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Original Research Article

Comparison of the acute effects of Tulbaghia violacea William Henry Harvey (Alliaceae) on blood pressure and heart rate of ageing male normotensive Wistar kyoto rats and adult male spontaneously hypertensive rats

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Abstract

Purpose: *To assess the effect of the crude methanol leaf extracts of Tulbaghia violacea William Henry Harvey (Alliaceae) on blood pressure (BP) and heart rate in ageing normotensive Wistar Kyoto rats (WKY), and compare the results obtained with those for adult spontaneously hypertensive rats (SHR).*
Methods: *T. violacea (5 – 150 mg/kg) and/or vehicle (dimethylsulfoxide and normal saline) were respectively and randomly administered intravenously to groups of ageing (15 months) WKY and adult (< 5 months) SHR, weighing 380 - 470 and 280 - 320 g, respectively. BP and heart rate (HR) were measured via a pressure transducer connecting the femoral artery and Powerlab equipment.*
Results: *T. violacea significantly and dose-dependently reduced systolic BP, diastolic BP, mean arterial pressure (MAP) and HR in both strains of rats. No statistically significant differences were however observed when the changes in BP and HR in the two rats strains were compared.*
Conclusion: *T. violacea was effective in reducing BP and HR in both age-induced and spontaneously hypertensive rats.*

Keywords: *Age, Wistar Kyoto rats, blood pressure, heart rate, Tulbaghia violacea*

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INTRODUCTION

Treatment of hypertension (HTN) has been one of the major successes of medicine in the past half-century [1,2]. However, the number of people with uncontrolled blood pressure is increasing, despite therapeutic advances [3]. Consequently, the prevalence of HTN is still on the rise in developing countries [4] and worldwide [3]. As a result of better management of both communicable and non-communicable diseases, the global population of the elderly is increasing, a trend that has been predicted to continue in the coming decades [3,5]. Therefore, the incidence and prevalence of HTN, as well as clinical and subclinical cardiovascular disease (CVD) in general, are expected to rise [3]. Hypertension is associated with considerable morbidity and an increased risk of CVD, stroke, decreased quality of life, and mortality in the elderly [1-3]. Furthermore, ageing and HTN play critical roles in both cardiovascular (CV) and cerebrovascular complications [2,3].

Tulbaghia violacea Harv. (Alliaceae) is common throughout Africa, with the highest concentration

in Southern Africa, where it is widely used as herbal remedy for various complaints, including HTN [6,7]. *T. violacea* has been suggested to have similar secondary metabolites and biological activities as garlic since they belong to the same plant family [6,7], and for these reasons, there has been a recent increase in the number of studies aimed at exploring and/ or confirming the therapeutic potential of *T. violacea* on the CV system [8-12].

The direct and indirect costs of HTN are high [4,13], and the incidence of HTN is relatively high among the elderly. Also, the elderly are largely excluded from many clinical trials, as they belong to the upper age limits and/or do not present with age-specific results [5,14]. A couple of studies have investigated the effect of *T. violacea* on different strains of rats, including the adult Wistar rats [9], the spontaneously hypertensive rat (SHR) [11,12] and the Dahl salt-sensitive rats (DSS) [8,10,15]. The inhibition of the angiotensin converting enzyme (ACE) [9,11] and the β1 adrenoceptors [12]; the reduction in the expression of angiotensin II type 1a (AT1a) mRNA [8], and levels of aldosterone in plasma [12]; and increases in diuresis and natriuresis [8,10] are some of the mechanisms that have been suggested to elicit the effects observed.

Therefore, the present study assessed the effect of the crude methanol leaf extracts of *T. violacea* on blood pressure (BP) and heart rate in ageing normotensive Wistar Kyoto rats (WKY), and compared the results obtained with those of adult SHR.

EXPERIMENTAL

Plant material

Fresh plants were purchased from the New Plant Nursery, George, South Africa in August and September, 2008; identified by a taxonomist at the University of the Western Cape (UWC), Bellville; and deposited at the herbarium at the UWC, with voucher numbers 6955 and 6956.

Plant extraction

To obtain the crude methanol leaf extract (MLE) of the plant, 2.4 kg of fresh plants were weighed, oven-dried, boiled in methanol in the Soxhlet apparatus. Excess solvent was removed from the resulting extract with a rotavapor and freeze-drying, and the direct extract stored in a brown bottle in a -4 ºC freezer. The final dried extract was 76.6 g or 3.2 % of the original weight of the fresh plant [12].

Animals

Healthy male WKY and SHR were obtained from the Animal Unit of the University of Cape Town, South Africa. The WKY weighed 380 - 470 g and aged just over 15 months, while the SHR weighed 280 - 320 g and aged less than 5 months. All rats were kept in the Animal house, School of Pharmacy, University of the Western Cape; and given water and normal rat pellet *ad libitum*. The room temperature was kept at 24 ºC, with a 12:12 h light-dark cycle. From literature, the median lifespan of the WKY is 21.5 months [16,17]. Therefore, 15 month old WKY were used in the present study. This is an age that is slightly older than those stated for aging groups in literature [18,19].

Drugs

Drops of dimethylsulfoxide (Merck Chemicals, South Africa) were used to dissolve *T. violacea*, and the resultant paste made up to the required dilution using 0.9 % saline.

In vivo experiments

The effects of different doses of the methanol leaf extracts (MLE) of *T. violacea* (5–150 mg/kg) on both anaesthetized WKY and SH rats were determined using the method previously described [12]. The femoral artery of the rats were cannulated to measure BP and HR, while the jugular vein were cannulated for intravenous infusion of *T. violacea*. The randomized dose-response experiments (DRE) for MLE of *T. violacea* (5–150 mg/kg) were carried out using 8 WKY and 8 SH rats for each dose of extract.

Data analysis

The paired and/or unpaired Student's T test was used to calculate statistical significance ($p < 0.05$) between values obtained at baseline with that obtained after infusing the dose of the extract. It was also used to compare the changes in BP and/or HR observed in the WKY with those obtained in the SHR.

Ethical considerations

The methodology and ethics adhered to in this study were approved by the Ethics Committee of the University of the Western Cape, with a registration number of 09/7/35. All experimental procedures used in the study were conducted in accordance with the guidelines provided by the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

RESULTS

The ageing WKY rats used in this study had a body weight ranging from 380 - 470 g and were over 15 months old. In the WKY, *T. violacea* (150 mg/kg) significantly ($p < 0.05$) and dose –dependently decreased SBP, DBP and MAP values (66.83 ± 10.84, 31.33 ± 7.79 and 44.00 ± 8.48 mmHg respectively; when compared to their respective values at baseline (139.25 ± 8.71, 95.00 ± 6.81 and 110.38 ± 7.44 mmHg) (Figures 1 to 3). This equates to 54.73 ± 4.36 %, 69.83 ± 7.79 % and 63.04 ± 6.05 % reduction in SBP, DBP and MAP respectively with the highest dose (Table 1). *T. violacea* (150 mg/kg) also significantly ($p < 0.05$) and dose –dependently decreased HR values obtained (250.00 ± 9.81 bpm) when compared to the value at baseline (379.38 ± 8.03 bpm) (Figure 4), i.e, a 33.72 ± 1.03 % reduction in HR with the highest dose (Table 1).

In the SHR, *T. violacea* (150 mg/kg) significantly ($p < 0.05$) and dose–dependently decreased the SBP, DBP and MAP values obtained (117.96 ± 7.98, 73.06 ± 7.18 and 88.14 ± 7.17 mmHg respectively) when compared to their respective values at baseline (199.97 ± 5.17, 147.68 ± 4.63 and 165.30 ± 4.55 mmHg) (Figures 1 to 3). This equates to 42.38 ± 3.68 %, 51.67 ± 4.33 % and 47.91 ± 3.82 % reduction in SBP, DBP and MAP respectively at the highest dose (Table 1). *T. violacea* (150 mg/kg) also significantly ($p < 0.05$) and dose–dependently decreased HR values obtained (309.71 ± 20.15 bpm) when compared to the value at baseline (389.44 ± 17.31 bpm) (Figure 4), i.e, a 20.82 ± 1.96 % reduction in HR at the highest dose (Table 1).

The percentage changes (%Δ) in BP and HR observed at the different MLE doses were similar between the two rat strains (Figures 1 to 4).

Table 1: Baseline biologic parameters in WKY and SHR

	AGE	WEIGHT	SBP	DBP	MAP	HR
	M	g	mmHg			bpm
WKY	15	421.6 ± 14.9	139.3 ± 8.7	95.0 ± 6.8	110.4 ± 7.4	379.4 ± 8.0
SHR	5	317. 9 ± 4.4*	200.0 ± 5.2*	147.7 ± 4.6*	165.3 ± 4.6*	389.4 ± 17.3

* Statistical significance. m = months. g = grams. bpm = beats per minute

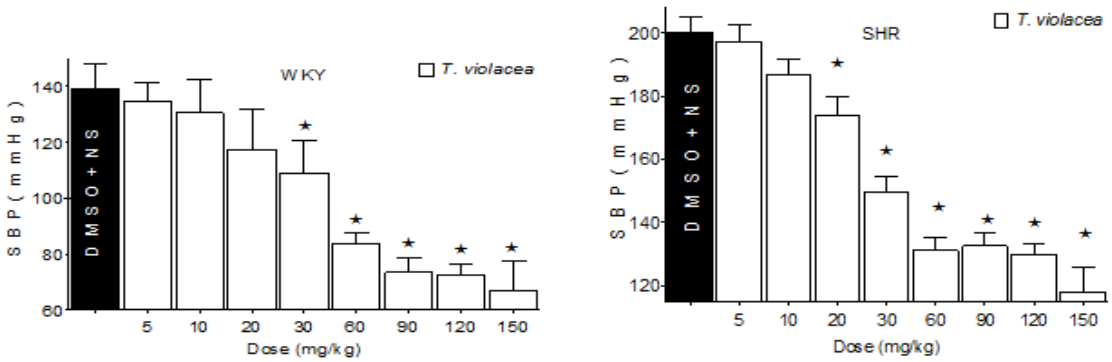

Figure 1: Effect of *T. violacea* (5 - 150 mg/kg) on SBP in WKY and SHR. Values are presented as mean ± SEM. ★ indicates statistical significance

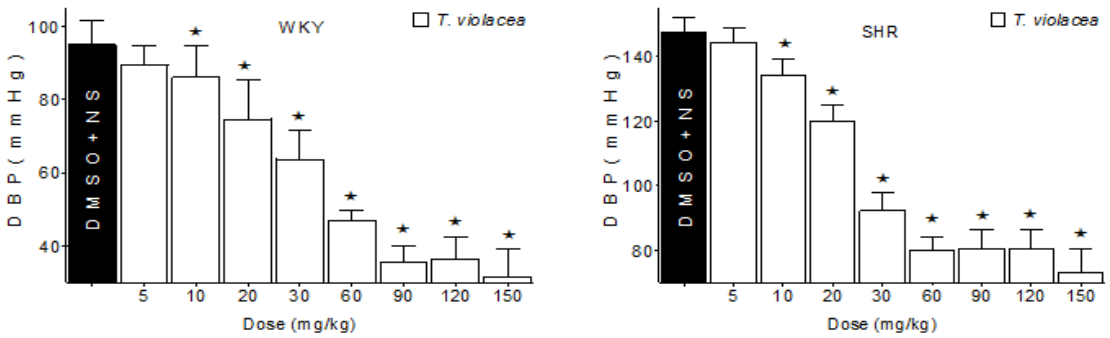

Figure 2: Effect of *T. violacea* (5 - 150 mg/kg) on DBP in WKY and SHR. Values are presented as mean ± SEM. ★ indicates statistical significance

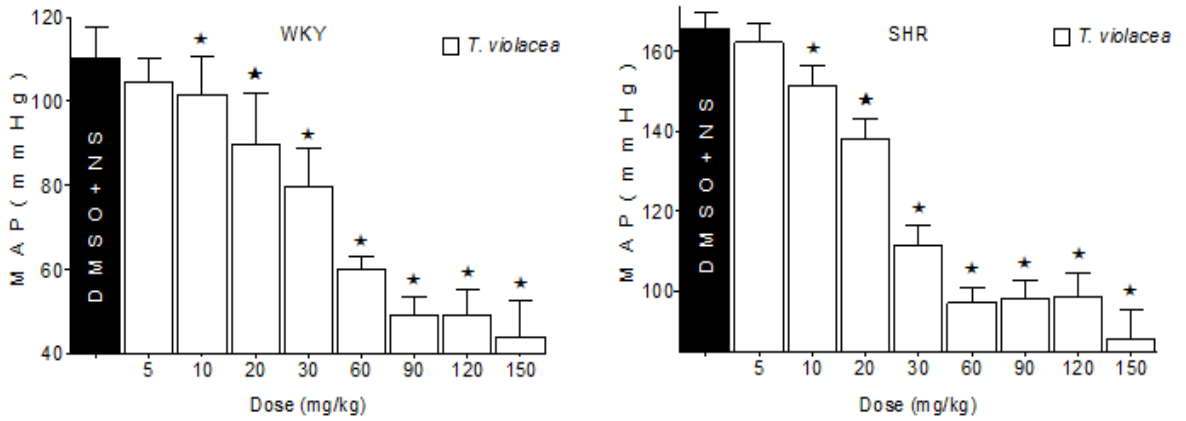

Figure 3: Effect of *T. violacea* (5 - 150 mg/kg) on MAP in WKY and SHR. Values are presented as mean ± SEM. ★ indicates statistical significance

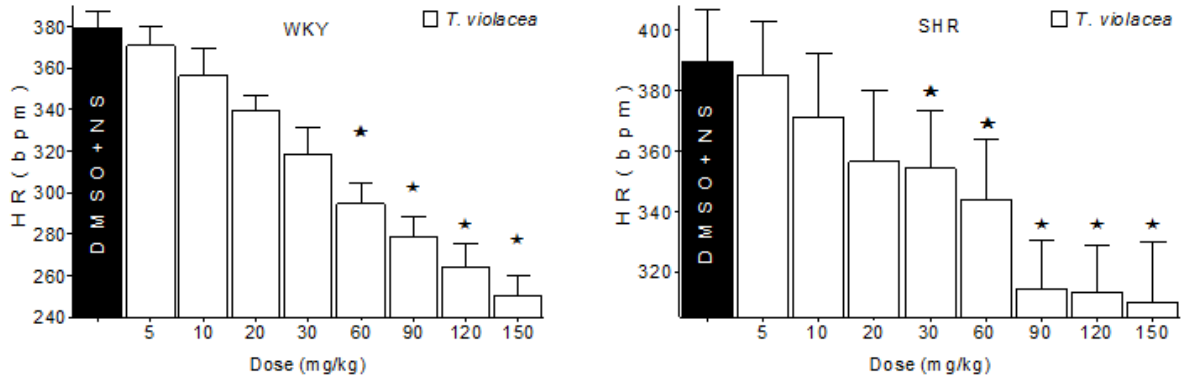

Figure 4: Effect of *T. violacea* (5 - 150 mg/kg) on HR in WKY and SHR. Values are presented as mean ± SEM; ★ indicates statistical significance ($p < 0.05$)

DISCUSSION

The age of the WKY rats (15 months) used in this study is equivalent to 45 years and above in humans [20,21]. The difference in basal HR between the ageing WKY and the adult SHR was not significant. However, the ageing WKY had significantly lower BP, and significantly higher body weight as compared to the adult SHR. The baseline SBP of the ageing rats used in this study was significantly higher than the normal value of 120 ± 14 mmHg as seen in adult (5 months) normotensive rats, while the SBP of the SHR used in the study was also higher than the normal value for normotensive rats, but within the normal range for hypertensive rats [17,22].

Rats of different strains and age were used in the study to compare the effect of the crude MLE of *T. violacea* on old age - related increase in BP, with spontaneous HTN [19,23]. *T. violacea* significantly and dose-dependently reduced the SBP, DBP, MAP and HR in both WKY (Figure 1) and SHR (Figure 2), with the percentage changes (%Δ) in both BP and HR being similar between the two strains of. The results obtained in the ageing WKY is consistent with that obtained by Ramesar *et al* [9] in adult Wistar rats, while that obtained in the SHR corroborates previous results in SHRs [11,12] and Dahl salt sensitive rats [8,10,15].

The mechanisms implicated in the pathogenesis of CVD include alterations in the renin angiotensin aldosterone system (RAAS) [2], the autonomic nervous system [2,24], the reactive oxygen species [25], and thrombosis [25]. Similarly, *T. violacea* has been reported to inhibit ACE [9,11] and the expression of AT1a mRNA [8]; reduce aldosterone levels in plasma [12], and consequently increase diuresis and natriuresis [8,10] in rats. It has also been found to stimulate the muscarinic receptors [12], block β1 adrenoceptors [12], and improve antioxidant activity [6]. Although the specific mechanisms by which *T. violacea* reduced BP and HR in the ageing WKY and SHR were not investigated in the current study, we propose that some of the mechanism previously mentioned, with other rats of different ages and genetic make-up [6,8-12,15], would have contributed to the antihypertensive effect observed.

CONCLUSION

Crude MLE of *T. violacea* reduces BP and HR in ageing normotensive WKY rats and in adult spontaneously hypertensive rats. No significant differences were observed with the BP and HR-lowering effect, despite the differences in the pathology of high blood pressure in the two strains used. Thus, *T. violacea* may not only be able to reduce BP and HR in rats, but may also be useful in the management of HTN in both young and elderly patients.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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