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The *in vivo* effects of *Tulbhagia violacea* on blood pressure in a salt-sensitive rat model

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Abstract

Aim of the study: The *in vivo* effects of *Tulbhagia violacea* on systemic arterial blood pressure and on the renin-angiotensin system in a Dahl salt-sensitive rat model were investigated.
Materials and methods: Animals were treated for 14 days intraperitoneally as follows: *Tulbhagia violacea* (Tvl) (50 mg/kg b.w.), captopril (Cap) (10 mg/kg b.w.) or DMSO (Con). Baseline blood pressures were recorded prior to the commencement of the study and biweekly during the experimental period. Urine volume and sodium concentration were measured during the experimental period. On day 15, animals were anaesthetized (sodium thiopentane, 50 mg/kg, i.p.), blood samples for aldosterone levels were taken and the kidneys removed for determining AT1a mRNA expression.
Results: Cap and Tvl groups showed significantly reduced AT1a mRNA expressions by 3.11- and 5.03-fold, respectively, when compared to the Con group ($p < 0.05$). When compared to baseline blood pressures (day 0); Cap and Tvl showed reductions in systolic blood pressure (SBP) of $7.76 \pm 0.41\%$ and $9.12 \pm 0.31\%$, respectively (mean% decrease from day 0 to day 14). In contrast, in the Con group the systolic blood pressure increased from day 0 to day 14 by $4.66 \pm 0.56\%$. Blood pressure changes in all treated groups differed from Con significantly. Systolic blood pressure decreased with the decrease in AT1a mRNA expressions in these groups. When comparing day 0 to day 14, urine output increased in the Cap and Tvl groups. In the Con group, urinary volume was reduced by day 14 as compared to day 0. Urinary sodium excretion was increased in the treated groups by day 14.
Conclusion: It can be concluded that *Tulbhagia violacea* reduces systemic arterial blood pressure in the Dahl rat by decreasing renal AT1 receptor gene expression and hence modulating sodium and water homeostasis.

Keywords: Dahl rats; AT1a receptor mRNA; Kidney; Hypertension; *Tulbhagia violacea*

1. Introduction

The use of medicinal plants forms an integral part of African culture (Hutchings et al., 1996) and is indeed one of the oldest and perhaps the most diverse in the world (Van Wyk and Wink, 2004b). In South Africa, a developing economy, indigenous African medicine coexists with Western allopathic medicine (Van Wyk and Gericke, 2003), catering for the needs of the diverse local populations. *Tulbhagia violacea*, also known as wild garlic, has been used traditionally over the years in the Southern African region for the treatment of fever, asthma, constipation oesophageal cancer and hypertension (Hutchings et al., 1996; Van Wyk and Wink, 2004a). More recently it has been investigated for its antifungal activity against *Candida albicans* (Motsei et al., 2003). There have been no reports on

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its potential cardiovascular effects *in vivo*. There has been one report on its ACE inhibitor activity (Duncan et al., 1999) *in vitro*.

Tulbhagia violacea Harv. (Alleacea), is a bulbous plant with hairless leaves arising from a white, fleshy stalk (Van Wyk et al., 1997). Perhaps, retarding its emergence as a major role player in the world of naturopathic remedies, is the strong smell of garlic when crushed, which has been ascribed largely to alliin, a compound found in true garlic (Van Wyk and Wink, 2004b) and perhaps in this plant as well. It has attractive mauve or purple flowers, which can be easily distinguished in the gardens of KwaZulu-Natal. *Tulbhagia violacea* is found in the Eastern Cape and Southern KwaZulu-Natal and is commonly known as wild garlic (English), wilde Knoffel (Afrikaans) and isihaqa (Zulu) (Van Wyk et al., 1997). Other species, such as *Tulbhagi alliacae*, were used as a remedy for fever, fits, rheumatism and paralysis; while *Tulbhagi simmerlerj* is often used as a substitute for *Tulbhagia violacea*.

The cardiovascular effects of true garlic have been widely reported, viz. the antiplatelet effects, lipid and blood pressure lowering effects (Van Wyk and Wink, 2004a). Interestingly, garlic has been reported to mediate its blood pressure lowering effects via the renin-angiotensin system (RAS) (Mcmahon and Vargas, 1993) The renin-angiotensin-aldosterone system is a well-known regulator of blood pressure (Dzau and Pratt, 1986) and its main effector is the octapeptide angiotensin II, which is thought to be one of the most potent vasoconstrictors, four times more than that of norepinephrine (Ganong, 2003). There are no studies regarding this particular plant with regard to its antihypertensive activity *in vivo*.

Clinically, the treatment of hypertension using the pharmacological interference of RAS is via angiotensin I-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs). As a distinct advantage, these drugs not only lower BP, but also offer further protection in organs such as the brain and heart since these are targets for end-organ damage (Yusuf et al., 2000); impacting positively on major diseases such as hypertension, stroke and diabetes (Volpe et al., 2002). In particular, ACEI itself has been shown to be renoprotective in patients with type 1 diabetes (Fabris et al., 1999; Hilgers and Mann, 2002). The exact molecular mechanism is still lacking with regard to how ACEI mediate their functions. In this study we address this issue by investigating the effect on AT1 gene expression which has not been performed before. In addition to identifying the medicinal effects of plants, it is also critical that its mechanism of action is elucidated. An understanding of the mechanism of action will lead to new drug development, identification of an ideal dosage form for drug delivery and also patient therapy and safety optimization.

In this paper we report the activity of Tvl *in vivo*, its blood pressure lowering effects (using a non-invasive, *in vivo* blood pressure monitoring), and its impact on the components of renin-angiotensin-aldosterone system in a model of salt-sensitive hypertension.

2. Materials and methods

2.1. Plant material

The plant (TVL) was collected from KwaZulu-Natal, South Africa by botanist, H. Baijnath. Voucher specimens are housed in the Ward Herbarium, University of KwaZulu-Natal, Westville Campus.

2.2. Extract preparation

Field collected fresh leaves were oven dried at 25 °C. Samples were ground and used to prepare aqueous plant extracts. Briefly 10 g of material was suspended in 100 ml of water and left to extract for 48 h. The solution was then filtered *in vacuo* and the filtrate was left to air dry resulting in the specific test compound. It was then reconstituted as required. The product yield was approximately 3.41%.

2.3. Animal study

Eighteen, 12-week-old, weight-matched, male Dahl salt-sensitive (DSS) rats were used in this study. Ethical approval was obtained from the Animal Ethics Committee of University of KwaZulu-Natal (UKZN). The animals were divided into three groups: control group (Con), ($n = 6$) received equivalent volume of vehicle (dimethyl sulfoxide); experimental control group (Cap), ($n = 6$) treated with captopril (Merck Inc.), 10 mg/kg b.w.; the experimental group (Tvl) treated with 50 mg/kg b.w. intraperitoneally.

2.4. Toxicity testing

Various methods were used to evaluate toxicity. Acute toxicity of Tvl was evaluated using a brine shrimp (*Artemia salina*) toxicity test (Meyer et al., 1982). Various concentrations of extracts (0.1–1 mg ml^{-1}) were made. Surviving nauplii were counted after 24 h. The data from the shrimp toxicity testing was used to estimate the dose of Tvl which would just be toxic in rats. In all cases where deaths had occurred in the control experiment, the data was corrected using Abbots formula (% deaths = [(test − control)/control] × 100). LC_{50} and 95% confidence intervals were determined by linear regression analysis using the GraphPad Instat (V.03) (GraphPad Software Inc., USA). Furthermore, 3T3 cells were used to determine the inhibitory concentration (IC_{50}) of the extract according to the method described by Singh and Ariatti (2006).

In addition, the OECD guideline 420 was used to test for acute toxicity in rats (OECD, 1999). Briefly, five male DSS rats of about the same weight and age were used. All animals were given a single dose of Tvl, 50 mg/kg b.w., i.p. and observed at regular intervals during the first 48 h for any signs of toxicity and then daily for 7 days. At the end of the 7th day the animals were weighed. If none of the five animals showed any signs of toxicity as stipulated in OECD guidelines (such as fur and mucous membrane changes, somatomotor activity and behaviour patterns, etc.) then this dosage was regarded to be non-toxic.