Antihypertensive effect of \textit{Gynura Procumbens} Water Extract in Spontaneously Hypertensive Rats

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\textbf{Summary.} Present study was designed to evaluate the antihypertensive effect of \textit{Gynura procumbens} water extract (GPWE) in spontaneously hypertensive (SH) rats. Short term fall of mean arterial pressure (MAP) and heart rate (HR) occur in Sprague Dawley (SD) and SH rats treated with GPWE $1\text{ g kg}^{-1}$ (single dose). Treatment of SH rats with repeated dose of 300 and 600 mg kg$^{-1}$ of GPWE and water (control) through gastric gavage for 4 weeks shows long lasting effects on MAP and HR along with increased urine flow rate (UFR). Pressor responses induced by different agonist’s acetylcholine (ACH), phenylephrine (PE), methoxamine (Mtx), angiotensin II (AngII), and isoprenaline (IsoP) were significantly inhibited, with more stability to chronotropic effects of agonists, in GPWE repeatedly treated anaesthetized SH rats compared to control rats. These data indicate, GPWE lower blood pressure through non selective pathway mediated via stimulation of vasodilation, heart stabilization and diuretic effect.

\textbf{Industrial relevance.} Healing powers of plants are known since ancient times, in this respect herbs have been used for medical treatment since the beginning of human civilization. The present study provides sound pharmacological basis for use of flavonoid rich \textit{Gynura procumbens} in hypertension and palpitation.

\textbf{Keywords:} Diuretic; \textit{Gynura procumbens} Merr.; heart rate; mean arterial pressure; spontaneously hypertensive rats

\section*{INTRODUCTION}

Cardiovascular disease is the leading and rising reason of the worldwide burden of disease. An estimated 17.3 million people died from CVDs in 2008, representing 30\% of all global deaths (WHO, 2012). Hypertension is a foremost contributor to cardiovascular diseases, stroke, heart attack and renal failure (Collins et al., 1990; Hardman et al., 2006; American Heart Association, 2013). Herbal treatments have been used since long for cardiovascular disorders like congestive heart failure, hypertension, atherosclerosis, cerebral and venous insufficiency, arrhythmia and ischemic heart disease (Mashour et al., 1998). \textit{Gynura procumbens} Merr. from the family Compositae, is found throughout the continental South-East Asia, Malaysia and also the western and central Africa. In Malaysia, this plant is called as “sambung nyawa” means extending life. The leaves of this plant are traditionally used for the treatment of kidney diseases, eruptive fever, rash, hypertension, diabetes mellitus and hyperlipidemia (Perry, 1980). Phytochemical investigation of this plant showed the presence of various classes of natural compounds kaempferol, quercetin, rutin (Akowuah et al, 2001), chlorogenic acid (Rosidah et al, 2008), sitosterol, stigmasterol, nucleic acid (Sadikun et al, 1996). Quercetin has been reported to affect Na$^+$ reabsorption in renal tubules and have potent vasodilating effect on noradrenaline precontracted vessels (Pérez-Vizcaíno et al, 2002) play role in regulation of blood pressure (Aoi et al, 2004). Dietary chlorogenic acid is found to reduce oxidative stress and improves nitric oxide bioavailability leading to the attenuation of hypertension in spontaneously hypertensive rats (Suzuki et al, 2006). Our previous study indicated that crude water extracts of \textit{Gynura procumbens} leaves have high content of flavonoids and exhibit \textit{in vitro} vasodilating and cardiorelaxing properties (Kaur et al, 2012). This investigation aimed to address the possible mechanisms underlying the antihypertensive effect of \textit{Gynura procumbens}. For that purpose, we investigated various pressor agonists effect on anaesthetized, GPWE treated, spontaneously hypertensive rats.
MATERIALS AND METHODS

**Animals.** Experimental animals consisted of adult male Sprague Dawley (SD) and spontaneously hypertensive (SH) rats, 250±15 g, which were bred and obtained from Animal Research And Service Centre (ARASC), USM, Malaysia. The animals were housed in standard environmental conditions (25°C, 60-70 % humidity) under natural lighting and fed with normal commercial rat chow (Gold Coin Feed Mills Sdn. Bhd., Malaysia) and water ad libitum. The rats were acclimatised for one week in transit room before used for experiments. Guidelines of the Animal Ethics Committee, USM, Malaysia {Animal ethic approval no: USM/Animal Ethics Approval/2011/ (70) (322)} were followed while handling animals. Experiments consisted of 6 animals for each group.

**Preparation of water extract.** *Gynura procumbens* Merr leaves were collected from Penang Island in the month of July, Malaysia. A voucher specimen 10833 was deposited at the Herbarium of the School of Biological Sciences, University Sains Malaysia, Penang. The dried leaves powder (200 g) was extracted by maceration process with water at 60°C in water bath.

**Single dose effect.** Two groups of both SD and SH male rats, (250±15 g), fasted overnight, were given deionised water (10 ml kg⁻¹) (control,SD and control,SH) and GPWE (1 g (10 ml)⁻¹, 10 ml kg⁻¹) (GPWE,SD and GPWE,SH) by gastric gavage. The MAP and HR were measured in the caudal artery by an indirect tail-cuff method before and after treatment, using Pulse Amplifier (model 29) and cuff pump (model 20NW) linked to computerized data acquisition system (ADInstruments PowerLab Software). The rats were initially trained for blood pressure measurement on at least three separate occasions to establish a baseline blood pressure.

**Repeated dose effect.** Three groups of SH rats (250±10 g) were treated orally once daily with deionised water, 10 ml kg⁻¹ (control), GPWE, 300 mg kg⁻¹ (GPWE300) and GPWE, 600 mg kg⁻¹ (GPWE600) for four weeks. The MAP and HR were measured in the caudal artery by an indirect tail-cuff method before and after 1, 2, 3, 4 weeks of treatment, using Pulse Amplifier (model 29) and cuff pump (model 20NW) linked to computerized data acquisition system (ADInstruments PowerLab Software). The rats were initially trained for blood pressure measurement on at least three separate occasions to establish a baseline blood pressure.

**Urine flow rate (UFR).** SH rats under repeated dose treatment were observed in terms of urine output (24 h). This was carried out by caging individual animals in custom made metabolic cages, with free access to water and food, and allowed to collect the urine. These data were collected before and on 14th and 28th day of treatment. UFR is the volume of urine excreted per unit time and was calculated using the formula:

\[
UFR(\mu l/min/100g of BW) = \frac{[UV(\mu l) \times 100]}{[T(min) \times BW(g)]}
\]

**Acute agonist responses.** Above, repeated dose, treated spontaneously hypertensive rats were anesthetized by intraperitoneal injection of urethane (12.5 g (10 ml)⁻¹, 10 ml kg⁻¹) and the rectal temperature was kept at 36.5-37.5°C with an overhead lamp. The trachea was cannulated with PP250 to facilitate spontaneous respiration. PE50 cannula containing heparin-treated saline (120 IU ml⁻¹), were inserted into the left jugular vein (for administration of agonists ACh, PE, Mtx, AngII and IsoP) and into right carotid artery (for arterial pressure and heart measurement, via a pressure transducer P23 ID Gould, Statham instrument connected to ADInstruments, Powerlab Software). Upon completion of the surgery, cardiovascular parameters were allowed to stabilize for 30 min. After stabilization, baseline mean arterial pressure (MAP), heart rate (HR), systolic blood pressure (SP) and diastolic blood pressure (DP) values were obtained. Three increasing doses followed by same decreasing doses of an agonist (ACh, AngII & IsoP 100, 200, 400 ng kg⁻¹, PE 2.5, 5, 10 µg kg⁻¹ and Mtx 12.5, 25, 50 µg kg⁻¹) as bolus injections were administered over 15 s at interval of 3 min, to test any modified cardiovascular responses induced by the agonists in the treated groups compared to control group. Different agonists were tested at minimum interval of 30 min, after haemodynamic parameters (MAP, HR, SP and DP) returned to basal values.

**Statistical analysis.** All results were expressed as Mean ± Standard Error Mean per experimental groups and latter were analysed by one way analysis of variance (ANOVA). Dunnett’s test was used to evaluate the significant differences between the control and the experimental groups. Differences with a p<0.05 and p<0.01 was considered statistically significant.

**RESULTS**

**Single dose effect in SD and SH rats.** Mean arterial pressure (MAP) and heart rate (HR), measured 5 times in 24 h period in control SD and SH rats, after administration of water, and were not significantly different (p>0.05). GPWE (1 g kg⁻¹) produced; significant (p<0.05), short duration fall in MAP (Figure 1) after 3 h accompanied by drop in HR in both SD and SH rats. The peak effect on MAP was reached in 6 h and return back to initial value after 12 h. Moreover, HR kept significantly (p<0.05) reduced in SD rats while, not significantly (p>0.05) in SH rats after 12 and 24 h.

**Repeated dose effect in SH rats.** Mean arterial pressure (MAP) and heart rate (HR), measured weekly for 4 weeks period in control SH rats, administered water, were not significantly (p>0.05) different. GPWE 300 showed fall in MAP (Figure 2) after 1st week and significant (p<0.05) reduction in MAP was noticed after 4th week. However, GPWE600 produced significant (p<0.05) fall in MAP after 2nd week and attained plateau in 3rd and 4th week. Moreover, both GPWE300 and GPWE600 showed significant (p<0.05) drop in HR after 1st until 4th week of treatment.
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**Figure 1.** Comparative reduction of mean arterial pressure (MAP) and heart rate (HR) during 24 h, in control, GPWE p.o., treated conscious SD and SH rats. Data presented as Mean ± SEM (n=6). * represents significant difference compared with control at *p*<0.05.

**Figure 2** Comparative lowering of mean arterial pressure (MAP) and heart rate (HR) in control, GPWE300, GPWE600, p.o., treated daily, conscious SH rats for four weeks. Data presented as Mean ± SEM (n=6). * represents significant difference compared with control at *p*<0.05.

**Urine flow rate.** Urine flow rate remained unchanged (*p*>0.05) in the control rats (Table 1). In GPWE300 rats, urine flow rate was not significantly increased on day 14, but was found to be significantly (*p*<0.05) higher on day 28. GPWE600 showed significant (*p*<0.05) increase in urine flow rate on day 14 until day 28.

**Table 1.** Average urine flow rate (UFR) in control, GPWE300, GPWE600 at day 0, 14 and 28 of treatment. Data presented as Mean ± SEM (n=6). * represents significant difference compared with control at *p*<0.01.

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<tr>
<th>Urine flow rate (µl/min/100g of BW)</th>
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<td><strong>Groups</strong></td>
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**Acute agonist responses.** The average baseline values of MAP, SP, DP, HR of control SH rats and GPWE treated SH rats were relatively, significantly \((p<0.05)\) different. The average baseline values of MAP in anaesthetized SH rats were 146±4.88 mmHg (control), 119±3.71 mmHg (GPWE300), 102±3.87 mmHg (GPWE600). The average baseline values of SP in SH rats were 185±5.6 mmHg (control), 167±4.3 mmHg (GPWE300), 147±4.5 mmHg (GPWE600). The average baseline values of DP in SH rats were 127±3.7 mmHg (control), 95±2.2 mmHg (GPWE300), 80±2.6 mmHg (GPWE600). The average baseline values of HR in anaesthetized SH rats were 337±15 beats per min (control), 293±6 beats per min, (GPWE300), 273±6 beats per min (GPWE600) respectively.

ACh (100, 200, 400 ng kg\(^{-1}\)) induced reduction of MAP (Figure 3) in control SH rats. The ACh induced fall in MAP in GPWE300 was less but not significantly \((p>0.05)\) different from control. Whereas ACh induced MAP reduction in GPWE600 was significantly less \((p<0.05)\) compared to control. The decrease in SP and change in HR, in all the groups were not significant. While drop in DP with ACh in GPWE600 rats was significantly less \((p<0.05)\) compared to control and GPWE300.

PE (2.5, 5, 10 μg kg\(^{-1}\)) induced increase in MAP (Figure 4) in control SH rats. The PE induced rise in MAP in GPWE300 and GPWE600 were not significantly different \((p>0.05)\) compared to control, except the effect at the 10μg kg\(^{-1}\) of GPWE600 induced significantly \((p<0.05)\) higher rise in MAP. GPWE600 showed significant \((p<0.05)\) increase in rise in DP at 10 μg kg\(^{-1}\) and SP with 10 μg kg\(^{-1}\) PE. No significant changes were noticed in HR with PE in different groups.

Mtx (12.5, 25, 50 μg kg\(^{-1}\)) induced increase in MAP and DP (Figure 5) in control SH rats. The Mtx induced rise in MAP and DP in GPWE300 and GPWE600 was not significantly \((p>0.05)\) different to control. The rise in SP was significantly \((p<0.05)\) reduced in GPWE600 compared to control. While the drop in HR was significantly \((p<0.05)\) reduced in GPWE300, GPWE600 compared to control group.

The Ang II induced rise in MAP, SP and DP (Figure 6) in GPWE300 and GPWE600 treated rats were not significantly different \((p>0.05)\) compared to control. However the induced decrease in HR with AngII (100, 200, 400 ng kg\(^{-1}\)) was significantly \((p<0.05)\) less in GPWE600 as compared to decrease in control.

IsoP (100, 200, 400 ng kg\(^{-1}\)) induced fall in MAP (Figure 7) in control SH rats. The IsoP induced fall in MAP in GPWE300 and GPWE600 was significantly \((p<0.05)\) reduced. The induced increase in SP in control was significantly \((p<0.05)\) reduced in GPWE300, GPWE600. The induced increase in HR in control was significantly \((p<0.01)\) reduced in GPWE300 and GPWE600. While no significant difference \((p>0.05)\) was noticed in decrease of DP between control and GPWE300 or GPWE600.

**Figure 3.** Comparative average reduction in mean arterial pressure (MAP), systolic pressure (SP), diastolic pressure (DP) and heart rate (HR) with acetylcholine (ACh) in control (water), GPWE300, GPWE600 p.o., treated for four weeks, anaesthetized SH rats. Data presented as Mean ± SEM (n=6). * and # represents significant difference compared with control at \(p<0.05\) and \(p<0.01\) respectively.
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**Figure 4.** Comparative average reduction in mean arterial pressure (MAP), systolic pressure (SP), diastolic pressure (DP) and heart rate (HR) with phenylephrine (PE) in control (water), GPWE300, GPWE600 p.o., treated for four weeks, anaesthetized SH rats. Data presented as Mean ± SEM (n=6). † represents significant difference compared with control at *p*<0.05 respectively.

**Figure 5.** Comparative average reduction in mean arterial pressure (MAP), systolic pressure (SP), diastolic pressure (DP) and heart rate (HR) with methoxamine (Mtx) in control, GPWE300, GPWE600 p.o., treated for four weeks, anaesthetized SH rats. Data presented as Mean ± SEM (n=6). # represents significant difference compared with control at *p*<0.01 respectively.
Figure 6. Comparative average reduction in mean arterial pressure (MAP), systolic pressure (SP), diastolic pressure (DP) and heart rate (HR) with angiotensin II (AngII) in control, GPWE300, GPWE600 p.o., treated for four weeks, anaesthetized SH rats. Data presented as Mean ± SEM (n=6). # represents significant difference compared with control at \( p<0.01 \) respectively.

Figure 7. Comparative average reduction in mean arterial pressure (MAP), systolic pressure (SP), diastolic pressure (DP) and heart rate (HR) with isoprenaline (IsoP) in control, GPWE300, GPWE600 p.o., treated for four weeks, anaesthetized SH rats. Data presented as Mean ± SEM (n=6). * and # represents significant difference compared with control at \( p<0.05 \) and \( p<0.01 \) respectively.
DISCUSSION AND CONCLUSION

Basic mechanisms regulating blood pressure are control of blood vessel diameter, heart rate and contractility, and regulation of blood volume. Previous studies from our laboratory revealed the in vitro cardio-vascular relaxing potentials of the water extract of *Gynura procumbens* (Kaur et al, 2012). In present studies, marked, short term fall in MAP with single dose of GPWE (1 g kg\(^{-1}\)) in conscious normotensive (SD) rats and hypertensive (SH) rats indicate hypotensive/antihypertensive activity of *G. procumbens* water extract. Furthermore, significant lowering of MAP in two weeks in GPWE600 and four weeks in GPWE300 SH rats infer antihypertensive effect is dose dependent. However, significant long lasting bradycardia observed with single dose of GPWE and also after 1st week of GPWE300/600 administration indicates cardio-relaxing activity in addition to hypotensive/antihypertensive activity of GPWE.

In hypertension, the tone and reactivity of the vessels is changed due to the alteration in the structures and functions of large and small arteries throughout the systemic circulation. This modification can alter the compliance characteristics of the arterial blood vessels (McVeigh, 1996). In order to elucidate effects of GPWE in vivo, a series of experiments in anaesthetized SH rats, treated with GPWE were designed. The effects of GPWE treatment on the cardiovascular response induced by the different vasoconstrictor/relaxant agents used (ACh, PE, Mtx, AngII, IsoP) were compared with that of control SH rats. ACh a cholinomimetic drug produces vasodilatation and reduces the blood pressure by acting on endothelial muscarinic (M\(_3\)) receptor, producing nitric oxide (NO) that diffuses from the endothelium into the adjacent vascular smooth muscle cells causing relaxation. Markedly low baseline MAP and DP values, and also significantly less graded reduction in MAP and DP with administration of ACh in GPWE600 treated rats compared to control, indicate vasorelaxation in GPWE treated rats. This view was further supported by increased NO levels in serum of baseline MAP and DP values, and also significantly less graded reduction in MAP and DP with administration of ACh in GPWE600 treated rats compared to control; indicate vasorelaxation in GPWE treated rats. This view was further supported by increased NO levels in serum of *G. procumbens* water extract treated SH rats (Kim et al, 2006). \(\alpha_1\)-Adrenoceptors on vascular smooth muscle control BP by action of vasoconstrictor neurotransmitter (Reid, 1986). Upregulation of \(\alpha_1A\) and \(\alpha_1D\)-adrenoceptors has been shown in SH rats (Ye and Colquhoun, 1998), PE, \(\alpha_1\)-adrenoceptor agonist, has higher affinity for the \(\alpha_1A\) and \(\alpha_1D\) than \(\alpha_1\)-adrenoceptor (Goetz et al, 1995). Significant increase in pressor response induced by 10 \(\mu\)g kg\(^{-1}\) PE in GPWE600 compared to control however, Mtx a selective \(\alpha_1A\)-adrenoceptor agonist (Tsujimoto et al, 1989) administration to GPWE600 showed significant reduction in increase in SP as compared to control with marked reduction in bradycardiac effect of Mtx. AngII, a potent vasoconstricting peptide binds with AT1 receptor (Ruan and Arenshorst, 1996). Vascular reactivity to AngII is exaggerated when plasma concentration of endogenous AngII is low and vice versa (Arendshorst and Finn, 1977). AngII receptors frequently exhibit physiological adaptation, an increased circulating AngII and low sodium salt diet leads to downregulation of AngII receptor (Aguilera and Catt, 1981). No significant modifications were noticed in pressor effects of AngII in GPWE treated rats as compared to control SH rats. However, drop in HR with AngII was markedly modified in GPWE treated rats. IsoP, \(\beta\)-adrenergic agonist and structurally similar to adrenaline (Shen, 2008) increases force of contraction and heart rate. Positive chronotropic effect of IsoP was significantly reduced in GPWE300 & 600 treated rats accompanied by significant reduction in increased SP and decreased MAP.

In hypertension, diuretics, frequently known as "water pills," helps body to remove extra water and salt through the urine. The elimination of excess salt and water helps in lowering blood pressure and facilitates heart to pump blood properly (Krakoff, 2005). Diuretics are used to treat various heart-related conditions, including hypertension, congestive heart failure, kidney and liver problems, and glaucoma. Diuretics often are prescribed in conjunction with the other high blood pressure medicines (Ernst and Moser, 2009; Grossman et al, 2009). Use of diuretic along with another antihypertensive or cardiac ailment related medicine often works well, as diuretics can improve the efficacy of the other medicines and moreover prevent the fluid retention that can occur in many conditions. Significant increase in urine output after 2 weeks treatment in GPWE600 treated rats and after 4 weeks in GPWE300 treated rats, indicate the diuretic property of the GPWE. This property may be helping the rats to get rid of unwanted sodium & water and resulting in longer lasting lowering of blood pressure.

REFERENCES


Reid JL. 1986. Alpha-adrenergic receptors and blood pressure control. Am J Cardiol 57(9): 6E-12E.


