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Effect of *Tulbaghia violacea* on the blood pressure and heart rate in male spontaneously hypertensive Wistar rats

Ismaila A. Raji*, Pierre Mugabo, Kenechukwu Obikeze

Discipline of Pharmacology, School of Pharmacy, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa

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ABSTRACT

Ethnopharmacological relevance: Tulbaghia violacea Harv. (Alliaceae) is a small bulbous herb which belongs to the family Alliaceae, most commonly associated with onions and garlic. In South Africa, this herb has been traditionally used in the treatment of various ailments, including fever, colds, asthma, paralysis, hypertension and stomach problems. The aim of this study was to evaluate the effect of methanol leaf extracts (MLE) of *Tulbaghia violacea* on the blood pressure (BP) and heart rate (HR) in anaesthetized male spontaneously hypertensive rats; and to find out the mechanism(s) by which it acts.

Materials and methods: The MLE of *Tulbaghia violacea* (5–150 mg/kg), angiotensin I human acetate salt hydrate (ang I, 3.1–100 μg/kg), angiotensin II human (ang II, 3.1–50 μg/kg), phenylephrine hydrochloride (phenylephrine, 0.01–0.16 mg/kg) and dobutamine hydrochloride (dobutamine, 0.2–10.0 μg/kg) were infused intravenously, while the BP and HR were measured via a pressure transducer connecting the femoral artery and the Powerlab.

Results: Tulbaghia violacea significantly ($p < 0.01$) reduced the systolic, diastolic, and mean arterial BP; and HR dose-dependently. Ang I, ang II, phenylephrine and dobutamine all increased the BP dose-dependently. The hypertensive effect of ang I and the HR-increasing effect of dobutamine were significantly ($p < 0.01$) decreased by their co-infusion with *Tulbaghia violacea* (60 mg/kg). However, the co-infusion of ang II or phenylephrine with *Tulbaghia violacea* (60 mg/kg) did not produce any significant change in BP or HR when compared to the infusion of either agent alone in the same animal.

Conclusions: Tulbaghia violacea reduced BP and HR in the SHR. The reduction in BP may be due to actions of the MLE on the ang I converting enzyme (ACE) and β_1 adrenoceptors.

1. Introduction

Cardiovascular diseases (CVD) account for 29.2% of the total global deaths, with 80% of these occurring in low and middle-income countries (World Health Organization, 2007). Sixty per cent of the burden of CVD and about half of that of coronary heart disease (CHD) globally is caused by hypertension (HTN) (Seedat, 2007). Uncontrolled blood pressure (BP) has also been reported in a high number of hypertensive patients who adhere to the available antihypertensive drugs and/or therapy (Mensah, 2002; Chobanian et al., 2003), given impetus to intensive research towards discovering better, cheaper and equally effective medicines, including herbs. Presently, natural products and their derivatives represent more than 50% of all the drugs in clinical use (Gurib-Fakim, 2006).

Tulbaghia violacea Harv. (Alliaceae) is a small bulbous herb with hairless leaves arising from a white, fleshy stalk (van Wyk et al., 1997); belonging to the family Alliaceae, commonly associated with onions and garlic (Ramesar et al., 2008). It is commonly known as wilde knoffel (Afrikaans), isihaqa (Zulu) or itswele lomlambo (Xhosa) in South Africa. The plant is indigenous to Natal, Transvaal and the Eastern Cape region in South Africa. The evergreen leaves of *Tulbaghia violacea* exhibit a garlic-like smell when bruised and have been used in some cultures as a substitute for garlic and chive (van Wyk et al., 1997; Van Wyk and Gericke, 2000) probably for its antihypertensive properties. The constituents of *Tulbaghia violacea* include several odour forming compounds (Kubec et al., 2002) and bioflavonoids (Hutchings et al., 1996). Its medicinal uses include the treatment for fever, colds, asthma, tuberculosis, oesophageal cancer, rheumatism, paralysis, HTN and stomach problems. Literature on the biological activities of *Tulbaghia violacea* are few (Bungu et al., 2008), but the plant has been postulated to have similar biological activities and secondary metabolites as garlic since they belong to the same family and have similar characteristic sulfur smell (van Wyk et al., 1997; Van Wyk and Gericke, 2000; Bungu et al., 2006; Thamburan et al., 2006).

Tulbaghia violacea has been reported to inhibit angiotensin converting enzyme (ACE) *in vitro* (Duncan et al., 1999; Mackraj and Ramesar, 2007; Ramesar et al., 2008). It has also been reported to (a)

* Corresponding author. Tel.: +27 794538421; fax: +27 219593407.
E-mail addresses: ismailaraji@gmail.com, smilerays1@yahoo.com (I.A. Raji).

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limit the maximum rise in mean arterial pressure (MAP) associated with infusion of angiotensin I (ang I) in anaesthetized normotensive rats (Mackraj and Ramesar, 2007) and (b) reduce systolic BP(SBP) in the Dahl salt sensitive (DSS) rat by decreasing the expression of the renal angiotensin II type 1 (AT_1) receptor gene (Mackraj et al., 2008). Meanwhile, garlic (*Allium sativum*) and wild garlic (*Allium ursinum*) have both been reported to reduce circulating angiotensin II (ang II) levels, increase nitric oxide synthase activity and decrease SBP in spontaneously hypertensive rats (SHR) (Mohamadi et al., 2000; Preuss et al., 2001). These studies, put together, suggest that disruption(s) in the renin angiotensin aldosterone system (RAAS) may be involved in the antihypertensive effect of *Tulbaghia violacea*. The RAAS is a key physiologic regulator of vascular tone, salt and water balance, and BP (Gradman, 2009; Lacolley et al., 2009). None of the previous studies involved the use of *Tulbaghia violacea* Harv. (Alliaceae) in SHR, nor did they take cognizance of the effect of the plant extract on HR, ACE, ang II receptors, α_1 adrenoceptors or β_1 adrenoceptors *in vivo*. Therefore, the motivation for this study is to further elucidate possible mechanisms by which *Tulbaghia violacea* may reduce BP and HR in SHR.

2. Materials and methods

2.1. Plant material

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

2.2. Plant extraction

Leaves weighing 2.378 kg were washed before being dried in an oven at 30 °C till constant mass. Dried leaves were ground into powder, MLE was prepared by Soxhlet extraction and the excess solvent removed at 40 °C using a rotavapor. The dried black paste obtained was placed in a −20 °C freezer before being dried further using a freeze-drier. The final dried extract (76.6 g or 3.22% yield) was stored in a brown bottle in a refrigerator at −4 °C. Fresh MLE was dissolved with drops of dimethyl sulfoxide (DMSO) and the required concentration made up with 0.9% normal saline and filtered before infusion into the rat to prevent the formation of emboli.

2.3. Animals

Healthy male SHR weighing 300–350 g and <5 months old obtained from the Animal Unit at the University of Cape Town, South Africa; were kept under laboratory conditions in the animal room, School of Pharmacy, UWC; and given water and feed *ad libitum*. Room temperature was kept at 24 °C, with a 12:12-h light-dark cycle.

2.4. Drugs and chemicals

Angiotensin I human acetate salt hydrate, angiotensin II human, phenylephrine hydrochloride, dobutamine hydrochloride (racemic dobutamine), captopril, losartan, prazosin and propranolol were purchased from Sigma–Aldrich, South Africa. Solvents were 0.9% saline (Adcock Ingram, South Africa) and dimethyl sulfoxide (DMSO, Merck Chemicals, South Africa). Fresh drug solutions were made at the beginning of each experiment and kept on ice during the course of the experiment.

2.5. In vivo experiment

Rats were anaesthetized with 6% sodium pentobarbitone (Kyron Laboratories, South Africa) at a dose of 40 mg/kg intraperitoneally, and fastened in a supine position on a heated rat operating table (BioScience). A temperature probe (AD Instruments) was inserted into the rectum to monitor the body temperature. The table temperature was maintained at 37.3 ± 0.5 °C by a thermostat. The trachea was cannulated to maintain air flow during the experiment. An oxygen mask was placed close to the opening of the tracheal cannula to maintain adequate supply of oxygen (Afrox, South Africa) to the animal. The right external jugular vein was cannulated with a small polyethylene cannula to allow intravenous infusion of drugs via a syringe placed on the two-way injection Ascor AP 22 syringe pump (Poland). The left femoral artery was cannulated with a small polyethylene cannula filled with 100 IU/ml heparinized (Intramed, South Africa) saline (Hearse and Sutherland, 2000). The femoral cannula was connected to a BP transducer attached to a BP amplifier (AD Instruments) and Power Lab 4/20T (AD Instruments) for recordings of the BP and HR onto a Chart 5.0 for Windows software (Lasec, South Africa). Rats were allowed a 15-min stabilization period to ensure that BP and HR parameters were stable before any further experiment, and each group consisted of 8 animals. Drugs were infused, flushed with 0.1 ml of normal saline and results recorded within 3 min of infusion. Pressures and HR were allowed to return to baseline values (10–15 min) before further doses were infused.

2.6. Experimental protocol

(i) Dose response experiments (DRE) for methanol leaf extracts (MLE) of *Tulbaghia violacea* (5–360 mg/kg), ang I (3.1–400 μg/kg), ang II (3.1–400 μg/kg), phenylephrine (0.01–5.12 mg/kg), dobutamine (0.01–0.32 mg/kg), captopril (0.3–20 mg/kg) and propranolol (0.1–12.8 mg/kg). The dose at which 80% of the maximum effect was obtained was noted.
(ii) DRE for ang I (3.1–100 μg/kg) co-infused with *Tulbaghia violacea* (60 mg/kg).
(iii) DRE for ang I (3.1–100 μg/kg) infused after infusion of captopril (10 mg/kg) (Mackraj et al., 2008; Ramesar et al., 2008).
(iv) DRE for ang II (3.1–50 μg/kg) co-infused with *Tulbaghia violacea* (60 mg/kg).
(v) DRE for ang II (3.1–50 μg/kg) infused after pre-treatment with losartan (30 mg/kg) (Matys et al., 2000; Zhang and Leenen, 2001; Choi et al., 2009; Susic et al., 2009).
(vi) *Tulbaghia violacea* (60 mg/kg) was infused, during ang II (0.39 mg/kg/hr) infusion. Losartan (30 mg/kg) was thereafter infused into the same animal after the BP and HR returned to the values observed prior to the infusion of the MLE.
(vii) DRE for phenylephrine (0.01–0.16 mg/kg) co-infused with *Tulbaghia violacea* (60 mg/kg).
(viii) DRE for phenylephrine (0.01–0.16 mg/kg) infused after pre-treatment with prazosin (1 mg/kg) (Antunes et al., 2006; Wang et al., 2006; Braga et al., 2008; Pinterova et al., 2009).
(ix) DRE for dobutamine (0.2–10.0 μg/kg) co-infused with *Tulbaghia violacea* (60 mg/kg).
(x) *Tulbaghia violacea* (60 mg/kg) was infused, during dobutamine (2.3 mg/kg/h) infusion. Propranolol (1.6 mg/kg) was thereafter infused into the same animal after the BP and HR returned to the values observed prior to the infusion of the MLE.

2.7. Ethical considerations

All the experimental procedures used in this study were carried out in accordance to the guidelines provided by the Ethics