลล์สู่ภายในเซลล์ ผลการศึกษานี้เป็นข้อมูลพื้นฐานสำหรับการศึกษาทางคลินิกเพื่อนำสารสกัดไพลในแพทย์แผนไทยโบราณเป็นยาลดความดันหลอดเลือดแดง

คำสำคัญ: ไพล; ความตึงตัวของหลอดเลือด; หลอดเลือดเอออร์ตา

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Effects of *Cassumunar Ginger* extract on the vascular tone of rat aortic rings

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Abstract

Cassumunar ginger has been widely used in Thai traditional medicine for the treatment of various diseases. A recent study reported that *Cassumunar ginger* extract (CGE) could decrease mean arterial blood pressure. However, there is currently no evidence regarding the effects of CGE on vascular tone or the underlying mechanisms involved. So, this study aims to investigate the effects of CGE on vascular tone and vascular responses to certain vasoactive agents in the rat aorta. Our findings indicate that CGE (0.1-10 μg/ml) induces relaxation of rat aortic rings. Additionally, pretreatment with CGE reduced methoxamine- and CaCl2-induced contractions. However, the vasorelaxant responses to carbachol and sodium nitroprusside were not affected by CGE pretreatment. These results suggest that the vasorelaxant effects of CGE in the rat aorta are mediated, at least in part, through the inhibition of Ca^{2+} influx from the extracellular space. The findings from this study provide fundamental data that support the potential clinical use of CGE in Thai traditional medicine to reduce arterial blood pressure.

Keywords: *Cassumunar ginger*; vascular tone; aortic rings

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Introduction

Cassumunar ginger (Zingiber cassumunar or current scientific name as Zingiber montanum) belongs to the Zingiberaceae family. It is known locally as "Plai" in Thai[1,2]. In Thailand, Plai has been used as a single plant or a constitutive of herbal recipes to treat inflammation, bruises, sprain and strain, rheumatism, musculoskeletal pain, wounds, asthma, cough, and respiratory problems[2].

Previous studies have reported the antioxidant and anti-inflammatory properties of cassumunar ginger[1,3-5]. A recent study has shown that Plai oil has antioxidant activity that inhibits 2,2-diphenyl-1-picrylhydrazyl radicals. This essential oil consists of four active compounds, sabinene, γ-terpinene, terpinene-4-ol, and (E)-1-(3,4-dimethoxy phenyl) butadiene[3]. Moreover, curcuminoids isolated from cassumunar ginger showed protective effect on living cells suffering from oxidative stress[4]. A study in rats induced by a high-fat diet showed that the extract of cassumunar ginger increased superoxide dismutase activity[5].

Cassumunar ginger has antimicrobial activities against a wide range of gram-positive and gram-negative bacteria, dermatophytes, and yeasts[6,7]. A recent study has demonstrated that oils extracted from cassumunar ginger exhibit antibacterial and antifungal activities. Major constituents of this essential oil were sabinene, (E)-1-(3', 4'-dimethoxy phenyl) buta-1,3-diene, terpinen-4-ol, γ-terpinene and β-phellandrene[7].

The mechanism of vasorelaxation induced by herbal medicine mainly involves endothelium-dependent and endothelium-independent pathways or direct effects on vascular smooth muscle cells. The vascular endothelium, the inner lining of blood vessels, plays a vital role in regulating vasomotor tone via synthesizing and releasing endothelium-derived relaxing factors. Nitric oxide, for instance, is a potent vasodilator produced by endothelial cells in response to various stimuli, including sheer stress from blood flow. It diffuses to the smooth muscle cells in the vessel wall, causing relaxation and subsequent vasodilation, which decreases vascular resistance and lowers blood pressure. Dysfunction of the endothelium can lead to various cardiovascular disorders, including hypertension[8]. In vascular smooth muscle cells, calcium ions play a significant role in regulating vascular tone. Vasorelaxation is due to inhibiting extracellular calcium influx that reduces the availability of calcium ions within smooth muscle cells[9].

Concerning the vascular effects of cassumunar ginger, a recent study reported that the extract of cassumunar ginger decreased mean arterial blood pressure, which was about four times that of prazosin hydrochloride, an alpha-1 blocker[10]. However, there is no evidence for the vascular effects of cassumunar ginger and its underlying mechanisms.

Objectives

The present study aims to investigate the effect of the dried extract of cassumunar

ginger (CGE) on vascular tone in rat aortic rings. Moreover, the vascular effects of CGE on endothelium-dependent and endothelium-independent vasorelaxants were evaluated. Finally, the involvement of extracellular Ca^{2+} influx in the vascular effects of CGE was examined.

Materials and methods

Extraction of cassumunar ginger

The dried milled rhizomes of *Z. cassumunar* (100 g) were boiled in the distilled water for 2 hours and subsequently filtered through Whatman No.1 filter paper by using vacuum filtration. The filtrates were then freeze-dried for 2 days at -50°C (Labconco freeze dryer Model 700611130). The dried extracts (3.8 g) were stored at -20°C until use.[10]

Tissue preparation

Experiments were performed using aorta obtained from male Wistar rats (300 - 350 g). Rats were housed in standard environmental conditions (25°C) under a 12-hour light/dark cycle and fed with standard laboratory rat chow and tap water *ad libitum*. All experimental procedures were reviewed and approved by the Animal Research Ethics Committee of Srinakharinwirot University.

Male Wistar rats were anaesthetized with Zoletil® 50 mg/kg (tiletamine and zolazepam) into quadriceps muscle. Then, rats were sacrificed by cervical dislocation. Following a thoracotomy, the thoracic aorta was carefully removed and cleaned out connective tissue and fat. Next, the aortae were cut into 5 mm ring segments. Each ring was transferred to a jacketed organ bath filled with 20 mL of modified Krebs-Henseleit solution, composed of (mM) NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, D-glucose 10 that was maintained at 37°C, and bubbled continuously with 95 % O_2 and 5 % CO_2 mixture. The solution in the organ bath was exchanged every 15 minutes for one hour. The rings were mounted between two triangular stainless-steel hooks that were passed through the lumen and stretched to an optimal passive tension of about 1 g and maintained at this tension for one hour. The upper hook was connected to an isometric force transducer (MLT0210). Changes in tension were measured by isometric force and recorded on a PowerLab recording system[11].

Experimental protocol

Following a 1-hour equilibration period, methoxamine (60-100 μM) was used to induce tone by approximately 1 g. Once a stable contraction was achieved, CGE (0.1-10 μg/ml) was added cumulatively. In vehicle-control experiments, dimethyl sulphoxide (DMSO) alone was added in the same volumes as those used in the experiments with CGE.

To investigate the effect of CGE on extracellular Ca^{2+} influx, concentration-response curves to $CaCl_2$ (10 μM-30 mM) were constructed in the presence and absence of CGE (1 and 10 μg/ml) for 30 minutes. After aortic rings were allowed to equilibrate for

30 minutes at 1 g tension, normal Krebs solution was replaced with Ca^{2+}-free Krebs solution. The rings were washed three times at 10-minute intervals with Ca^{2+}-free Krebs solution. Then, the rings were bathed with Ca^{2+}-free, high KCl (100 mM) buffer with or without CGE. In the vehicle-control study, DMSO was added in the same volume as that used in the experiments with CGE. After 30 minutes of incubation with CGE or DMSO as vehicle control, concentration-response curves for the contractile responses to $CaCl_2$ were established[12].

To examine the vascular effects of CGE on endothelium-dependent and -independent vasorelaxants, aortic rings were incubated with CGE (1 and 10 μg/mL) for 30 minutes. Then, concentration-responses curves were constructed for an endothelium-dependent vasodilator, carbachol (1 nM-100 μM), and an endothelium-independent vasodilator, sodium nitroprusside (0.1 nM-30 μM)[12]. DMSO was used as the vehicle control instead of CGE.

Data and statistical analysis

The maximal responses (R_{max}) were obtained from the concentration-response curve fitted to a sigmoidal logistic equation using the GraphPad Prism package described by Tep-areenan et al. (2003)[13]. R_{max} was compared by analysis of variance (ANOVA) with statistically significant differences between groups being determined by Bonferroni's post-hoc test.[13] Data were expressed as mean±SEM. The results were considered statistically significant when the p-value was less than 0.05. The aortic rings in each group are represented by n.

Chemicals

All drugs and chemicals were purchased from Sigma Chemical Company (St. Louis, Missouri, USA), but Zoletil® was purchased from Virbac (Carros Cedex, France). CGE was dissolved in DMSO. Methoxamine, carbachol, and sodium nitroprusside were dissolved in distilled water. All drugs were made up on the day of the experiment.

Results

The effects of CGE on aortic rings pre-contracted with methoxamine

In the rat-isolated aorta, CGE (0.1-10 μg/mL) caused vasorelaxation in a concentration-dependent manner (Figure 1). However, CGE induced low relaxant responses (R_{max}: 0.1 μg/ml CGE = 2.33 ± 0.49 %, n = 6; 0.3 μg/ml CGE = 3.22 ± 0.28 %, n = 6; 1 μg/ml CGE = 4.63 ± 0.39 %, n = 6; 3 μg/ml CGE = 5.79 ± 0.53 %, n = 6; 10 μg/ml CGE = 7.62 ± 0.67 %, n = 6; Figure 1).

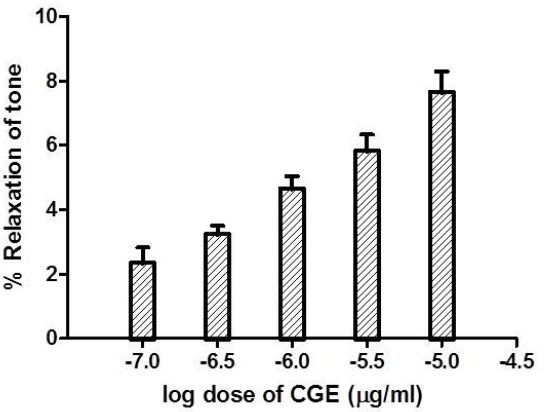

Figure 1 The effects of CGE on vascular tone in rat aortic rings. Data are shown as mean ± SEM.

The effects of CGE on extracellular Ca^{2+} influx in rat aortic rings

As shown in Figure 2, pre-treatment with 10 μg/mL CGE significantly inhibited maximal contractions to methoxamine (R_{max}: control = 2.34 ± 0.09 g, n = 6; 10 μg/ml CGE = 1.41 ± 0.21 g, p<0.05, n = 6). However, maximal contractions to methoxamine were unaffected by pre-treatment with 1 μg/ml CGE (R_{max}: control = 2.34 ± 0.09 g, n = 6; 1 μg/ml CGE = 2.31 ± 0.24 g, n = 6).

$CaCl_2$ (10 μM-30 mM) induced concentration-dependent contraction of KCl (100 mM) depolarized rings in a Ca^{2+}-free medium. CGE at a concentration of 1 μg/ml significantly reduced contractions to $CaCl_2$ (R_{max}: control = 1.55 ± 0.04 g, n = 6; 1 μg/ml CGE = 1.29 ± 0.06 g, p<0.01, n = 6; Figure 3). Moreover, contractile responses to $CaCl_2$ were significantly reduced by 10 μg/ml CGE (R_{max}: control = 1.55 ± 0.04 g, n = 6; 10 μg/ml CGE = 1.13 ± 0.04 g, p<0.001, n = 6; Figure 3).

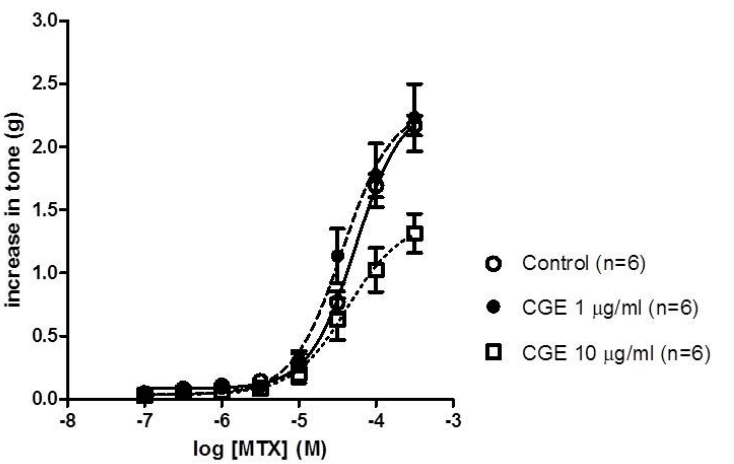

Figure 2 The effects of pre-treatment with CGE (1 and 10 μg/ml) on methoxamine-induced contraction in rat aortic rings. Data are shown as mean ± SEM. n indicates the number of aortic rings.

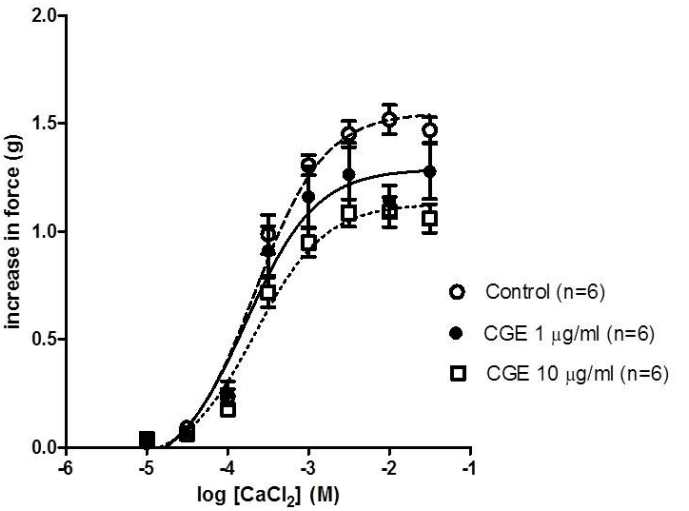

Figure 3 The effects of pre-treatment with CGE (1 and 10 μg/ml) on $CaCl_2$-induced contraction in rat aortic ringsdepolarized by 100 mM KCl. Data are shown as mean ± SEM. n indicates the number of aortic rings.

The effects of CGE on endothelium-dependent and -independent vasorelaxants in rat aortic rings

Maximal relaxations to carbachol were not affected by pre-treatment with 1 and 10 μg/mL CGE (control: R_{max} = 106 ± 2%, n = 5; 1 μg/ml CGE: R_{max} = 110 ± 3%, n = 5; 10 μg/ml CGE: R_{max} = 108 ± 2%, n = 5; Figure 4). In addition, vasorelaxations induced by sodium nitroprusside were not affected by pre-treatment with CGE (control: R_{max} = 144 ± 3%, n = 6; 1 μg/ml CGE: R_{max} = 143 ± 3%, n = 6; 10 μg/ml CGE: R_{max} = 145 ± 4%, n = 6; Figure 5).

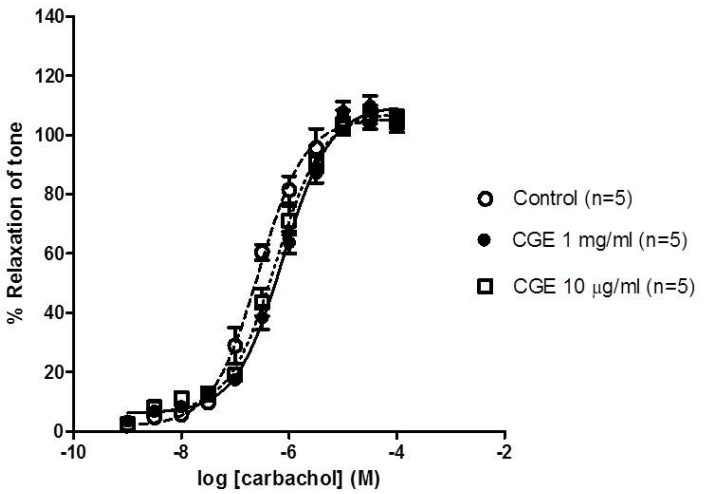

Figure 4 The effects of pre-treatment with CGE (1 and 10 μg/ml) on vasorelaxation to carbachol in rat aortic rings. Data are shown as mean ± SEM. n indicates the number of aortic rings.

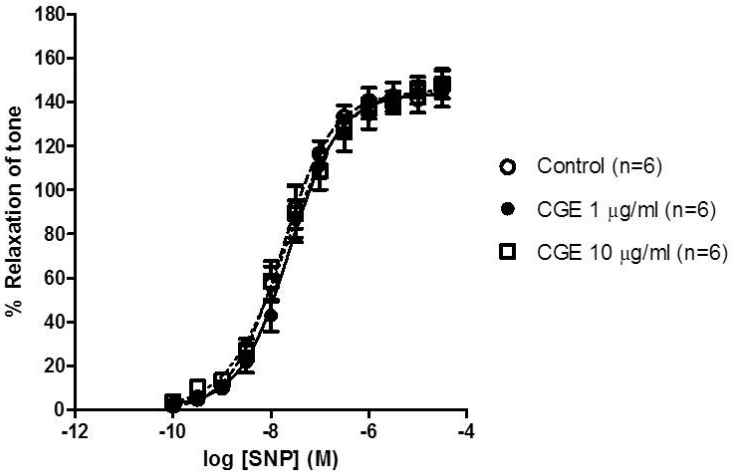

Figure 5 The effects of pre-treatment with CGE (10 and 10 μg/ml) on vasorelaxation to sodium nitroprusside in rat aortic rings. Data are shown as mean ± SEM. n indicates the number of aortic rings.

Discussion

Findings from the present study in the rat aorta have clearly demonstrated that CGE (0.1-10 μg/ml) causes acute concentration-dependent relaxations. Interestingly, CGE reduces contractile responses to $CaCl_2$ and methoxamine. However, vasorelaxant responses to carbachol and sodium nitroprusside are unaffected by CGE.

A previous study by Manosri et al. (2013) has showed that CGE exerts anti-hypertensive effect as it could reduce mean arterial blood pressure. Its effect was more potent than prazosin hydrochloride, an alpha 1 blocker[10]. In the present study, we found that CGE caused vasorelaxation in a concentration-dependent manner. These findings support that CGE acts as a vasodilator to reduce mean arterial blood pressure.

In the present study, we investigated whether inhibition of extracellular Ca^{2+} influx is involved in vasorelaxant responses to CGE. We found that contractile responses of rat aortic rings to $CaCl_2$ in Ca^{2+} -free medium containing high KCl were inhibited by CGE. Methoxamine, an α_1-adrenoreceptor agonist, causes vasoconstriction via activation of protein kinase C to increase extracellular Ca^{2+} influx through receptor-operated Ca^{2+} channels, and/or Ca^{2+} release from the intracellular store[14-15]. We found that CGE inhibits methoxamine-induced vasoconstriction. Taken together, these findings support the notion that CGE inhibits Ca^{2+} influx from the extracellular space.

To characterize the vascular actions of CGE, we investigated its effects on responses to carbachol, a muscarinic receptor agonist that induces vasorelaxation via the endothelium-dependent pathway[16]. In addition, endothelium-independent relaxation to sodium nitroprusside, a nitric oxide donor[16], was examined in the presence and the absence of CGE. We found that pre-treatment of rat aortic rings with CGE did not affect relaxant responses to carbachol and sodium nitroprusside. These results suggest that CGE does not participate in mechanisms contributing to vasorelaxations induced by these vasodilators.

Conclusions

The present findings in the rat aorta have shown that the vasorelaxant effects of CGE are mediated, at least in part, via inhibition of Ca^{2+} influx from extracellular space. However, further investigations need to be done to investigate other possible mechanisms involved in the vasorelaxant effects of CGE. Moreover, the active ingredients involved in CGE-induced vasorelaxation need to be examined.

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