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Research Article

Vascular Reactivity Concerning *Orthosiphon stamineus* Benth-Mediated Antihypertensive in Aortic Rings of Spontaneously Hypertensive Rats

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Orthosiphon stamineus Benth has been traditionally used to treat hypertension. The study aimed to investigate the vascular reactivity of water extract (WOS) and water : methanolic (1 : 1) extract (WMOS) of *Orthosiphon stamineus* Benth and AT_1 receptors blocker in the mechanisms of antihypertensive mediated by α_1-adrenergic receptor and EDNO and PGI_2 releases in the SHR aortic rings. SHR (230–280 g) were divided into four groups: control, WOS, WMOS, and losartan. After being fed orally for 14 days, the aorta was harvested and subjected to PE (10^{-9} to 10^{-5} M) and ACh (10^{-9} to 10^{-5} M) with and without L-NAME (100 μM) and indomethacin (10 μM), respectively. WOS, WMOS, and losartan significantly reduced the contractile responses to PE intact suggesting the importance of endothelium in vasorelaxation. Losartan significantly enhanced the ACh-induced vasorelaxation. L-NAME significantly inhibited the ACh-induced relaxation in all groups. Indomethacin enhanced ACh-induced vasorelaxation in WMOS. Collectively, *Orthosiphon stamineus* leaves extract reduced vasoconstriction responses by the alteration of α_1-adrenergic and AT_1 receptors activities. The involvement of EDNO releases was clearly observed in this plant. In WOS, PGI_2 releases might not participate in the ACh-induced vasorelaxation. However, in WMOS, enhancement of vasorelaxation possibly due to continuous release of PGI_2.

1. Introduction

Orthosiphon stamineus Benth (syn.: *O. aristatus* (Bl.) Miq., *O. grandiflorus* Bold., *O. spicatus* (Thumb) Bak.; Lamiaceae) [1], or locally known as "Misai Kucing," leaves extracts have been used as traditional medicine [2] and possess benefits such as antidiabetic, ability to increase plasma triglyceride and plasma HDL-cholesterol concentrations [3], anti-lithiatic and hypouricemic effects [4, 5], antifungal [6], and ability to treat kidney stone and urinary tract diseases [7–9]. It has traditionally been used in Java for the treatment of hypertension and diabetes [1]. Hypertension has been reported to be associated with endothelium dysfunction in both human and animal studies [10]. Endothelium regulates vascular tone by releasing vasoconstrictors such as endothelins, prostanoids and oxygen reactive species, and vasodilators such as nitric oxide (NO), prostacyclin (PGI_2), and endothelial hyperpolarizing factor (EDHF). These vasodilators were a great discovery by Furchgott and Zawadzki in 1980 [11], known as endothelium derived relaxing factors (EDRF). It has been reported by Peach et al. [12] that releases of EDRF caused vasorelaxant effects of acetylcholine (ACh), which is dependent on the presence of the endothelial cells [11, 13].

Phenylephrine (PE) is a selective α_1-adrenergic receptor agonist that increases arterial blood pressure by peripheral vasoconstriction. α_1-Adrenergic receptors which exist

postsynaptically are G-protein-coupled receptors, and thus activation of cellular signaling is subsequent to the interaction with a G-protein. Activation of these receptors on vascular smooth muscle leads to vasoconstriction. PE has predominantly α_1-postjunctional receptors in rat's aorta [14]. Since PE is a selective α_1-adrenergic receptor agonist and losartan is AT_1 receptor blocker, there is possible relationship between AT_1 and α_1 receptors [15]. In addition, crosstalk between AT_1 and α_1 receptors in the smooth muscle of rabbit aorta is endothelium dependent [16]. It has been reported that MRC A isolated from *Orthosiphon stamineus* causes continuous decreases in systolic blood pressure (SBP) and heart rate (HR) after subcutaneous administration in conscious SHR [17]. However, studies on the antihypertensive mechanisms by *Orthosiphon stamineus* still remain unclear. The present study aimed to investigate the vascular reactivity of water extract (WOS) and water : methanolic (1 : 1) extract (WMOS) of *Orthosiphon stamineus* Benth and AT_1 receptors blocker in the mechanisms of antihypertensive effects mediated by α_1-adrenergic receptor and prostacyclin (PGI_2) and endothelium-derived nitric oxide (EDNO) releases in the SHR aortic rings.

2. Materials and Methods

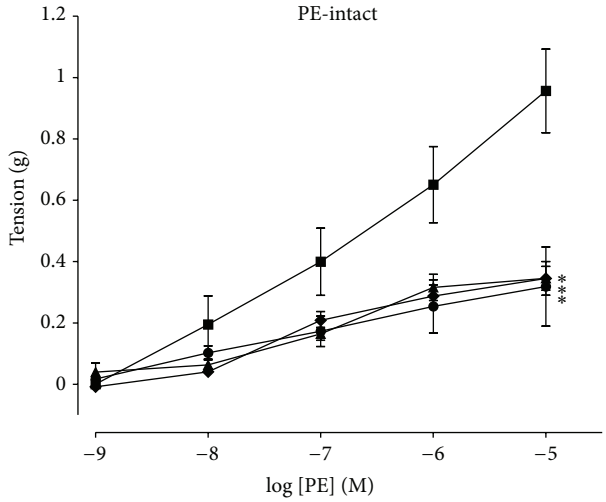
2.1. Preparation of Orthosiphon stamineus Leaves Extracts. Voucher specimen (no. 11009) of the plant material was deposited at Herbal Room, School of Pharmaceutical Sciences, Universiti Sains Malaysia (USM). WMOS was prepared by having dried and ground *Orthosiphon stamineus* leaves extracted by a mixture of methanol : water (1 : 1) using a Soxhlet extractor for a period of 12 hours, whereas preparation of WOS involved hot maceration of the dried and ground *Orthosiphon stamineus* leaves at 50°C for 6 hours and was repeated thrice. Each extract was bulked and concentrated in a rotary evaporator under vacuum and then freeze-dried and kept in a freezer until used [18]. WOS and WMOS were freshly prepared in distilled water prior to the feeding of the animals.

2.2. Animals. Male spontaneously hypertensive rats (SHR, 230–280 g) were housed in individual cages with free access to foods and water and maintained at Animal Transit Facility of School of Pharmaceutical Sciences, USM. All procedures involving animals were conducted according to the ethical guidelines by the Animal Ethics Committee, USM. The animals were divided into four groups: (1) WOS, 1000 mg/kg; (2) WMOS, 1000 mg/kg; (3) losartan, 10 mg/kg; and (4) control (vehicle). All animals were given daily treatment orally for 14 days before being subjected to vascular reactivity studies.

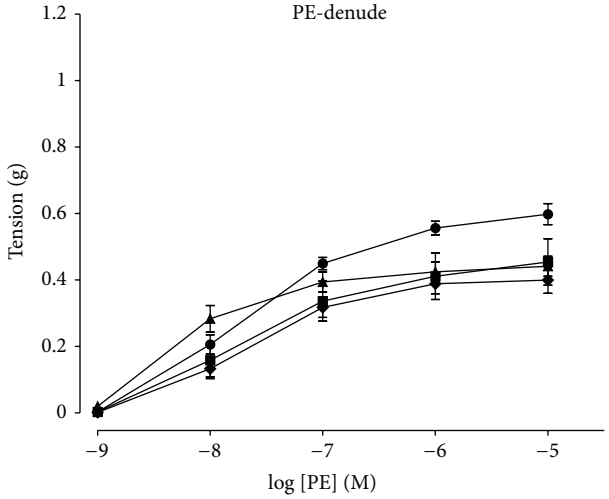
2.3. Drugs and Chemicals. Phenylephrine hydrochloride (PE), acetylcholine (ACh), indomethacin, and N^{ω}-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma-Aldrich, Germany, while sodium chloride (NaCl), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), magnesium sulphate ($MgSO_4{\cdot}7H_2O$), glucose, sodium hydrogen carbonate ($NaHCO_3$), and calcium chloride dehydrate ($CaCl_2{\cdot}H_2O$) were purchased from R&M Chem., UK. All drugs were freshly prepared in normal saline, except indomethacin in 0.5% (w/v) sodium carbonate, prior to use.

2.4. Vascular Reactivity Using Aortic Rings. The rat was anesthetized with sodium pentobarbital (60 mg/kg, i.p.). A midline abdominal incision was performed to expose the aorta. The thoracic aorta was carefully isolated, cleaned from the adherent fat and connective tissues, and cut into 3–5 mm rings. The aortic rings were then suspended horizontally in tissue chambers containing 10 mL of Kreb's solution (mmol/L: NaCl 118.6, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4{\cdot}7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.1, and glucose 11.0). The tissue-bath solution was bubbled incessantly with 95% O_2 and 5% CO_2 (carbogen) at 37°C. Aortic rings were then allowed to equilibrate at an optimal tension of 1 g for 45 min. Kreb's solution was replaced every 15 min, and the tension was readjusted to 1 g when necessary. At the beginning of the experiment, the presence of intact endothelial cells was confirmed by precontracting the tissues with PE (1 μM) and followed by relaxation with ACh (1 μM). Relaxation not less than 60% indicated the presence of intact endothelial cells. Responses were recorded isometrically via a force transducer (Grass FT03D) connected to a computerized data acquisition system (PowerLab; ADInstruments Pty Ltd., Australia). For vasoconstriction study, the concentration-response curves for PE (cumulative final chamber concentration of 10^{-9} to 10^{-5} M) were recorded. The contraction effects of PE were recorded in two different preparations, intact and denude endothelium. Denude endothelium of aortic rings was obtained by gently rubbing the intimal layer of the tissue with a blunt needle for a few times. The aortic rings were considered denuded when there were less than 10% relaxations to ACh (1 μM) precontracted with PE (1 μM) whereas in order to obtain the concentration-response curves of relaxation, ACh (10^{-9} to 10^{-5} M) was added cumulatively to the chamber at the plateau of the PE (1 μM) precontracted aortic rings at 3-minute intervals. To further assess the involvement of EDNO and prostacyclin (PGI_2) releases, relaxations of aortic rings were performed in WOS, WMOS, and losartan groups preincubated for 30 minutes with L-NAME (100 μM), a nonspecific NO synthase inhibitor, and indomethacin (10 μM), a nonselective cyclooxygenase inhibitor, respectively.

2.5. Data Analysis. All data are given as mean ± standard error means (SEM). PE-induced contraction and ACh-induced relaxation were analysed using one-way ANOVA followed by Dunnett's post hoc test, whereas the effects of ACh-induced relaxation after preincubated by L-NAME and indomethacin were analysed using Student's t-test. E_{max}, R_{max}, and pD_2 values were derived from nonlinear regression analysis. All analyses were using the computer software GraphPad Prism 5.0 for Windows (GraphPad Software Inc., USA). Values of $P < 0.05$ were considered statistically significant.



(a)



(b)

Figure 1: PE-induced contraction responses in intact (a) and denude (b) aortic rings from SHR treated with control (■), losartan (▲), and WOS (•) and WMOS (♦). Responses to PE are expressed as difference between absolute tension developed and baseline tension. Values are mean ± SEM of 5 to 8 SHRs in each group. *Denotes $P < 0.05$ compared to control analyzed by one-way ANOVA followed by Dunnett post hoc test.

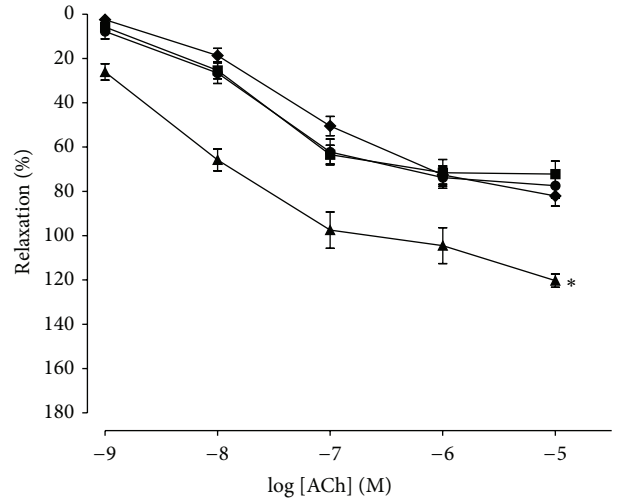


Figure 2: ACh-induced relaxation responses of aortic rings precontracted with PE (1 μM) in control (■), losartan (▲), WOS (•), and WMOS (♦). Responses to ACh are expressed as percentage of relaxation. Values are mean ± SEM of 5 to 8 SHRs in each group. *Denotes $P < 0.05$ compared to control.

3. Results

3.1. Vasoconstriction Effects of PE on Aortic Rings. Cumulative additions of PE (10^{-9} to 10^{-5} M) produced a concentration-dependent contraction of aortic rings in all groups. In PE intact, WOS, WMOS, and losartan significantly decreased ($P < 0.05$) the contractile responses as compared to control whereas, in PE denude endothelium, no significant changes were obtained (Figure 1). Maximal contractile responses (E_{max}) in PE intact were significantly decreased in WOS, WMOS, and losartan (0.30 ± 0.06, 0.33 ± 0.03, 0.35 ± 0.03 versus 0.90 ± 0.10). In contrast, the E_{max} of WMOS significantly enhanced the contraction responses in PE denude (0.57 ± 0.02 versus 0.43 ± 0.04). The pD_2 values from both PE intact and denude endothelium were unaltered as shown in Table 1.

3.2. Vasorelaxant Effects of ACh Precontracted with PE on Aortic Rings. ACh (10^{-9} to 10^{-5} M) produced dose-dependent relaxation in all groups in aortic rings precontracted with PE (1 μM). Only losartan significantly enhanced ($P < 0.05$) the relaxant effect of ACh as compared to control (R_{max} 111.20 ± 4.08 versus 73.15 ± 3.03). Both extract groups did not significantly alter the vasorelaxant effects of ACh as shown in Figure 2 and Table 2.

3.3. Effects of L-NAME on ACh-Induced Relaxation in Aortic Rings in WOS, WMOS, and Losartan Groups. To assess the contribution of EDNO, the aortic rings were preincubated with L-NAME (100 μM), a NO synthase inhibitor for 30 minutes. ACh-induced relaxations in all groups were significantly inhibited ($P < 0.05$) by L-NAME as shown in Figure 3. R_{max} and pD_2 values were tabulated in Table 2.

3.4. Effects of Indomethacin on ACh-Induced Relaxation in Aortic Rings in WOS, WMOS, and Losartan Groups. To investigate the role of prostacyclin (PGI_2) releases, the aortic rings were preincubated with indomethacin (10 μM), a COX inhibitor for 30 minutes. Indomethacin significantly reduced ($P < 0.05$) the ACh-induced relaxations in losartan and in contrast, significantly improved vasorelaxation in WMOS (Figure 4). R_{max} and pD_2 values were tabulated in Table 2.

3.5. Role of Intracellular and Extracellular Calcium Mobilization on the PE-Induced Contraction. To assess the role of intracellular and extracellular calcium mobilization, the aortic rings were incubated in Ca^{2+}-free medium containing

Table 1: Maximal contractile (E_{max}) responses and sensitivity (pD_2) for PE intact- and PE denude-induced contraction's in aortic rings.

Group	PE intact		PE denude	
	E_{max} (g)	pD_2 ($-\log EC_{50}$)	E_{max} (g)	pD_2 ($-\log EC_{50}$)
Control (vehicle)	0.90 ± 0.10	6.67 ± 0.31	0.43 ± 0.04	7.73 ± 0.32
Losartan (10 mg /kg)	0.35 ± 0.03*	6.84 ± 0.22	0.43 ± 0.02	8.39 ± 0.19
WOS (1000 mg/kg)	0.30 ± 0.06*	7.05 ± 0.62	0.39 ± 0.02	7.72 ± 0.18
WMOS (1000 mg/kg)	0.33 ± 0.03*	7.24 ± 0.22	0.57 ± 0.02*	7.72 ± 0.10

Each value represents the mean ± SEM of 5 to 8 SHRs. *Denotes $P < 0.05$ compared to control for each drug.

Table 2: Maximal relaxant effects (R_{max}) and sensitivity (pD_2) for ACh-induced relaxation in aortic rings in the absence and presence of indomethacin and L-NAME.

Treatment groups	ACh		ACh + indomethacin		ACh + L-NAME	
	R_{max} (% of relaxation)	pD_2 ($-\log EC_{50}$)	R_{max} (% of relaxation)	pD_2 ($-\log EC_{50}$)	R_{max} (% of relaxation)	pD_2 ($-\log EC_{50}$)
Control	73.15 ± 3.03	7.72 ± 0.15	91.74 ± 2.39	7.33 ± 0.08	NI	NI
Losartan (10 mg/kg)	111.20 ± 4.08#	7.98 ± 0.17	90.20 ± 7.64*	7.31 ± 0.28	29.55 ± 2.46*	6.73 ± 0.30
WOS (1000 mg/kg)	76.42 ± 3.39	7.62 ± 0.17	91.04 ± 3.07	7.26 ± 0.11	7.12 ± 1.35*	8.17 ± 0.88
WMOS (1000 mg/kg)	79.56 ± 2.97	7.24 ± 0.12	96.37 ± 6.33*	7.32 ± 0.24	NI	NI

Values are mean ± SEM of 5 to 8 SHRs in each group. #Denotes $P < 0.05$ compared to control and *denotes $P < 0.05$ compared to ACh without inhibitors. NI: not identified by nonlinear regression analysis by GraphPad Prism 5.0.

0.1 mM EGTA. Under this condition, PE induced transient contraction mainly from sarcoplasmic reticulum. In endothelium-denuded aortic rings, a transient contractile response in Ca^{2+}-free medium was elicited by 10^{-6} M PE. A second contraction known as sustained contraction was then induced again by PE. The percentage contractile responses to PE were significantly reduced ($P < 0.05$) in losartan (30.69 ± 4.41%) and WOS (25.35 ± 1.61%) as compared to control (48.69 ± 7.59%) in response to PE in Ca^{2+}-free medium (Figure 5). When the same procedure was repeated in normal $Ca2^{+}$-containing medium which contained 2.5 mM $CaCl_2$, no significant difference was seen in the treatment groups.

4. Discussion

The present study demonstrated that, in intact endothelium, the contractile response to phenylephrine (PE), a selective agonist for a_1-adrenergic receptor, was significantly lowered in SHR treated with WOS and WMOS as compared to control. No significant change was seen in denude endothelium. These results showed that 14-day oral treatment of WOS and WMOS affected the α_1-adrenergic receptors activities in this preparation. The use of PE as a vasoconstrictor in the present study because the rat's aorta has predominantly α_1-postjunctional receptors [14]. Furthermore Griffith et al. [19] and Martin et al. [20] demonstrated that suppression of constrictor responses to several agonists such as PE in the intact vascular endothelium may be due continuously basal release of endothelium-derived relaxing factor (EDRF) from endothelial cells. As seen in the present study, WOS and WMOS inhibited the contraction induced by PE as comparable to losartan. There were studies found that possible crosstalk between AT_1 and α_1-adrenoceptors existed [21–23]. Furthermore, Maeso et al. [24] reported that losartan reduced vasoconstrictor responses to PE in SHR aortic rings via endogenous Angiotensin II (AngII) acting on AT_1 receptors. Activation of AT_1 receptors results in increasing systolic blood pressure (SBP), blood vessels growth, and associated vascular smooth muscle cells (vsmc) apoptosis [25]. Blockade of AT_1 receptors which inhibit the effects of AngII may promote good prognosis in pathological conditions such as hypertension and to inhibit vasoconstriction [26]. Hence, we may suggest that (1) *Orthosiphon stamineus* leaves extracts exert their antihypertensive effects by blunting the increase of blood pressure in SHR; and (2) *Orthosiphon stamineus* leaves extracts may play their role in reducing vasoconstriction similar as AT_1 receptor blocker. WOS and WMOS may possibly possess antihypertensive properties and exert similar effects through these interactions.

Our results showed that the presence of endothelium is very important in vasorelaxation. It is likely that contribution by the variable EDRF such as NO, prostacyclin, and EDHF caused vascular smooth muscle cells to relax. The necessary endothelial cells for the relaxation by acetylcholine (ACh) to be occurred have been discovered since 1980 by Furchgott and Zawadzki. They demonstrated that loss of endothelium by rubbing the intimal surface of aorta caused no relaxation induced by ACh. ACh acts on muscarinic receptors of these cells thus stimulates substances that caused relaxation of vascular smooth muscle cells. In the present study, aortic rings from both WOS and WMOS showed essentially similar relaxant effects with control by dose-response manner to ACh (10^{-9} to 10^{-5} M). It may speculate that both extracts given orally did not alter the endothelium of the rats; thus

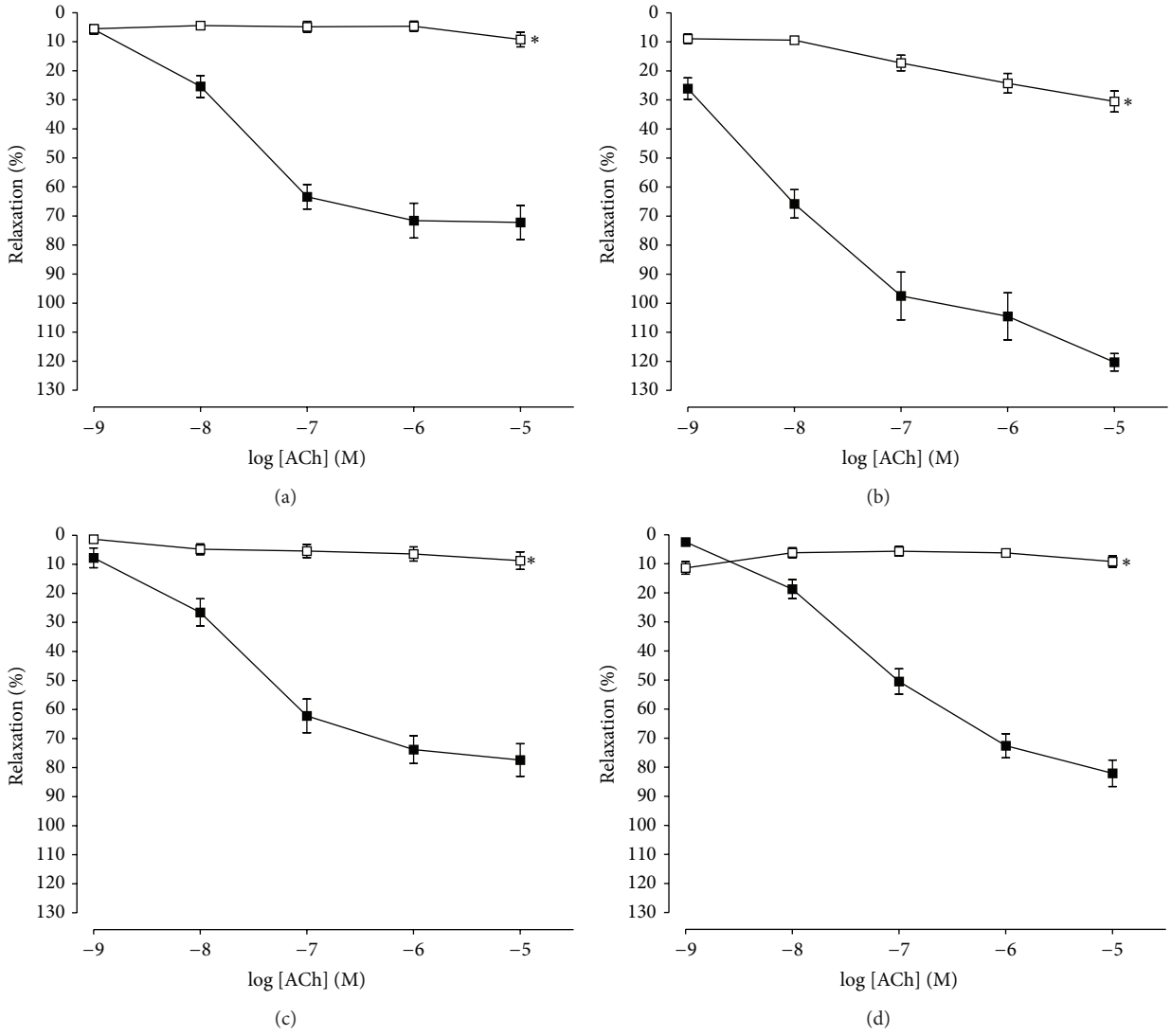


Figure 3: ACh-induced relaxation responses of aortic rings precontracted with PE (1 μM) in the presence of L-NAME (100 μM) in control (vehicle) (a), losartan (10 mg/kg) (b), WOS (1000 mg/kg) (c), and WMOS (1000 mg/kg) (d). Responses to ACh are expressed as percentage of relaxation. Values are mean ± SEM of 5 to 6 SHRs in each group. *Denotes $P < 0.05$ compared to ACh without L-NAME (■).

the aortic rings isolated from these rats showed no effect to the ACh-induced relaxation. In contrast, losartan showed significantly greater relaxation. It is plausible that blockade of AT_1 receptor further enhanced the relaxation caused by ACh. Furthermore, Schiffrin and Touyz [27] demonstrated that losartan enhanced the endothelium-dependent relaxation to ACh in SHR aortic rings. In this preparation, the relaxation to ACh was probably due to the production or release of EDRF [12] and the endothelial vasorelaxant factors derived from cyclooxygenase (COX) pathways (prostacyclin PGI_2 released from endothelial cells).

WOS showed similar result of ACh-induced relaxation after preincubation with indomethacin. This similar effect has been reported by Luscher and Vanhoutte [28]. However, WMOS improved vasorelaxation to ACh after blockade of COX pathways. In this case, there was plausibility because the vasodilator PGI_2 was continuously released as indicated by its tonic effects on platelet cyclic adenosine monophosphate (cAMP) [29]. In contrast, losartan significantly reduced the ACh-induced relaxations, which may be due to the attenuation of PGI_2 production which was compensated for by the enhanced release of another vasodilator, for example, nitric oxide (NO). In this point of view, we might suggest that WMOS and blockade of AT_1 receptors modulate the derived endothelial vasorelaxant factors such as PGI_2 from COX pathways.

The release of NO by endothelial cells to vascular smooth muscle cells causes vasorelaxation. NO to play a vital role in the maintenance of vascular tone [30]. In order to assess the contribution of NO releases in the vasorelaxant effects elicited by *Orthosiphon stamineus* leaves extracts, we preincubated the rat aortic rings with L-NAME (100 μM), NO synthase inhibitor. Our study showed that the ACh-induced vasorelaxation in all treatment groups reduced significantly after

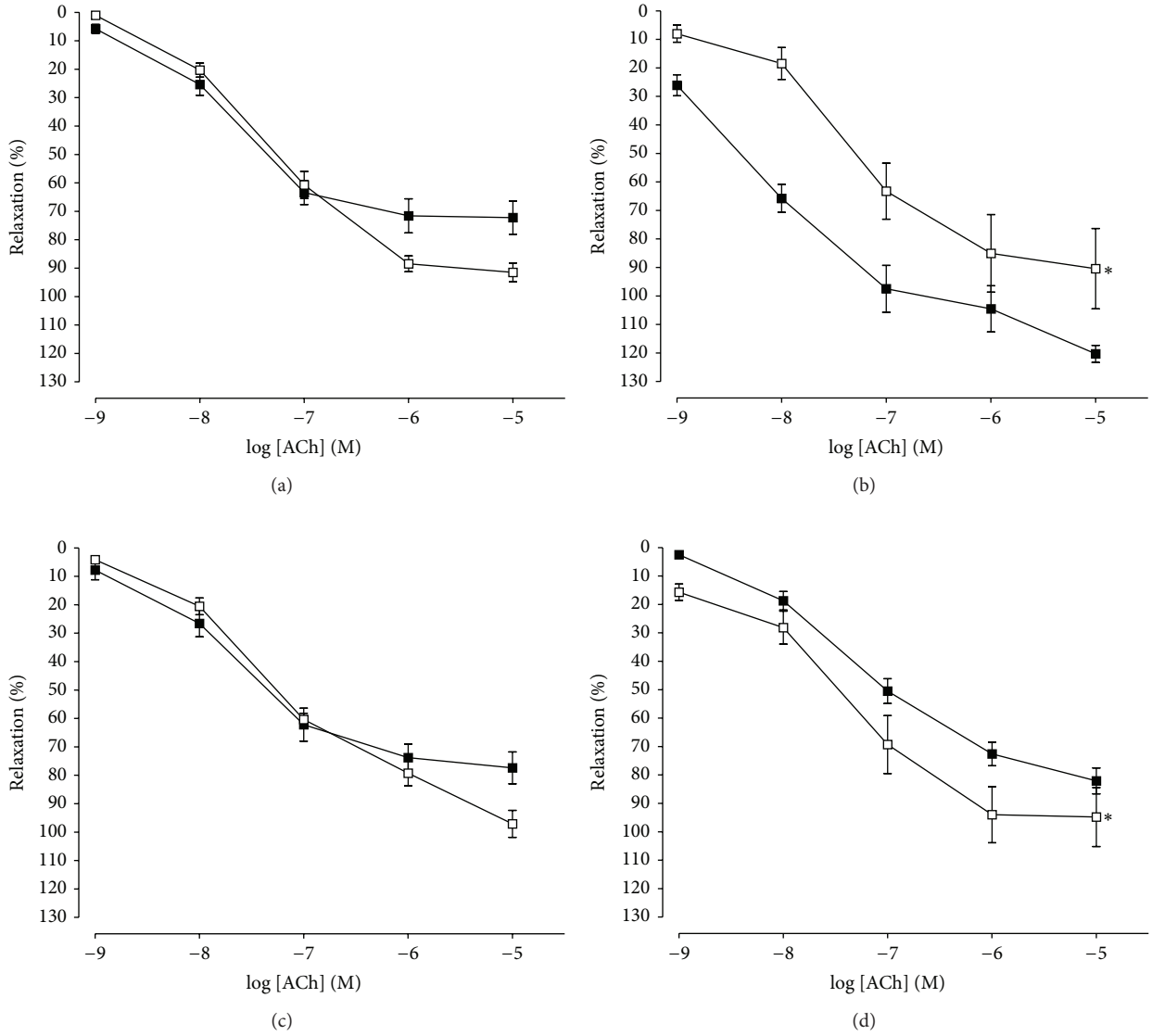


FIGURE 4: ACh-induced relaxation responses of aortic rings precontracted with PE (1 μM) in the presence of indomethacin (10 μM) in control (vehicle) (a), losartan (10 mg/kg) (b), WOS (1000 mg/kg) (c), and WMOS (1000 mg/kg) (d). Responses to ACh are expressed as percentage of relaxation. Values are mean ± SEM of 5 to 6 SHRs in each group. *Denotes $P < 0.05$ compared to ACh without indomethacin (■).

inhibition of NO synthase pathways. From the present data, it could be clearly proven that NO synthase pathways were involved in the vasorelaxation in the SHR.

In view of the present study, it is plausible that the vasorelaxant activities produced by *Orthosiphon stamineus* extracts may take place in the vascular smooth muscle cells. To investigate the effects of both extracts and losartan on the role intracellular Ca^{2+} on the contractility of the vascular smooth muscle cells of the aortic rings, media absent of and with Ca^{2+} were used. Significantly reduced contraction response to PE in denude aortic rings observed in the WOS and losartan were possibly due to inhibition of intracellular Ca^{2+} release from the sarcoplasmic reticulum at the level of vascular smooth muscle cells. Decreased intracellular Ca^{2+} concentration and increased myosin light chain phosphatase activity may had caused the smooth muscle to undergo weaker vascular contractility. Also, inhibition of receptor- and voltage-operated Ca^{2+} channels in the plasma membrane reduced Ca^{2+} influx may contribute as well [31].

5. Summary

In conclusion, our studies showed that water extract (WOS) and water : methanolic (1 : 1) extract (WMOS) of *Orthosiphon stamineus* Benth leaves promote antihypertensive effects by reducing vasoconstriction through the alteration of α_{1-}adrenergic and AT_1 receptors activities. Vasorelaxant effects of both WOS and WMOS may possibly involve mainly the release of EDNO. In WOS, PGI_2 releases might not be participated in the ACh-induced vasorelaxation. However

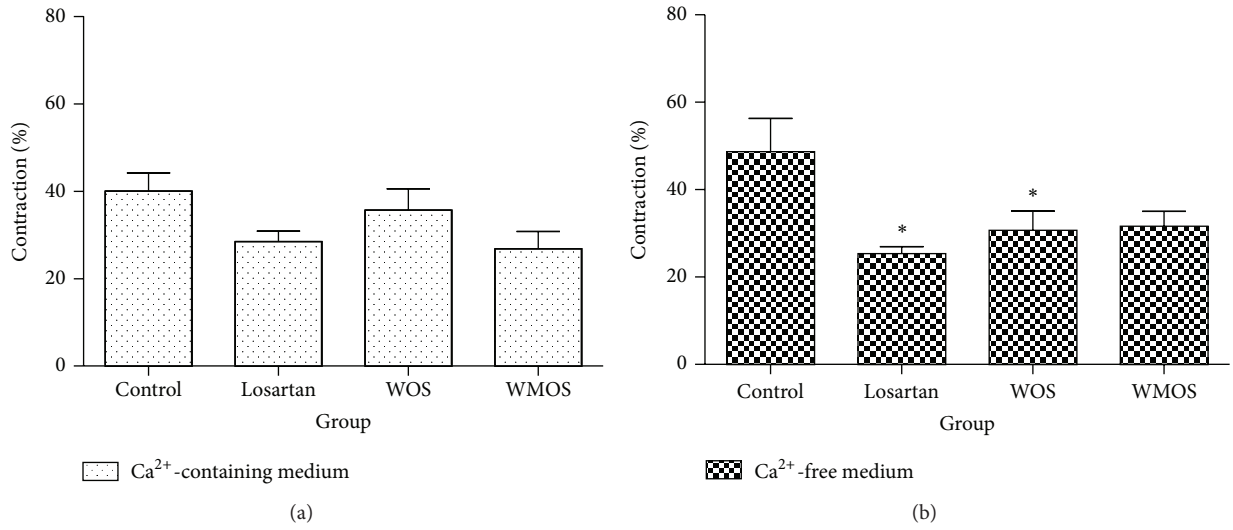


Figure 5: Histograms showing the mean of the response induced by 10^{-6} M PE without endothelium in Ca^{2+}-containing medium or Ca^{2+}-free medium. Results are expressed as mean ± SEM of 6 experiment sets. $^{*}P < 0.05$ compared to control.

in WMOS, enhancement of vasorelaxation might be due to the fact that vasodilator PGI_2 is continuously released as indicated by its tonic effects on platelet cAMP. In addition, WOS inhibited the contraction of aortic rings induced by PE, implying that WOS inhibits the release of intracellular Ca^{2+} and/or blocks ROCC.

Acknowledgments

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Conflict of Interests

The authors declare that they have no conflict of interests.

References

[1] S. Awale, Y. Tezuka, A. H. Banskota, S. Shimoji, K. Taira, and S. Kadota, "Norstaminane- and isopimarane-type diterpenes of *Orthosiphon stamineus* from Okinawa," *Tetrahedron*, vol. 58, no. 27, pp. 5503–5512, 2002.

[2] C. Wiart, "*Orthosiphon stamineus* Benth," in *Medicinal Plants of Southeast Asia*, F. K. Wong, Ed., pp. 264–265, Prentice Hall, Kuala Lumpur, Malaysia, 2002.

[3] K. Sriplang, S. Adisakwattana, A. Rungsipipat, and S. Yibchokanun, "Effects of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats," *Journal of Ethnopharmacology*, vol. 109, no. 3, pp. 510–514, 2007.

[4] G. A. Schut and J. H. Zwaving, "Pharmacological investigation of some lipophilic flavonoids from *Orthosiphon aristatus*," *Fitoterapia*, vol. 64, no. 2, pp. 99–102, 1993.

[5] O. M. Arafat, S. Y. Tham, A. Sadikun, I. Zhari, P. J. Haughton, and M. Z. Asmawi, "Studies on diuretic and hypouricemic effects of *Orthosiphon stamineus* methanol extracts in rats," *Journal of Ethnopharmacology*, vol. 118, no. 3, pp. 354–360, 2008.

[6] M. A. Hossain, Z. Ismail, A. Rahman, and S. C. Kang, "Chemical composition and anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth," *Industrial Crops and Products*, vol. 27, no. 3, pp. 328–334, 2008.

[7] D. D. Doan, N. H. Nguyen, H. K. Doan et al., "Studies on the individual and combined diuretic effects of four Vietnamese traditional herbal remedies (Zea mays, Imperata cylindrica, Plantago major and *Orthosiphon stamineus*)," *Journal of Ethnopharmacology*, vol. 36, no. 3, pp. 225–231, 1992.

[8] Y. Tezuka, P. Stampoulis, A. H. Banskota et al., "Constituents of the Vietnamese medicinal plant *Orthosiphon stamineus*," *Chemical and Pharmaceutical Bulletin*, vol. 48, no. 11, pp. 1711–1719, 2000.

[9] S. Awale, Y. Tezuka, A. H. Banskota, K. Kouda, K. M. Tun, and S. Kadota, "Five novel highly oxygenated diterpenes of *Orthosiphon stamineus* from Myanmar," *Journal of Natural Products*, vol. 64, no. 5, pp. 592–596, 2001.

[10] S. Taddei, A. Virdis, L. Ghiadoni, G. Salvetti, and A. Salvetti, "Endothelial dysfunction in hypertension," *Journal of Nephrology*, vol. 13, no. 3, pp. 205–210, 2000.

[11] R. F. Furchgott and J. V. Zawadzki, "The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine," *Nature*, vol. 288, no. 5789, pp. 373–376, 1980.

[12] M. J. Peach, A. L. Loeb, H. A. Singer, and J. Saye, "Endothelium-derived vascular relaxing factor," *Hypertension*, vol. 7, no. 311, pp. 94–100, 1985.

[13] R. F. Furchgott, J. V. Zawadzki, and P. D. Cherry, "Role of endothelium in the vasodilator response to acetylcholine," in

Vasodilatation, P. M. Vanhoutte and I. Leusen, Eds., pp. 49–66, Raven Press, New York, NY, USA, 1981.

[14] L. Alosachie and T. Godfraind, "The modulatory role of vascular endothelium in the interaction of agonists and antagonists with α-adrenoceptors in the rat aorta," *British Journal of Pharmacology*, vol. 95, no. 2, pp. 619–629, 1988.

[15] M. H. Abdulla, M. A. Sattar, N. A. Abdullah et al., "Inhibition of Ang II and renal sympathetic nerve influence dopamine-and isoprenaline-induced renal haemodynamic changes in normal Wistar-Kyoto and spontaneously hypertensive rats," *Autonomic and Autacoid Pharmacology*, vol. 28, no. 4, pp. 95–101, 2008.

[16] S. Jerez, P. De Bruno María, and C. Alfredo, "Cross talk between angiotensin II and alpha 1 adrenegic receptors in rabbit aorta:role of endothelium," *Journal of Cardiovascular Pharmacology*, vol. 43, no. 3, pp. 402–409, 2004.

[17] T. Matsubara, T. Bohgaki, M. Watarai, H. Suzuki, K. Ohashi, and H. Shibuya, "Antihypertensive actions of methylripariochromene A from *Orthosiphon aristatus*, an Indonesian traditional medicinal plant," *Biological and Pharmaceutical Bulletin*, vol. 22, no. 10, pp. 1083–1088, 1999.

[18] M. J. A. Siddiqui and Z. Ismail, "Simultaneous analysis of bioactive markers from *Orthosiphon stamineus* benth leaves extracts by reverse phase high performance liquid chromatography," *Tropical Journal of Pharmaceutical Research*, vol. 10, no. 1, pp. 97–103, 2011.

[19] T. M. Griffith, D. H. Edwards, and M. J. Lewis, "The nature of endothelium-derived vascular relaxant factor," *Nature*, vol. 308, no. 5960, pp. 645–647, 1984.

[20] W. Martin, R. F. Furchgott, G. M. Villani, and D. Jothianandan, "Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor," *Journal of Pharmacology and Experimental Therapeutics*, vol. 237, no. 2, pp. 529–538, 1986.

[21] M. H. Abdulla, M. A. Sattar, I. M. Salman, O. Z. Ameer, N. A. Abdullah, and E. J. Johns, "The interaction between renin-angiotensin and sympathetic systems in the renal vasculature of Wistar-Kyoto rats," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 2, no. 2, pp. 1–5, 2009.

[22] M. H. Abdulla, M. A. Sattar, N. A. Abdullah, M. A. H. Khan, H. H. AbdAllah, and E. J. Johns, "Chronic treatment with losartan and carvedilol differentially modulates renal vascular responses to sympathomimetics compared to treatment with individual agents in normal Wistar Kyoto and spontaneously hypertensive rats," *European Journal of Pharmacology*, vol. 612, no. 1–3, pp. 69–74, 2009.

[23] M. H. Abdulla, M. A. Sattar, N. A. Abdullah, M. A. H. Khan, K. R. L. Anand Swarup, and E. J. Johns, "The effect of losartan and carvedilol on vasopressor responses to adrenergic agonists and angiotensin II in the systemic circulation of Sprague Dawley rats," *Autonomic and Autacoid Pharmacology*, vol. 31, no. 1-2, pp. 13–20, 2011.

[24] R. Maeso, J. Navarro-Cid, R. Muñoz-García et al., "Losartan reduces phenylephrine constrictor response in aortic rings from spontaneously hypertensive rats: role of nitric oxide and angiotensin II type 2 receptors," *Hypertension*, vol. 28, no. 6, pp. 967–972, 1996.

[25] Q. N. Diep, J.-S. Li, and E. L. Schiffrin, "*In vivo* study of AT_1 and AT_1 angiotensin receptors in apoptosis in rat blood vessels," *Hypertension*, vol. 34, no. 4, pp. 617–624, 1999.

[26] T. Unger, "The role of the renin-angiotensin system in the development of cardiovascular disease," *American Journal of Cardiology*, vol. 89, no. 2, supplement 1, pp. 3–9, 2002.

[27] E. L. Schiffrin and R. M. Touyz, "From bedside to bench to bedside: role of renin-angiotensin-aldosterone system in remodeling of resistance arteries in hypertension," *American Journal of Physiology*, vol. 287, no. 2, pp. H435–H446, 2004.

[28] T. F. Luscher and P. M. Vanhoutte, "Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat," *Hypertension*, vol. 8, no. 4, pp. 344–348, 1986.

[29] U. Pohl, C. Nolte, A. Bunse, M. Eigenthaler, and U. Walter, "Endothelium-dependent phosphorylation of vasodilator-stimulated protein in platelets during coronary passage," *American Journal of Physiology*, vol. 266, no. 2, pp. H606–H612, 1994.

[30] S. Moncada, R. M. J. Palmer, and E. A. Higgs, "Nitric oxide: physiology, pathophysiology, and pharmacology," *Pharmacological Reviews*, vol. 43, no. 2, pp. 109–142, 1991.

[31] R. C. Webb, "Smooth muscle contraction and relaxation," *American Journal of Physiology*, vol. 27, no. 1–4, pp. 201–206, 2003.