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Research Article

Anti-hypertensive and cerebral blood flow improving actions of *Centella asiatica* (L.) Urban leaves juice in deoxycorticosterone acetate-salt hypertensive rats

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ABSTRACT

The cardiovascular effects of *Centella asiatica* (CA) leave juice were studied using hypertensive animal model.Single oral administration of lyophilized powder of CA leave juice at doses 16, 24 and 32 g of fresh leaves/kg (equivalent to 0.26, 0.38 and 0.52 g of lyophilized powder/kg) on blood pressure, heart rate and regional cerebral blood flow (rCBF) were investigated in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. CA lyophilized juice powder contained asiaticoside 0.42% w/w. The results showed that CA leave juice had blood pressure lowering and slight negative chronotropic effect in DOCA-salt hypertensive group, but not in normal. CA leave juice at the doses of 24 and 32 g/kg BW significantly decreased blood pressure from 30 to 90 and from 45 to 60 min with the maximal reduction of 11% and 12%, respectively, at 45 min in DOCA-salt hypertensive group. In addition, these two doses of CA leave juice significantly decreased HR at 60 and 45 min, respectively. Captopril at dose 25 mg/kg BW significantly reduced blood pressure at 30 to 120 min with a maximal reduction of 20% at 60 min and 19% at 90 min. in normal and DOCA-salt hypertensive groups, respectively. The significant effect on heart rate were not observed after captopril administration in both groups.

Prior administration of CA leave juice, the rCBF level of DOCA-salt hypertensive group was significantly low, compared to that of the normal. After CA leave juice administration at the dose of 32 g/Kg BW, rCBF increased significantly at 5-90 and 5-120 min in normal and DOCA-salt hypertensive groups, respectively. The increased rCBF was greater in DOCA-salt hypertensive than normal group (maximum increased rCBF: 52.27% vs 37.37 %). The increased rCBF was accompanied with significant decreased blood pressure at 15 to 120 min only in DOCA-salt hypertensive group. The maximum decrease of SBP and DBP were 13.86and 14.10 % at 60 minutes. These results demonstrates that CA leave juice possesses the actions as anti-hypertensive and regional cerebral blood flow enhancement in both normal and hypertensive rats. The present study suggested the beneficial use of CA in elderly especially in hypertensive condition.

1. INTRODUCTION

Centella asiatica (L.) Urban, known in Thai as bua-bok, is an important medicinal herb used in traditional and alternative medicine with a wide spectrum of indications and widely used among the Asian countries[1-3]. In Thailand, CA leaves are also used as drinking juice and fresh vegetable in several Thai dishes. The main active compounds of CA include triterpenic acids, asiatic acid, medecassic acid, and their sugar esters (total triterpenine glycosides): asiaticoside and madecassoside[1,4,5]. Several studies have demonstrated the various pharmacological actions of CA extract and their triterpenine glycosides and asiaticoside, including wound healing[6-9], venous insufficiency improvement[1,10-13], anti-inflammatiory[14,15], anti-gastric ulcer[16,17], and enhanced memory and cognition[18-21].

Regarding the cardiovascular effect, previous studies have reported that CA has cardioprotective [22], blood pressure and heart rate (HR) reduction[23-27], and vasodilation activities[28,29]. However, the blood pressure lowering effect of CA is still unclear. In addition, the CA effect on peripheral blood flow to vital organs has never been studied. The present study investigated the effect of a single oral administration of CA leave juice on blood pressure, HR, and peripheral blood flow, specifically regional cerebral blood flow (rCBF), in hypertensive condition by using DOCA-salt hypertensive rats.

2. MATERIALS AND METHODS

2.1. Plant Material

The CA leaves, bought from market in Bangkok, were identified by Professor Wongsatit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University. Voucher specimen (Serial number PBM 03380) was deposited at the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.

2.1.1. Preparation of CA leave juice

The fresh leaves were washed and grounded in distilled water with an electrical blender, then filtered through cotton and muslin cloth. The juice was lyophilized and maintained in airtight containers at -20 °C. One gram of CA leaves yielded 0.0158 g of lyophilized powder. For oral administration, CA leave juice was prepared by dissolving the lyophilized powder in distilled water, with the concentration expressed as gram of CA fresh leaves per kilogram of body weight.

2.1.2. Determination of Asiaticoside

Several studies reported the various pharmacological actions of the different extracts containing total triterpenoid fractions and asiaticoside from CA leaves [1, 5-7, 9-13, 16]. In present study, the lyophilized juice powder was analyzed for asiaticoside content as a marker. For the quantitation of asiaticoside, a Waters HPLC system equipped with 510 pump, UV detector and Symmetry® C_{18} column (4.6×250 mm, 5 µm) was used. Standardized asiaticoside was dissolved in methanol and injected into the column as external standard compounds. The column was eluted with acetonitrile: water (29:71) at flow rate 1ml/min and monitored at UV 210 nm. CA lyophilized juice had asiaticoside 0.42% w/w. One gram of CA leaves contained 0.0663 mg of asiaticoside.

2.2. Chemicals

Deoxycorticosterone acetate and captopril were purchased from Sigma Chemical Co., USA. Nembutal® was obtained from Abbot, USA. Other chemicals were of analytical grade.

2.3. Animals

Male Wistar rats, weighing between 140 and 160 g, were obtained from the National Laboratory Animal Center at Salaya Campus, Mahidol University. They were housed in a temperature-controlled (23±2°C) animal room at Faculty of Pharmacy, Mahidol University, on a 12 h light/dark cycles, with free access to standard pellet diet (C.P. Mice Feed; SWT Co., Ltd. Samut Prakan, Thailand) and drinking water.

2.3.1. Induction of DOCA-salt hypertensive rats

DOCA-salt hypertension was induced in rats by subcutaneous implantation of 1g/kg BW DOCA pellets at the dorsal area of neck under anesthesia with aseptic technique. DOCA-pellets were prepared by pushing DOCA powder with pellet generator (Perkin-Elmer, USA). After DOCA implantation, the rats were given 1% sodium chloride solution as drinking water. Systolic blood pressure (SBP) and HR were measured weekly by non-invasive tail-cuff method for 8 weeks. The rats exhibiting a SBP ≥190 mmHg were considered hypertensive.

2.4. Experimental protocol

Eight DOCA-salt hypertensive rats and eight normal rats were used. The change in SBP and HR were measured by non-invasive tail-cuff method before (0 min) and at 15, 30, 45, 60, 90, and 120 min after a single oral gavage of the angiotensin converting enzyme inhibitor captopril at dose 25 mg/kg BW and CA leave juice at doses16, 24, and 32 g/kg BW (equivalent to 0.26, 0.38 and 0.52 g of lyophilized powder/kg). Then, the anesthetized rats were used to examine the change in rCBF before (0 min) and at 15, 30, 45, 60, 90, and 120 min after single oral gavage of CA leave juice at dose 32 g/kg BW. The SBP and diastolic blood pressure (DBP) were also simultaneously monitored from femoral artery during rCBF measurement. Each administration was measured in the same rat with at least 2 days interval.

2.4.1. Determination of SBP and HR

SBP (mmHg) and HR (beats/min) were simultaneously measured in conscious rats using a noninvasive tail-cuff plethysmography with a piezoelectric transducer and indirect blood pressure recorder (Ugo Basile, Varese, Italy). The method is analogous to sphygmomanometry in human. Rat was placed in a restrainer and a cuff with a pneumatic pulse sensor was attached to the tail. The pneumatic tail cuff was inflated to occlude the tail arterial blood flow, then the cuff was deflated slowly. The cuff pressure at which the blood flow resumption, the pulse signals reappearance and automatically taken as the tail systolic blood pressure in mmHg[30]. Values are presented as the mean of three measurements.

2.4.2 Determination of rCBF

The rat was anesthetized with sodium pentobarbital (60 mg/kg BW intraperitoneal). After a tracheotomy was performed, the rat was ventilated mechanically with an air- oxygen mixture. A catheter was inserted into the femoral artery for monitoring the arterial blood pressure and blood gas tension. The arterial blood pressure was measured using a pressure transducer coupled to PowerLab system (AD instrument, USA).

The arterial blood gas tensions and pH levels were maintained within the normal limits of $PaCO_2$ = 35-45 mmHg, PaO_2 = 95-100 mmHg, and pH = 7.35-7.45. A craniotomy was performed to expose the anterior cerebral cortex. The dura matter was opened and a stainless steel metal frame with a circulation window (7 mm diameter) was fixed to the cranial bone. An artificial cerebrospinal fluid was infused into the cranial space for 30 minutes after the surgical procedure was completed. The rCBF was monitored using a Laser Doppler Flow meter (DRT4, Moor instrument, USA) with a fiber optic needle probe (wavelength 780 nm; 1 mm diameter) the needle probe was fixed 1 mm above the brain tissue[31]. Laser Doppler flowmetry technique is relative and measured in blood perfusion unit, which is related to the velocity and concentration of red blood cells (RBCs) within the tissue of studied. This technique is based on measuring the Doppler shift in the Laser light which induced by movement of RBCs[32]. The rCBF was measured at 5 points of the cranial tissue, and the average value was calculated for each rat. The rCBF and arterial blood pressure (SBP and DBP) were continuously recorded during 120 minutes after administration of CA.

2.5. Statistical analysis

Data are presented as mean±standard error of the mean (SEM). Differences within group were analyzed by paired t-test (two-tailed) and between 2 groups by unpaired *t*-test (two-tails). One-way analysis of variance (ANOVA) was used to compare differences among all groups and Duncan's Student-Newman-Keuls (SNK) test was used to differentiate between statistically significant groups. A *p*-value of less than 0.05 ($p<0.05$) was considered to be statistically significant.

3. RESULTS

3.1. Induction of hypertension

The DOCA-salt treatment successfully induced hypertension. The SBP, HR, and BW values of normal and treated groups are shown in Table 1. At week 8, DOCA-salt treated rats had SBP between 190-210 mmHg compared to 130-140 mmHg in normal rats. The SBP increased from week 0 by 65.25% in DOCA-salt and by 20.18% in normal rats. DOCA-salt hypertensive rats had a significant increased HR on weeks 5 and 7. However, the percentage change of weekly HR during 8 weeks was not significantly different (data not shown). Changes in body weight were not significantly different between DOCA-salt and normal groups during 8 weeks.

Table 1. Weekly systolic blood pressure, heart rate and body weight during 8 weeks of normal and deoxycorticosterone acetate (DOCA) – salt treated rats (n=8 in each group)

Time	Systolic blood pressure (mmHg)		Heart rate (beats/min)		Body weight (gram)	
	Normal	DOCA	Normal	DOCA	Normal	DOCA
Week 0	109.38±11.48	118.13±11.32	327.50±15.81	337.50±14.88	150.13±5.94	140.25±6.14
Week 1	113.75±6.94	117.50±8.45	328.75±1.53	333.75±10.61	209.00±16.18	171.75±12.31
Week 2	115.63±4.96	126.88±6.51^{++}	336.25±22.64	336.25±15.06	236.63±25.63	220.88±12.68
Week 3	122.50±3.78	136.88±7.53$^{**\ ++}$	337.50±19.82	338.75±11.50	259.63±25.24	231.50±23.23
Week 4	126.88±5.30	155.00±5.98$^{**\ ++}$	327.50±10.35	348.75±14.58^{++}	268.00±21.54	265.88±32.36
Week 5	126.25±3.54	159.38±10.84$^{**\ ++}$	337.50±23.15	355.00±11.95*	262.50±25.46	284.50±23.58
Week 6	126.88±5.30	174.38±13.48$^{**\ ++}$	342.50±11. 65	350.00±13.09	278.25±11.09	258.25±12.12
Week 7	130.63±1.77	191.88±7.04$^{**\ ++}$	351.25±6.41	356.25±13.02**	275.13±5.59	254.50±9.83
Week 8	131.88±2.59	195.63±6.78$^{**\ ++}$	332.50±15.81	360.00±19.27 $^{+}$	284.00±6.50	256.25±4.56

*(p<0.05), * *(p<0.01): significant difference from their starting values at week 0
+(p<0.05), ++(p<0.01): significant difference from the normal at the corresponding week

3.2. Effect of CA leave juice and captopril on SBP and HR

In normal rats, all three doses of CA leave juice had no significant effect on the SBP. The animals treated with captopril 25 mg/kg, however, had a significantly reduced SBP at 30 to 120 min with a maximal reduction of 20% occurring at 60 min. (Figure 1, A).

Similar to normal rats, captopril significantly decreased SBP at 30 to 120 min in DOCA-salt hypertensive rats. Captopril maximally

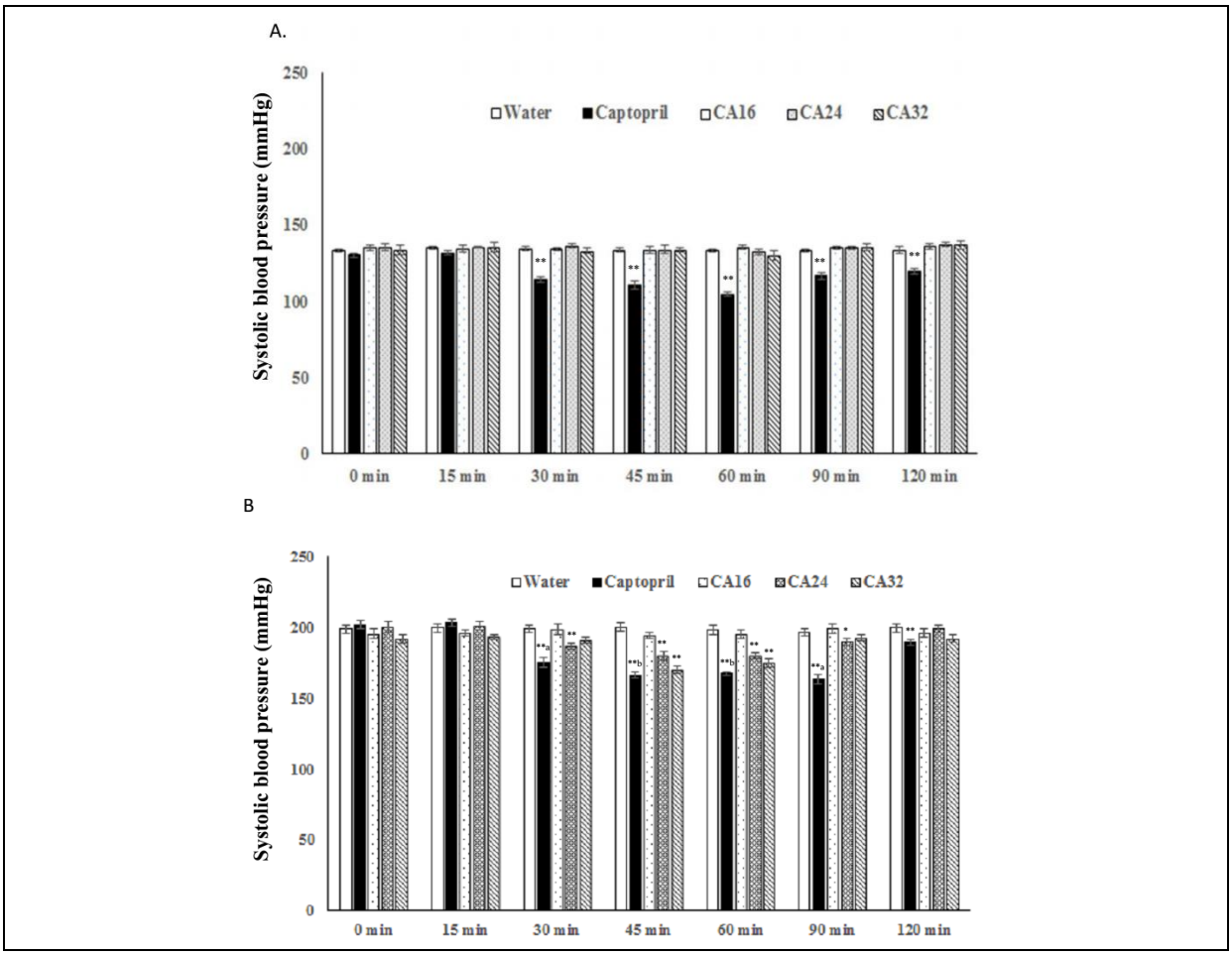

Figure 1. Systolic blood pressure after single-dose oral administration of *Centella asiatica* (CA) at doses 16, 24, and 32 g/kg BW, captopril 25 mg/kg BW and distilled water, in normal rats (A) and deoxycorticosterone acetate (DOCA) –salt hypertensive rats (B) at 15, 30, 45, 60, 90 and 120 min. (n=8 in each group). **(p<0.01) : significant difference from their starting values at 0 min; ^{a}(p<0.05) : significant difference from the control, CA at doses of 16, 24, 32 g/Kg treated group at the corresponding time; ^{b} (p<0.05) : significant difference from the control, CA at doses of 16 and 24 g/Kg treated group at the corresponding time

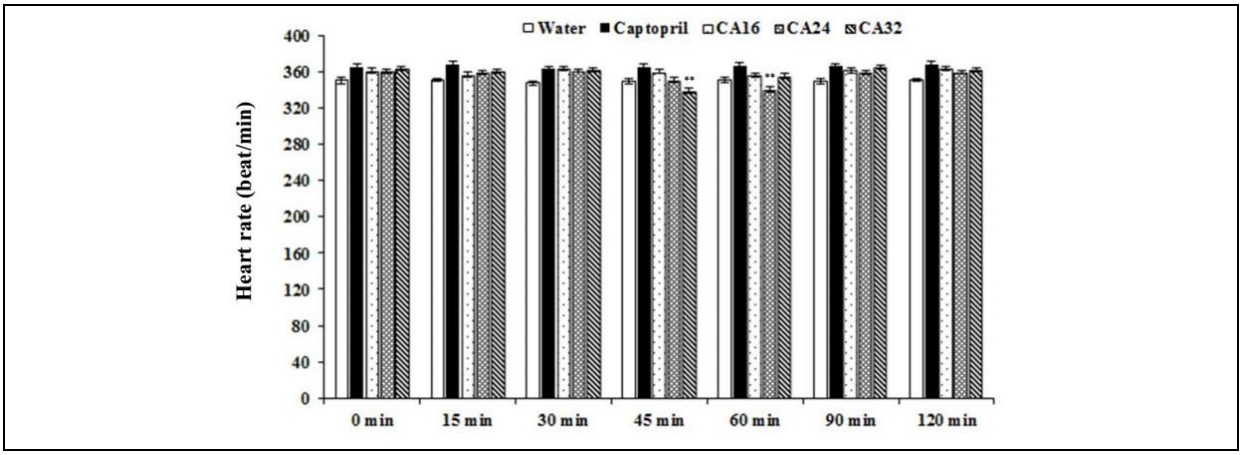

Figure 2. Heart rate after single-dose oral administration of *Centella asiatica* (CA) at doses 16, 24, and 32 g/kg BW, captopril 25 mg/kg BW and distilled water, in deoxycorticosterone acetate – salt hypertensive rats at 15, 30, 45, 60, 90 and 120 min. (n=8 in each group) **($p<0.01$): significant difference from their starting values at 0 min

reduced SBP by 19% at 90 min after the administration. Only two high doses of CA leave juice, 24 and 32 g/kg BW, significantly decreased SBP from 30 to 90 and from 45 to 60 min with the maximal reduction of 11 and 12%, respectively, occurring at 45 min in both groups. (Figure 1, B).

HR values were not altered after administration of either captopril or the three doses of CA leave juice in normal rats (data not shown). In DOCA-salt hypertensive rats, only CA leave juice at doses 24 and 32 g/kg BW significantly decreased HR at 60 and 45 min, respectively (Figure 2).

3.3. Effect of CA leave juice on rCBF, SBP and DBP

The rCBF measured at 7 periods (0, 15, 30, 45, 60, 90, and 120 minutes) after oral administration of CA leave juice at a dose 32 g/kg BW. Apparently, the rCBF levels of DOCA-salt hypertensive rats were significantly low, compared to that of the normal rats before the administration of CA leave juice. After administering CA leave juice, the rCBF significantly increased between 5-90 min in normal and 5-120 min in DOCA-salt hypertensive rats. The maximum increased rCBF of normal and DOCA-salt hypertensive rats were 37.37 % and 52.27%, respectively, occuring at 45 minutes after CA leave juice administration (Figure 3). Similar to the previous experiment, CA leave juice lowered the blood pressure only in DOCA-salt hypertensive rats. Both SBP and DBP significant decreased during 15 to 120 and 45 to 120 min, respectively (Figure 4 A and B).

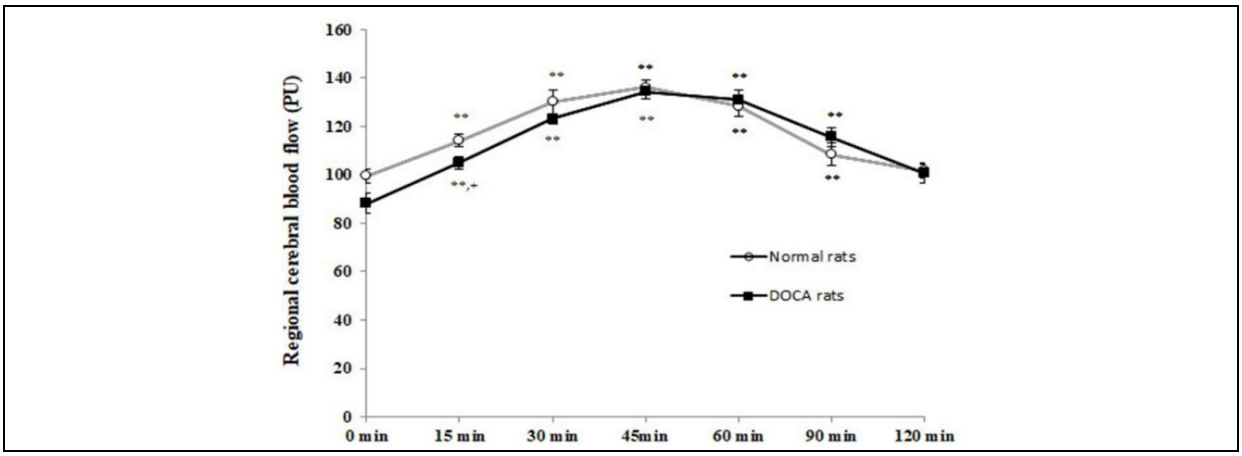

Figure 3. Regional cerebral blood flow after single-dose oral administration of *Centella asiatica* at dose 32 g/kg BW in normal and deoxycorticosterone (DOCA)-salt hypertensive rats at 15, 30, 45, 60, 90 and 120 min (n=8 in each group). *($p<0.05$), **($p<0.01$): significant difference from their starting values at 0 min; $^{+}$($p<0.05$): significant difference from the normal at the corresponding time

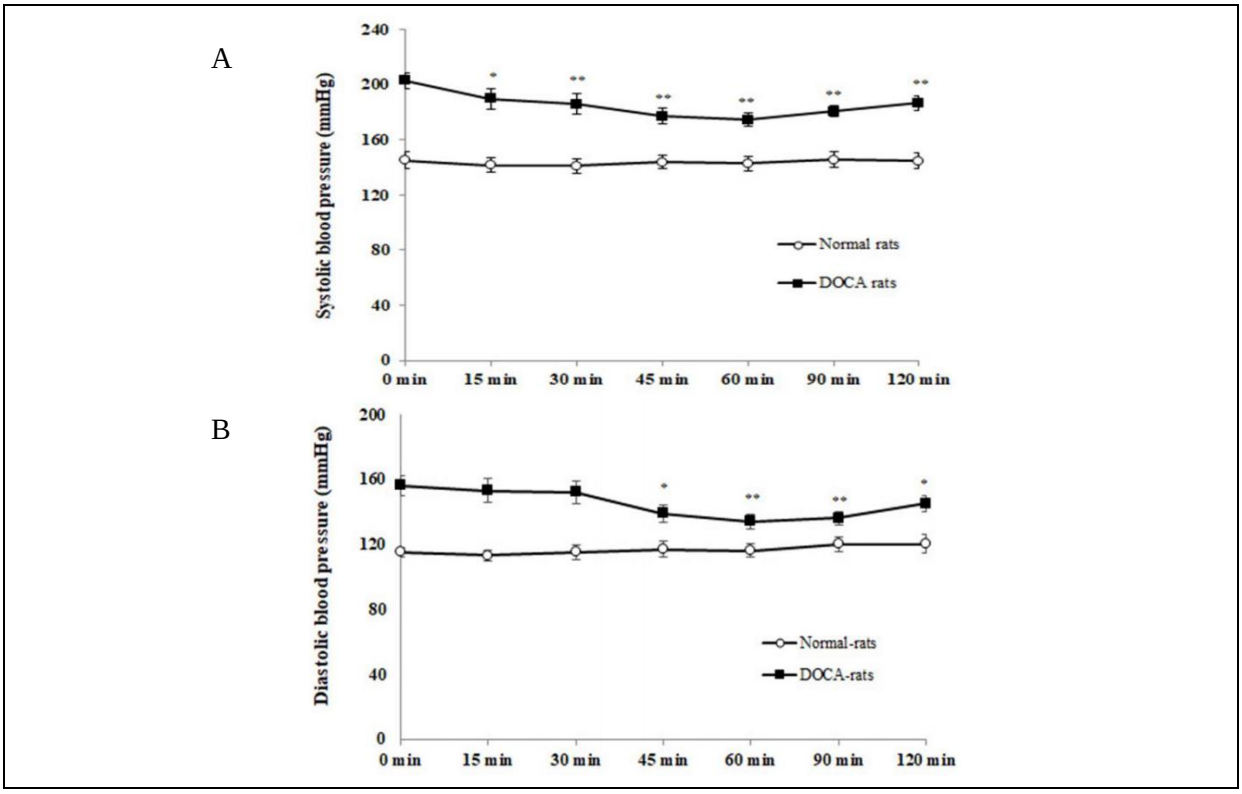

Figure 4. Systolic blood pressure (A) and diastolic blood pressure (B) after single-dose oral administration of Centella asiatica at dose 32 g/kg BW in normal and deoxycorticosterone (DOCA)-salt hypertensive rats at 15, 30, 45, 60, 90 and 120 min (n=8 in each group). *($p<0.05$), **($p<0.01$) : significant difference from their starting values at 0 min

4. DISCUSSION

Previous studies, using intravenous administration of various extracts of CA, demonstrated different effects on blood pressure and HR in normotensive animals [23-26]. Fresh juice and hot water extracts, shortly decreased blood pressure and HR in dogs. The 95% alcoholic extract slightly decreased blood pressure in dog and rats [24, 26], while 50% ethanol extract of whole plant and leaves had no effect in dogs [23, 25]. The alkaloid fraction neither changed the blood pressure nor the HR [26]. While the glycoside fraction decreased both blood pressure and HR. In isolated rabbit heart, the glycoside fraction also decreased force of contraction and heart rate with grater response on force than on rate[26]. However, when oral administration was studied in normotensive dogs using fresh juice, the effects on blood pressure and HR were not observed[26]. These studies suggested a weak effect of CA on cardiovascular functions in the normotensive state. A study in hypertensive rats, using 95% ethanol extract of CA at dose 2 g/kg BW daily for 7 days, found a decrease in blood pressure with no change in the HR. Hexane and dicholoromethane extracts of CA at dose 1g/kg BW showed similar effect [27].

In our study, CA leave juice given orally to rats showed alleviating effect on SBP and HR in hypertensive but not in normal rats. The angiotensin converting enzyme inhibitor captopril decreased SBP at similar levels in both hypertensive and normal rats without an effect on HR. Captopril inhibited the formation of angiotensin II and reduced its circulating levels. There were no evidence that angiotensin had a significant direct chronotropic effect. The mechanism of action of ACEI in lowering blood pressure was largely due to a withdrawal of vasoconstrictor role of angiotensin II with minimal changes in heart rate [33-34]. CA leave juice at a dose 32 g/kg BW showed a SBP reduction with delayed onset and short duration comparable to that of captopril. In addition, the same dose of CA leave juice lowered SBP and DBP only in hypertensive rats during rCBF determination. These results confirm the anti-hypertensive effect of CA leave juice. They demonstrate an anti-hypertensive activity with mild HR depression.

The present study provides the first evidence that CA enhances rCBF in both normal and DOCA-salt hypertensive rats. We found that while the maximum decrease of SBP (13.86%) and DBP (14.10 %) in DOCA-salt hypertensive

rats occurred at 60 minutes, the maximum increase in rCBF (52.12%) occurred at 45 minutes after the CA administration. In addition, the maximum increase in rCBF of CA in normal rats was 36.72% at 45 minutes, while CA had no effect on SBP and DBP. In the present study, the PaO_2 and $PaCO_2$ were controlled within the normal ranges throughout the period of cerebral blood flow measurement. Therefore, it is reasonable to regard the increased rCBF as occurring independent of the arterial blood pressure or metabolic changes. These results imply that CA may be useful in protecting brain damage from cerebral ischemia especially in hypertensive condition.

Several studies have demonstrated that CA had vasodilation activity. The chloroform extract of the whole plant decreased the maximal response to adrenaline-induced contraction of aortic strip from normal rats [28]. The vasodilation also has been demonstrated using a hexane extract in phenylephrine-induced contraction of aortic strip from hypertensive rats [29]. The blood flow at rabbit ears increased when perfused with a glycoside fraction [26]. CA significantly improved microcirculation in patients with moderate to chronic venous hypertension after oral administration for 6 to 12 months[35-36]. Vasodilation is the important mechanism in alleviating blood pressure as well as enhancing blood flow to various organs. We suggested that vasodilation may be responsible for the anti-hypertensive and increased rCBF actions of CA leave juice.

Hypertension constitutes an important risk factor for cerebrovascular diseases including stroke and the development of vascular cognitive impairment and vascular dementia [37]. From our results, CA leave juice lowered the elevated blood pressure and also increased rCBF suggesting a protective effect on hypertensive brain damage.

Alzheimer's disease, a common cause of dementia which is the major cause of disability and dependency among elderly worldwide, seems to involve oxidative stress. Several studies have demonstrated enhancing cognitive function as well as antioxidant activities of CA. The antioxidant activity of CA is comparable to that of α-tocopherol [38, 39]. Antioxidant activity of CA has also been observed in the brain [18, 40]. In the brain of normal rats, the whole plant aqueous extract decreased the levels of malonaldehyde with simultaneous increase of endogenous anti-oxidant enzymes, glutathione and catalase. That extract also improved rats learning and memory [18]. These actions of CA were also observed in various models of cognitive impairment and oxidative stress-induced rats [19-21, 41]. Orally administration of the water extract of CA also attenuated β-amyloid-associated behavioral abnormalities in mouse model of Alzheimer's disease [42]. In human study, CA improved mild cognitive impairment in elderly patients after 6 months of administration [43]. The enhanced working memory and mood improvement were also observed in elderly volunteer following the CA treatment [44]. CA was found to be safe by oral administration. The standardized CA extract containing 45. 74% of asiaticoside had LD50 >2 g/ kg by oral administration in rats. In addition, subchronic administration for 90 days at daily dose of 1 g/ kg produced no significant toxic effects and did not show any adverse effects after cessation of treatment for 3 weeks [45]. Oral administration of the acetone extract of CA showed LD50 > 4 g/kg and did not shows any toxic effects after subacute treatment for 15 days in mice [46].

Taken together from these previous studies and our results suggested the potential use of CA to alleviate the age-related decline in cognitive function and probably delay the development of dementia in both healthy and hypertensive elderly.

5. CONCLUSION

The present study demonstrates that CA leave juice lower blood pressure and slow heart rate only in hypertensive rats, and increased rCBF in both hypertensive and normal rats. The enhanced rCBF effect was more prominent in hypertensive than normotensive condition. We propose that CA leave juice has a vasodilation action capable of alleviating blood pressure as well as improving blood flow to the brain. Our results support the beneficial use of CA leave juice in elderly especially in those with a hypertensive condition. However, the precise mechanisms underlying these actions would require further investigation.

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Conflict of interest disclosure

The authors declare no personal or professional conflicts of interest regarding any aspect of this study.

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Ethical approval

The experimental protocol for this study was approved by the Institutional Animal Care and Use Committee of Faculty of Pharmacy, Mahidol University in accordance with the Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes recommended by The National Research Council of Thailand.

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