EFFECT OF *TULBAGHIA VIOLACEA*

ON THE BLOOD PRESSURE AND HEART RATE

IN MALE SPONTANEOUSLY HYPERTENSIVE WISTAR RATS

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ABSTRACT

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

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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 (SA), this herb has been traditionally used in the treatment of various ailments, including fever, colds, asthma, paralysis, hypertension (HTN) and stomach problems. The aim of this study was to evaluate the effect of methanol leaf extracts (MLE) of *T. 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.

The MLE of *T. violacea* (5 - 150 mg/kg), angiotensin I (ang I, 3.1 - 100 µg/kg), captopril (10 mg/kg), angiotensin II (ang II, 3.1 - 50 µg/kg), losartan (30 mg/kg), phenylephrine (0.01 – 0.16 mg/kg), prazosin (1 mg/kg), dobutamine (0.2 – 10.0 µg/kg), propranolol (0.1 - 12.8 mg/kg), muscarine (0.16 -10 µg/kg), and atropine (0.02 - 20.48 mg/kg) were administered intravenously into male spontaneously hypertensive rats (SHR) weighing between 300 g and 350 g and aged less than 5 months. The MLE of *T. violacea* and/or the standard drugs were infused alone, simultaneously, or separately into each animal. The BP and HR were measured via a pressure transducer connecting the femoral artery and the Powerlab. The vehicle (0.2 mls of a mixture of dimethylsulfoxide and normal saline), *T. violacea* (60 mg/kg) and captopril (10 mg/kg) were injected intraperitoneally into

some SHR for 21 days to investigate the chronic effect of these agents on plasma levels of aldosterone. The mean change, the mean of the individual percentage changes and the percentage difference (in mean) observed with each intervention was calculated and statistically analyzed using the Student's t test for significant difference ($p < 0.05$). The Microsoft Excel software was used for statistical analysis.

T. violacea significantly ($p < 0.05$) reduced the systolic, diastolic, and mean arterial BP; and HR dose-dependently. In a dose-dependent manner, ang I, ang II, phenylephrine significantly ($p < 0.05$) increased the BP, while propranolol, muscarine and atropine reduced the BP. The increases in BP due to dobutamine were not dose-dependent. In a dose dependent manner, phenylephrine and propranolol reduced the HR, while dobutamine increased the HR. The effect of ang I, ang II, muscarine and atropine on HR were not dose-dependent; with both increases as well as decreases observed with ang I, and II and atropine, while decreases were seen with muscarine. Captopril produced significant ($p < 0.05$) reduction in BP which were not associated with any change in HR. The co-infusion of ang I with the MLE produced significant ($p < 0.05$) reduction in BP, which were not associated with significant changes in HR. The co-infusion of ang II with the MLE did not produce any significant changes in BP or HR when compared to the infusion of the standard drug alone. The co-infusion of phenylephrine with the MLE did not produce any significant change in BP or HR when compared to the values obtained with the infusion of the standard drug alone, in both the absence and presence of prazosin. The co-infusion of dobutamine with *T. violacea* produced siginificant ($p < 0.05$) increases in DBP which were associated with significant ($p < 0.05$) reductions in HR,

when compared to the values obtained with the infusion of the standard drug alone. The co-infusion of atropine with the MLE did not produce any significant change in BP or HR when compared to the values obtained with the infusion of atropine alone. However, the infusion of *T. violacea*, 20 minutes after pre-treating animals with atropine (5.12 mg/kg) lead to dose dependent significant ($p < 0.05$) increases in BP, which were associated with dose-dependent increases in HR. The chronic treatment of animals with *T. violacea* or captropril produced (a) signicant ($p < 0.05$) reductions in the plasma levels of aldosterone when compared to the values obtained in the vehicle-treated group, (b) produced signifiant ($p < 0.05$) reduction in BP in the captopril treated group when compared to the vehicle-treated, (c) did not produce any signficant change in BP in the *T. violacea*-treated group when compared to the vehicle-treated group and (d) did not produce any signifiant change in HR or body weight in any of the groups.

The result obtained in this study suggests that *T. violacea* reduced BP and HR in the SHR. Secondly, the BP and HR reducing effect of the MLE may involve a) the inhibition of the ACE, b) the inhibition of the β_1 adrenoceptors, c) the stimulation of the muscarinic receptors and d) the reduction of the levels of aldosternone in plasma. The results also suggest that the MLE may not act through the angiotensin II receptors or the α_1 adrenergic receptors.

Key words: *Tulbaghia violacea*, spontaneously hypertensive rats, blood pressure, heart rate, renin angiotensin aldosterone system, angiotensin converting enzyme, angiotensin II

receptors, α_1 adrenergic receptors, β_1 adrenergic receptors, muscarinic receptors, aldosterone.

DECLARATION

I declare that *Effect of Tulbaghia violacea on the blood pressure and heart rate in male spontaneously hypertensive Wistar rats* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name: **Raji, Ismaila Adebayo**

Signed: _____

Date: **30/08/11**

DEDICATION

Glory to thy Lord, the Lord of honour and power,

From what they ascribe,

And peace on the messengers,

And praise to Allah, the Lord and Cherisher of the Worlds.

ACKNOWLEDGEMENT

I am grateful to my parents (Mr and Mrs S. Raji), my wife (Maryam), my siblings (Abdul Gafar, Shakira, Risqiyah, Rahma and Abdul Hamid) and my kid (Abu Bakr); for their love, support and patience during this journey.

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LIST OF ABBREVIATIONS

2KIC	two-kidney-one-clip
ACE	angiotensin 1 converting enzyme
ACE 2	angiotensin 1 converting enzyme type 2
ACE-I	angiotensin 1 converting enzyme inhibitor
ang I	angiotensin I
ang II	angiotensin II
Ang-(1-7)	angiotensin-(1-7)
ANS	autonomic nervous system
ARB	angiotensin II receptor blocker
AT_1	angiotensin II receptor type 1
AT_2	angiotensin II receptor type 2
BP	blood pressure
bpm	beats per minute
CAM	complementary and alternative medicine
CCB	calcium channel blockers
CHD	coronary heart disease

CKD	chronic kidney disease
CO	cardiac output
CV	cardiovascular
CVD	cardiovascular disease
CVS	cardiovascular system
DBP	diastolic blood pressure
DM	diabetes mellitus,
DMSO	dimethylsulfoxide
DRC	dose- response curve
DRE	dose- response experiment
DSS	Dahl salt sensitive
ECF	extracellular fluid
EDHF	endothelium-derived hyperpolarizing factor
EDRF	endothelium-derived relaxing factor
EDTA	ethylenediaminetetraacetic acid
eNOS	endothelial nitric oxide synthase
HR	heart rate

HRV	heart rate variability
HTN	hypertension
IHD	ischemic heart disease
JGA	juxta-glomerular apparatus
LVH	left ventricular hypertrophy
MAP	mean arterial pressure
MI	myocardial infarction/heart attack
MLE	methanol leaf extract
mmHg	millimetres of mercury
NO	nitric oxide/endothelium-derived relaxing factor/EDRF
NOS	nitric oxide synthase
NS	normal saline
phen	phenylephrine
PNS	parasympathetic systems
PP	pulse pressure
PPAR-γ	peroxisome proliferator-activated receptor gamma
RAABS	renin angiotensin aldosterone and bradykinin system

RAAS	renin angiotensin aldosterone system
SA	South Africa
SBP	systolic blood pressure
SEM	standard error of mean
SHR	spontaneously hypertensive rats
SNS	sympathetic nervous system
T. violacea	*Tulbaghia violacea*
TM	traditional medicine
TPR	total peripheral resistance
UWC	University of the Western Cape
VMC	vasomotor centre
VSM	vascular smooth muscle
VSMC	vascular smooth muscle cells
WHO	World Health Organisation
WKY	Wistar-Kyoto rats
α_1	alpha 1
β_1	beta 1

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CHAPTER ONE

INTRODUCTION

Cardiovascular disease (CVD) is a complex multi-factorial disease (Rahman & Lowe, 2006) that accounts for 16.7 million or 29.2% of the total global deaths; with around 80% of deaths occuring in middle to low-income countries. At least, 20 million people survive myocardial infarction (MI, i.e., heart attack) and stroke each year, many requiring costly clinical care (WHO, 2007). In Africa alone, CVD affects 1.3 million people yearly. It however seems that stroke rather than ischemic heart disease (IHD), is a unique feature of the health transition associated with urbanization of black South Africans (Steyn *et al.*, 2005; Pieters & Vorster, 2008). Africans (van Rooyen *et al*., 2002) and African Americans (Hinderliter *et al*., 2004; Suarez *et al*., 2004) have been observed to respond with heightened vascular reactivity when exposed to stress.

Epidemiologic studies indicate that hypertension (HTN), elevated serum lipids, increased plasma fibrinogen and coagulation factors, increased platelet activation, alterations in glucose metabolism (diabetes mellitus, DM), and smoking are factors positively associated with CVD (Wood & Joint European Societies Task, 2001; Seedat, 2007). Sixty per cent of the burden of CVD and about 50% of that of coronary heart disease (CHD) globally is caused by HTN (Seedat, 2007). Age (Chalmers, 1999), gender (WHO, 1995), urbanisation (van Rooyen *et al*., 2000), obesity (Stampfer *et al*., 1991) and certain dietary factors (Appel *et al.*, 1997) strongly influence the occurrence of essential HTN. Meanwhile, stroke which is a common complication of uncontrolled blood pressure (BP) kills more people than communicable

diseases such as acquired immunodeficiency disease syndrome (AIDS) (Ezzati *et al.*, 2002; Connor *et al.*, 2005; Mayosi, 2007).

Throughout the ages, man has relied on nature for his basic needs of food, shelter, clothing, means of transportation, fertilizers, flavours and fragrances, and, not the least, medicines (Gurib-Fakim, 2006). Plants have formed the basis of sophisticated traditional medicine (TM) systems that have been in existence for thousands of years and continue to provide mankind with new remedies, and WHO estimates that 80% of the population of the world still rely on plant-derived medicines for their healthcare (Samuelsson, 2004; Gurib-Fakim, 2006). These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick & Cox, 1997; Samuelsson, 2004).

Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world. Drug discovery from medicinal plants led to the isolation of early drugs such as cocaine, codeine, digitoxin, quinine, diosgenin, reserpine, pilocarpine and morphine, of which some are still in use (Farnsworth *et al.*, 1985; Newman *et al.*, 2000; Butler, 2004; Samuelsson, 2004; Gurib-Fakim, 2006). There has also been a rekindling of interest in 'rediscovering natural products' (Cragg & Newman, 2005) and it seems only 6% of potential medicinal plants has been investigated for biological activity and 15% of these for their chemical constituents (Gurib-Fakim, 2006).

In contrast to chemical drugs, herbs have sometimes been erroneously claimed to be non-toxic, due to their natural origin and long-term use as folk medicines (Street *et al.*, 2008).

However, problems may arise due to intrinsic toxicity, adulteration, substitution, contamination, misidentification, drug-herb interactions, and lack of standardization (Gagnier *et al.*, 2006). Both adverse drug reactions and poisonings associated with the use of herbal medicines are increasingly being reported; and medicinal plants behave as authentic medicines because the chemical substances of which they are formed can have a biological activity in humans (Ernst, 2002; Fennell *et al.*, 2004; Zhou *et al.*, 2004; Rodriguez-Fragoso *et al.*, 2008). Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. On the other hand, modern medicine usually aims to develop a patentable single compound or a 'magic bullet' to treat specific conditions (Gurib-Fakim, 2006). Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products which contain as active ingredients parts of plants, or other plant materials, or combinations thereof. "Traditional medicine" is often used when referring to Africa, Latin America, South-East Asia, and/or the Western Pacific, whereas "complementary and alternative medicine" (CAM) is used when referring to Europe and/or North America (and Australia) (Wicke, 1995; WHO, 2002; Hanrahan, 2006; Makunga *et al*., 2008).

Africa has a high rate of endemism, and the Southern African region is exceptionally rich in plant diversity with some 30,000 species of flowering plants of which 80% are endemic. Famous African medicinal plants include *Acacia senegal* (Gum Arabic), *Agathosma betulina* (Buchu), *Aloe ferox* (Cape Aloes), *Aloe vera* (North African Origin), *Artemisia afra* (African wormwood), *Aspalanthus linearis* (Rooibos tea), *Hibiscus sabdariffa* (Hibiscus, Roselle), *Hypoxis hemerocallidea* (African potato) and *Catharanthus roseus* (Rosy Periwinkle) (Good, 1974; Goldblatt & Manning, 2000; Gurib-Fakim, 2006). As seen in other parts of Africa, it is, therefore, not surprising that TM also forms the backbone of rural healthcare in South Africa

(McGaw *et al.*, 2005) and an estimated 27 million of the population depend on TMs and traditional healers for some or all of their healthcare needs (Cunningham, 1993; Meyer *et al.*, 1996; Mander, 1998; McGaw *et al.*, 2005). Traditional medicine coexists with modern medicine (van Wyk & Gericke, 2000) and caters for the health needs of diverse local populations (Mackraj *et al.*, 2008), and is most prevalent in more rural areas where western medicines are inaccessible due to unavailability and/or their comparably high cost. The sustained high popularity of TM may also be due to it being closely interwoven with the cultural and spiritual ideology in South Africa (Hutchings *et al.*, 1996; McGaw *et al.*, 2005; Makunga *et al.*, 2008); and more recently, the fact that South Africa has also mirrored the paradigm shift towards CAM in developed countries serving a diverse range of consumers (Makunga *et al.*, 2008). Plants which have been previously reported to have potential beneficial effects against CVD include *Allium sativum* (garlic), (Dillon *et al.*, 2003; Zahid Ashraf *et al.*, 2005), *Rauwolfia serpentina* (Snake-root) (Gurib-Fakim, 2006), turmeric (Deters *et al.*, 2003), Hawthorn (*Crataegus laevigata*) leaves, flowers and berries (Walker *et al.*, 2006) and *Tulbaghia violacea* (Mackraj *et al.*, 2008; Ramesar *et al.*, 2008). The known cardiovascular (CV) effects of these plants will be discussed in more details in the second chapter of this thesis.

Tulbaghia violacea (*T. violacea*) is widely used as herbal remedy for various ailments such as fever and colds, asthma, tuberculosis, rheumatism, paralysis, HTN and stomach problems (MacDonald *et al.*, 2004; van Wyk & Wink, 2004; Bungu *et al.*, 2006; Bungu *et al.*, 2008). *T. violacea* has been postulated to have similar biological activity as garlic since they belong to the same family (Order Asparagales, Family Alliaceae) (van Wyk *et al.*, 1997; van Wyk & Gericke, 2000; Bungu *et al.*, 2006; Thamburan *et al.*, 2006). However, only few publications have reported on the biological activities of *T. violacea* (Jacobsen *et al.*, 1968; Sparg *et al.*,

2005; Bungu *et al.*, 2006; Thamburan *et al.*, 2006; Bungu *et al.*, 2008; Naidoo *et al.*, 2008; Ramesar *et al.*, 2008; van den Heever *et al.*, 2008; Kirby & Meyers, 2010; Ebrahim & Pool, 2010). Ramesar *et al.* (2008) reported that crude leaf extracts of *T. violacea* inhibited angiotensin I converting enzyme (ACE) and also blocked the rise in mean arterial pressure (MAP) associated with infusion of exogenous angiotensin I in normotensive male Wistar rats; while Mackraj *et al* (2008) observed a reduction in systemic arterial BP associated with decreased renal angiotensin II type 1 (AT_1) receptor gene expression in DSS rats.

Pharmacological disruption of the renin angiotensin aldosterone system (RAAS) using ACE inhibitors (ACEIs) and angiotensin II (Ang II) receptor blockers offer primary and secondary protection to the cardiovascular system (CVS), brain, and kidneys (Nagata *et al.*, 2002). The renin angiotensin aldosterone and bradykinin system is a key physiologic regulator of vascular tone, salt and water balance, and BP (Gradman, 2009; Lacolley *et al.*, 2009). *T. violacea* has also been reported to have anticoagulant (Bungu *et al.*, 2008), antithrombotic (Bungu *et al.*, 2008) and antioxidant (Naidoo *et al*., 2008) properties. These properties are equally beneficial towards the prevention and treatment of HTN and other CVDs. Therefore, the motivation for this study is to further elucidate the mechanism(s) by which *T. violacea* may reduce BP in spontaneously hypertensive rats (SHR), and by inference propose mechanisms by which the extracts of the plant can help treat HTN in humans.

CHAPTER TWO

LITERATURE REVIEW

1. PLANTS WITH CARDIOVASCULAR EFFECTS

1.1. ***Allium sativum* (Liliaceae) Garlic**: Although results obtained from clinical studies have been conflicting (Banerjee & Maulik, 2002; Tanamai *et al.*, 2004; Turner *et al.*, 2004), the results from *in vitro* (preclinical) studies and epidemiologic studies indicate that garlic may be beneficial against cardiovascular disease (CVD) (Rahman & Lowe, 2006). Garlic and its derivatives have been reported to significantly decrease the diastolic BP (DBP) in humans (McMahon & Vargas, 1993), decrease both the circulating ang II levels and the systolic BP (SBP), while increasing the nitric oxide (NO) system activity in hypertensive rats (Mohamadi *et al.*, 2000; Preuss *et al.*, 2001), increase NO production in isolated pulmonary arteries of rats (Kim-Park & Ku, 2000), reduce the increases in ACE activity in serum and various tissues during the development of HTN in the two-kidney-one-clip (2K1C) hypertensive rats (Sharifi *et al.*, 2003), dilate blood vessels (Zahid Ashraf *et al.*, 2005), inhibit angiotensin converting enzyme (ACE) (Sendl *et al.*, 1992), possess anti-oxidative properties (Dillon *et al.*, 2003); increase fibrinolysis , inhibit blood coagulation, inhibit platelet aggregation and thrombus formation (Rahman & Lowe, 2006), reduce lipid levels in blood (Augusti *et al.*, 2005), possess anti-atherogenic and anti-atherosclerotic effect (Elkayam *et al.*, 2003; Kempaiah & Srinivasan, 2005). Undesirable effects have also been reported with the consumption of large doses of garlic (Yadav & Verma, 2004).

1.2. ***Rauwolfia serpentina* (Apocynaceae) (*Radix Rauwolfiae*):** Snake-root is a small shrub, with snake shaped woody roots, and is a proven cure for snake bite. *R. vomitoria*, a

related species, contains high amounts of reserpine. A third species, *R. canescens* is also used in West Africa to treat HTN (Gurib-Fakim, 2006).

1.3. ***Crataegus monogyna*** **(Hawthorn) and** ***Crataegus laevigata*** **(Hawthorn thorny):** has been reported to improve cardiac function, reduce afterload, HTN, tachycardia and arrhythmia in both clinical and pharmacological studies (Walker *et al.*, 2006).

1.4. ***Curcuma longa*** **(Turmeric):** and compounds obtained from it have been reported to possess vasodilatory (Ashraf *et al.*, 2005); as well as anti-inflammatory, anti-carcinogenic, hypolipidemic and antioxidant properties (Deters *et al.*, 2003).

1.5. ***Salvia miltiorrhiza*** **(Red sage, Danshen):** has been reported to stimulate endothelial NO synthase (eNOS) production and reduced BP in the two-kidney, one-clip (2KIC) reno-vascular HTN model in hamsters (Kim *et al.*, 2007).

1.6. **Other herbs;** aside *Tulbaghia violacea*, with reported CVS protective effect include *Cissus assamica* (Laws.) Craib (Yang *et al.*, 1998), *Ocimum gratissimum* L. (Labiatae) (Lahlou *et al.*, 2004) and *Andrographis paniculata* (A. paniculata) (Yoopan *et al*., 2007).

2. *TULBAGHIA VIOLACEA*

Tulbaghia violacea Harv. (Alliaceae) is a small bulbous herb. It has attractive mauve or purple flowers and hairless leaves arising from a white, fleshy stalk. It belongs to the family, Alliaceae, most commonly associated with onions and garlic (figure 2.1); and is commonly known as wilde knoffel (Afrikaans), isihaqa (Zulu) or itswele lomlambo (Xhosa). It is also called “society garlic”, “sweet garlic” and “wild garlic” based on the assumption that, in spite of its garlic-like flavour, its consumption is not accompanied by the development of bad

breath as is the case with the real garlic (*Allium sativum* L). The plant is widespread throughout Africa, with the greatest concentration in Southern Africa, and is indigenous to Natal, Transvaal and the Eastern Cape region in South Africa where it grows in rocky grasslands. In Southern Africa, there are three indigenous genera within this family: *Agapanthus*, *Allium*, and *Tulbaghia*. The plant has been used in some cultures as a substitute for garlic and chive possibly for culinary and/or medicinal purposes. It is widely used as herbal remedy for various ailments such as fever, colds, asthma, tuberculosis, rheumatism, paralysis, HTN, stomach problems and oesophageal cancer; with its leaves and bulbs being the parts most commonly used. It is grown around homes to drive away moles and snakes by the Zulus of South Africa. Despite its glowing reviews, adverse effects such as gastroenteritis, abdominal pain, acute inflammation, sloughing of the intestinal mucosa, contraction of the pupils, as well as fatalities have been reported following treatment with extracts of the plant. Other related species include, *Tulbhagi alliacae* which is used to treat fever, fits, rheumatism and paralysis and *Tulbhagi simmerlerj* which is used as a substitute for *Tulbhagia violacea* (van Wyk & Wink, 2004; Bungu *et al.*, 2006; Bungu *et al.*, 2008).

T. violacea has been postulated to have similar secondary metabolites and hence, biological activities as garlic since they belong to the same family (Order Asparagales, Family Alliaceae) and both have the characteristic sulphur smell of garlic (Bungu *et al*., 2006). The medicinal properties of garlic have been attributed to the sulphur compounds which incidentally have been isolated in both plants (Burton & Kaye, 1992; Hirsch *et al*., 2000; Kubec *et al*., 2002). These sulphur containing compounds are produced when alliinase, an enzyme present in all plants belonging to the *Allium* species, reacts with alliin when the plant is bruised/ crushed (Burton & Kaye, 1992; Kubec *et al*., 2002). The pH optimum of alliinase

from both garlic and *T. violacea* are 6.5; and that for onion 8 (Nock & Mazelis, 1986, 1987). Numerous odour forming compounds (Kubec *et al.*, 2002) and bioflavonoids such as quercetin (Hutchings *et al.*, 1996) have been isolated from extracts of *T. violacea.*

Figure 2. 1: Picture of *Tulbaghia violacea* showing the green hairless leaves and purple flowers. The picture was taken by the author of this thesis, i.e., Raji, I. A.

Apart from the cardio-protective properties of *T. violacea* previously mentioned in Chapter 1 of this thesis, the plant has also been reported to have anti-proliferative and pro-apoptotic effects in human cancer cell lines (Bungu *et al*., 2006; Bungu *et al*., 2008); anti-thrombotic activity (Bungu *et al*., 2008); anti-oxidant properties (Naidoo *et al.*, 2008); anti-fungal activity against *Candida albicans* (Motsei *et al.*, 2003); anti-bacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (Gaidamashvili & van Staden, 2002); anti-helmintic activity (McGaw *et al.*, 2000) and potential benefits in treating coccidial infections (Naidoo *et al*., 2008).

3. MODELS IN CARDIOVASCULAR RESEARCH

The experimental models and preparations used in cardiovascular (CV) research include the models that are based on isolated cells or tissues or structures immersed in organ baths

(Chorro *et al.*, 2009). Animal models have allowed the study of CVD in the early stages, as well as the investigation of the mechanisms of the pathogenesis of CVD and the effects of drug intervention in humans (Doggrell & Brown, 1998). Rodent models are most widely used due to the possibility for (a) genetic and environmental standardization, (b) availability of a broad spectrum of strains tailored to specific scientific problems, and (c) their acceptance by the regulatory authorities (Aigner *et al.*, 2010). The use of rats is rational from economic viewpoint and many techniques have been developed to measure relevant functional parameters. However, there are some drawbacks. For instance, CVDs such as HTN and heart failure in humans are usually slowly developing with wide-ranging neurohumoral adaptations in contrast to the acute onset of symptoms in many surgical or drug-induced rat models. Secondly, CVD is uncommon in young humans but markedly increases with age while most models of HTN and heart failure only use young adult rats (Doggrell & Brown, 1998; Chorro *et al.*, 2009; Sweeney, 2010). Scientific studies typically begin at "the bench" with basic research, before progressing to the clinical level (Takata & Kato, 1996; Y. Suzuki *et al.*, 2008; Chorro *et al.*, 2009; Y Suzuki *et al.*, 2009). The spontaneously hypertensive rat (SHR) and the hereditary obese mice, ob/ob mice, are among the very few excellent animal models that excellently mimic the respective human disease, and are therefore, very useful for translational research into the common human diseases, HTN and obesity (Nakao *et al.*, 2009).

The anaesthetized SHR model allows the researcher to record changes in BP and heart rate (HR) in intact animals, in response to administered drugs. The rat is anaesthetized with sodium pentobarbitone, injected intra-peritoneally, and the trachea is exposed and cannulated for artificial respiration. To record changes in HR and BP, the carotid artery, the abdominal aorta or the femoral artery is exposed and cannulated. The cannula is connected to a pressure

transducer and thence to a suitable instrument to record changes in BP and HR. Drug substances are infused via a cannula inserted into either the jugular or femoral vein (Obikeze, 2009) (figure 2.2). Although there are some drawbacks in using this model, such as the effect of anaesthetic agents on BP and HR, and the possible dampening of pressure due to blood clotting. The dampening of pressure could be avoided by carefully infusing heparinised saline, with care taken to ensure that the volume infused is also removed to avoid volume expansion which will also increase the BP. Infusion of excess heparin in the animal must be avoided, as it can affect the BP (Hearse & Sutherland, 2000; Kurtz *et al.*, 2005)

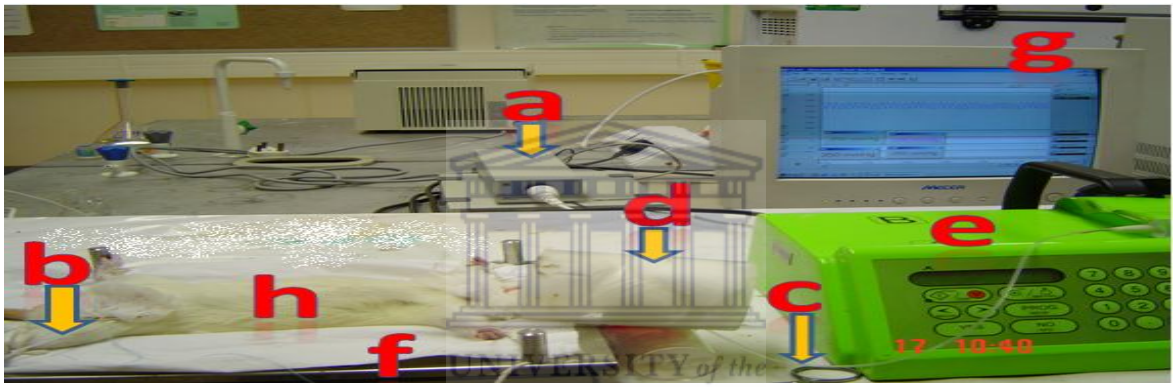

Figure 2. 2: The anaesthetized SHR rat model. (a= BP transducer; b= arterial catheter; c= venous catheter; d= Oxygen mask; e= syringe pump; f= small animal operating table; g= computer running chart 5 software; h= male SHR) (Obikeze, 2009). The above picture was adapted from Obikeze (2009), although the methods have been previously used by several researchers.

4. THE SPONTANEOUSLY HYPERTENSIVE RAT

The SHR initially bred in Kyoto, Japan is the most widely studied animal model of essential HTN (Doggrell & Brown, 1998; Choi *et al.*, 2009b; Fernandes-Santos *et al.*, 2009; Koprdova *et al.*, 2009; Shin *et al.*, 2009; Susic *et al.*, 2009a). The SHR strain was developed by Okamoto and Aoki (1963) and has been maintained by selective sibling mating. The

normotensive descendants of the Wistar rats, known as the Wistar-Kyoto rats, WKY, obtained from the colony in Kyoto from which the SHR strain was derived is often used as controls for the SHR (Kurtz & Morris Jr, 1987; Doggrell & Brown, 1998).

Hypertension is present in 100% of SHR. In many colonies, pre-HTN (100 to 120 mmHg) is present during the first month of life (Adams *et al.*, 1989; Dickhout & Lee, 1998). Increased BP (above 140 mm Hg) is present by 5 weeks of age in many rats, and by 10 weeks of age, it is maintained at over 180 mm Hg, frequently exceeding 200 mmHg (Anderson *et al*., 2006; Charles River Laboratories International, 2009). The HR values of the SHR are also usually significantly higher than those of the WKY (Valenti *et al.*, 2009). *In vivo* studies have shown that the SHR has an increased cardiac output (CO) with normal total peripheral resistance (TPR) in the early stages of HTN, but as the animal progresses into the established HTN state, the CO returns to normal while the hypertrophied blood vessels produce an increase in the TPR (Smith & Hutchins, 1979). The SHR also develops left ventricular hypertrophy (LVH), increased BP and BP variability; all of which are important indices of heart damage (Zamo *et al.*, 2010). An advantage of the SHR is that compounds which lower BP in this strain also lower BP in hypertensive humans. A criticism of the SHR model is that little is known about the cause of the onset of HTN, although it has been around for a long time (Doggrell & Brown, 1998).

Other strains of rats used in CV research, aside the SHR and WKY; include the New Zealand genetically hypertensive rats (GH); the Dahl salt sensitive (DSS) and Dahl salt-resistant (DSR) rats; the Obese SHR (Koletsky); the SHR-stroke prone (SPSHR); the Israel deoxycorticosterone acetate (DOCA) salt-sensitive (SBH) and Israel DOCA salt-resistant

(SBN) rats; the Lyon hypertensive (LH), Lyon normotensive (LN) and Lyon low BP (LL) rats (Doggrell & Brown, 1998; Anderson *et al.*, 2006; Charles River Laboratories International, 2009).

5. THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system (ANS) plays a vital role in the control of the CV activity, therefore, impairment of its activity will lead to a corresponding disruption in its function (McCorry, 2007; Scigliano *et al.*, 2008; Thayer *et al.*, 2010). The ANS has two major branches, i.e., the sympathetic system (SNS) and the parasympathetic systems (PNS). The SNS enables the body to respond to challenges to survival, situations of haemodynamic collapse or respiratory failure; and its responses include increases in HR, BP, and CO and a decline in metabolic activity. Meanwhile, the PNS regulates the conservation and restoration of energy by causing a reduction in HR and BP; and facilitating the digestion and absorption of nutrients and the discharge of wastes. The activities of the two systems are normally in a dynamic balance (Thayer *et al*., 2010). Interestingly, a large proportion of patients with HTN have increased sympathetic activity, associated with decreased parasympathetic activity (Dauphinot *et al.*, 2010; Joyner *et al.*, 2010).

6. THE CARDIOVASCULAR SYSTEM

The cardio-vascular system (CVS) generally consists of the heart (cardio) and blood vessels (vascular). Specifically, the CVS can be viewed as the pulmonary circulation and the systemic circulation. The two circulations are arranged in series and, the right and left heart chambers must pump identical volumetric flow rate in health. The primary function of the

CVS is to circulate the blood through the capillaries to within diffusion distances (less than or equal to 10 μm) of every tissue parenchyma. Blood serves as the vehicle for delivery and removal of nutrients and waste products respectively, while the vessels serve as the highways. These 'highways' have a passive capacitance function that maintains the BP in diastole, and an active auto-regulatory control that allows the organ to respond to local metabolic demands (Kassab, 2006).

6.1. THE VASCULAR SMOOTH MUSCLE CELLS

The vascular smooth muscle (VSM) cells (VSMCs) are the major cellular component of the vascular media and mediate vasodilatation and vasoconstriction. They are innervated by sympathetic and parasympathetic fibres and depend on the vasa vasorum located in the outer layer of the vessel wall which sends branches into the outer part of the media for oxygen and nutrients. Numerous hormones and growth factors act on the VSMCs to cause migration, proliferation, and the secretion of extracellular matrix (Günthner *et al*., 2009). Rapid changes in vascular diameter primarily depend on the contractile activation and interaction of actin with myosin in VSMCs (Martinez-Lemus *et al.*, 2009).

6.2. THE ENDOTHELIUM

The human vascular endothelium constitutes approximately 1% of the total body mass (1 kg) and has a surface area of approximately 5000 m^2. The endothelium is a multifunctional endocrine organ strategically placed between the vessel wall and the circulating blood, and has a key role in vascular homeostasis. The endothelium is both a target for and a mediator of CVDs such as HTN, diabetes, insulin resistance, obesity and hyperlipidaemia (Galley &

Webster, 2004; Khazaei *et al.*, 2008; Günthner *et al.*, 2009; Higashi *et al.*, 2009; Huang, 2009). Through flow-mediated release of vaso-active autacoids, endothelial cells are important participants in scenarios leading to remodelling of resistance vessel structure. Ultimately, the endothelium with neural, hormonal, and metabolic stimuli, collectively influence VSM function and the structure of the vessel wall (Günthner *et al*., 2009; Martinez-Lemus *et al*., 2009). The normal functions of the endothelium include mediating vasodilatation (Vanhoutte, 2009), haemostasis (Gross & Aird, 2000), angiogenesis (Lingen, 2001; Szekanecz & Koch, 2001), inflammation and immune response (Khazaei *et al*., 2008), and also acting as a "tissue-blood barrier" (Irie & Tavassoli, 1991; Toborek & Kaiser, 1999). The endothelium generates relaxing factors such as; the endothelium-derived relaxing factor (EDRF/NO) (Vanhoutte, 2003; Forstermann & Munzel, 2006); b) prostacyclin (Coleman *et al.*, 1994); endothelium-derived hyperpolarizing factor (EDHF) (Emerson & Segal, 2001; Ellis & Triggle, 2003). It also generates contracting factors; such as endothelin-1 (Shah, 2007); thromboxane A2, prostaglandin endoperoxide (Stankevicius *et al.*, 2003) and 20-hydroxyeicosatetraenoic acid (20-HETE) (McGiff & Quilley, 2001).

6.2.1. ENDOTHELIUM DYSFUNCTION

Endothelial dysfunction occurs when the endothelial cells do not carry out their normal physiologic and protective functions. This leads to progressive degeneration of the CVS, characterized by a decline in NO synthase (NOS) activity. There is also an exaggerated increase in the release of paracrine factors, which act either as growth factors to induce VSMC proliferation or as chemokines to recruit circulating inflammatory cells (Channick *et al.*, 2004; Deanfield *et al.*, 2007; Khazaei *et al.*, 2008; Günthner *et al.*, 2009).

6.3. THE CONTROL OF BLOOD VESSELS

The vascular tone (the active tension of smooth muscles in the tunica media) controls the width of blood vessels. This tone regulates local blood flow, arterial pressure, capillary filtration and central venous pressure; and is under the influence of intrinsic and extrinsic factors. The intrinsic factors include the a) myogenic response (arterial vessels contract when BP rises), b) endothelial secretions (NO, EDHF, prostacyclin, endothelin-1), c) vasoactive metabolites (adenosine, carbon (IV) oxide, oxygen, lactate, potassium ions), d) autacoids (histamine, bradykinin, serotonin, prostaglandins, thromboxane A_2), e) local pH and f) temperature. Meanwhile the external influences include the a) vasomotor nerves involved in short term regulation; and b) circulating hormones (such as adrenaline, noradrenaline, vasopressin, angiotensin II, histamine) involved in long term regulation (Levick, 2003; Ganong, 2005; Pocock & Richards, 2006).

6.4. THE ARTERIAL BLOOD PRESSURE

The pressure in the aorta and in the brachial and other large arteries in a young adult human rises to a peak value (systolic pressure, SBP) of about 120 mm Hg during each heart cycle and falls to a minimum value (diastolic pressure, DBP) of about 70 mm Hg (Joyner *et al.*, 2008). The SBP reflects how much the energy of ejection is able to compress the arterial contents downstream of the ventricles. The combination of the stroke volume (SV) and SBP in normal individuals can be used to assess cardiac workload, most commonly calculated by the product of HR and SBP, and termed rate-pressure product. The DBP is influenced by the period over which the pressure can fall, so the HR in itself is a major determinant of DBP,

with tachycardia predictably causing a pressure rise. A second major determinant of DBP is the speed at which the pressurized blood flows out through the resistance of peripheral blood vessels. Therefore, the TPR is also a major determinant of DBP (Bell, 2008). The pulse pressure (PP) is the difference between the SBP and DBP (Ganong, 2005). The mean arterial pressure (MAP) is the average pressure throughout the cardiac cycle, and its approximate value is obtained from the DBP plus one third of the PP (Ganong, 2005; Bell, 2008; Joyner *et al.*, 2008).

6.5. THE PERIPHERAL VASCULAR RESISTANCE

The peripheral resistance is determined mainly by the diameter of the lumen of resistant arterioles, and to a lesser extent, that of the medium and large arteries. Luminal diameter can be altered actively by the contraction of smooth muscles, or passively by remodelling; under the influences of the hemodynamic load, neuro-humoral regulation and the concentrations of sodium and potassium ions (Hulin *et al.*, 2009). The product of the TPR and CO gives the arterial BP (Levick, 2003).

6.6. BARORECEPTORS

These are stretch receptors in the walls of the heart and blood vessels that monitor blood flow. They are the input end of the arterial baroreflex and are located in the carotid sinus and the aortic arch. Afferent signals are sent via the glosso-pharyngeal and vagus nerves to the vasomotor centre (VMC) (of the nucleus tractus solitariis) in the medulla oblongata. Efferents from the VMC are inhibitory to the tonic discharge of vasoconstrictor nerves; and excitatory

to the vagal innervations of the heart leading to vasodilation, venodilation, hypotension, bradycardia and fall in CO (Ganong, 2005).

The ultimate goal of the arterial baroreflex is to maintain arterial BP homeostasis. The baroreceptors chronically reset to a higher "set point" when arterial BP is chronically elevated; and defends the higher BP, until the set point is again adjusted (Brunner *et al.*, 1979; Ganong, 2005; Bogachev *et al.*, 2009). Baroreceptor sensitivity is impaired in many experimental models of HTN, and appears to precede the onset of HTN (Peach, 1977). Impaired baroreflex is believed to lead to significant dysregulation of BP; leading to increased BP variability, orthostatic hypotension, aberrant pressure rises with major risk of fatal events such as myocardial infarction (MI) and stroke (Tozawa *et al.*, 1999; Mancia & Parati, 2003; Di Rienzo *et al.*, 2009); and has been reported in several models of HTN such as SHR, 2KIC hypertensive model and Lyon hypertensive rat. Angiotensin II inhibits baroreceptor function (Huang & Leenen, 1998; Huang *et al.*, 2006; Xie *et al.*, 2006; Parsons & Coffman, 2007; Carlson & Wyss, 2008; Cravo *et al.*, 2009).

6.7. CARDIAC OUTPUT (CO)

The volume of blood pumped out of each ventricle per minute = 5 litres/min in humans. It is influenced by venous return, HR, and cardiac contractility (Levick, 2003; Ganong, 2005; Pocock & Richards, 2006).

6.8. HEART RATE

This is the number of times the heart beats/contracts in a minute. Heart rate (HR) and stroke volume are the main factors used by animals to effectively adapt CO to metabolic demands (Levine, 1997); and in normal adults, it depends on the pacemaker activity of the sinoatrial (SA) node cells and constantly varies under the influence of a number of non-modifiable and modifiable factors (Valentini & Parati, 2009). The non-modifiable factors include age (Yamaguchi *et al*., 2005) and sex (with women generally having higher resting HR when compared with men) (Stolarz *et al.*, 2003). The modifiable (physiologic) factors include (i) circadian rhythm (HR is usually lower during sleep) (Ben-Dov *et al*., 2007); (ii) BP (HR is positively associated with BP) (Zhang & Kesteloot, 1999); (iii) physical activity (training reduces the resting HR (Borresen & Lambert, 2008); (iv) mental stress (increases catecholamines, and hence HR and BP) (Carter & Ray, 2009); (v) smoking (increases HR and BP) (Groppelli *et al.*, 1992); (vi) alcohol consumption (increases HR) (Kloner & Rezkalla, 2007) and (vii) excess body weight, (increases HR) (S. Julius *et al.*, 2000). The importance of the resting HR as a prognostic factor and potential therapeutic target is not yet generally accepted, even though recent large epidemiologic studies have confirmed earlier studies that showed resting HR to be an independent predictor of CV and all-cause mortality in men and women with and without diagnosed CVD. Studies have also found a continuous increase in CV risk with HR above 60 beat per min (bpm) (S Julius, 2009; Perret-Guillaume *et al.*, 2009; S Julius *et al.*, 2010). Among mammals, with the exception of the human species who has on the basis of his resting HR doubled his life time, there is a linear, inverse semi-logarithmic relation between HR and life expectancy (Levine, 1997).

6.8.1. HEART RATE VARIABILITY

Heart rate variability (HRV) is a non-invasive, practical and reproducible measure of the function of the ANS. A HR that is variable and responsive to demands is believed to bestow a survival advantage, whereas reduced HRV may be associated with poorer CV health and outcomes (Nunan *et al.*, 2010; Routledge *et al.*, 2010; Thayer *et al.*, 2010; Wheat & Larkin, 2010).

6.9. ACID- BASE BALANCE AND HAEMODYNAMICS

Although, the normal range of pH for clinical laboratories is 7.35-7.45, in vivo pH is maintained within a much narrower range. Blood pH varies in response to respiratory or renal impairment. A reduction in the peri-vascular pH decreases the responsiveness to vasoconstrictors and consequently, leads to difficulty in maintaining systemic BP (Wagner *et al.*, 2009).

7. HYPERTENSION

Hypertension (HTN) is a sustained elevation of the systemic arterial BP (Ganong, 2005). It can either be (a) essential HTN or (b) secondary HTN. Essential HTN is the HTN without any obvious predisposing organic cause (Levick, 2003). Approximately 93% of all HTN are of the essential type (Williams, 2008). Secondary HTN has identifiable pathological cause such as (a) hyperaldosteronism (b) renal artery stenosis, (c) phaeochromocytoma, (d) pre-eclamptic toxaemia (Levick, 2003). Sadly, HTN in childhood has now been recognized as a common and serious problem, with a prevalence of between 2% to 5% (Kavey *et al.*, 2010).

In humans, a daytime BP of less than 120/80 mm Hg is regarded as optimal, while BP less than 130/85 mm Hg is regarded as normal. Repeated measurements of resting BP above 140/90 mm Hg in humans under 50 years old, or 160/95 mm Hg in an older individual is considered to be HTN (Williams, 2008; Hulin *et al.*, 2009). In rats, BP below 125/83 mm Hg is considered to be normal, while a SBP above 140 mm Hg is generally considered to be HTN (Anderson *et al*., 2006; Charles River Laboratories International, 2009).

8. CONTROL OF HIGH BLOOD PRESSURE

Blood pressure is regulated by the CV, renal, endocrine, and central nervous systems and it in turn controls these systems via negative feedback mechanisms. Chronic rise in BP leads to vascular remodelling with resultant thickening and sclerosis of vessel walls, consequent endothelial dysfunction, loss of NO production, and irreversible rise in TPR (Aggarwal & Khan, 2006; Chang & Hollander, 2008). The failure to control high BP is multi-factorial in most patients, but usually includes lack of disease awareness, lack of consensus among treatment guidelines, diet and lifestyle factors, poor patient adherence to dietary/lifestyle initiatives and prescribed drug regimens (Sever *et al.*, 2009). Irrespective of the level of HTN, lowering of BP is always preferable by non-pharmacological means such as adequate sleep, low salt diet, weight loss, exercise and alcohol restriction (Williams, 2008; Kones, 2011).

9. PHARMACOLOGIC INTERVENTION

This can be done by (a) disrupting the RAAS, (b) reducing TPR, (c) reducing the CO and (d) reducing the extracellular fluid (ECF) volume (Levick, 2003; Hoffman, 2011).

9.1. THE RENIN ANGIOTENSIN, ALDOSTERONE AND BRADYKININ SYSTEM (RAABS)

The RAABS/RAAS is a key physiologic regulator of vascular tone, salt and water balance, BP, bradykinin system and pituitary gland hormones (Gradman, 2009; Lacolley *et al.*, 2009; Zamo *et al.*, 2010). Short term activation of the system is geared towards maintaining intravascular volume, BP, and tissue repair; however, chronic activation leads to chronic vasoconstriction and elevation of BP, VSMC growth/migration, endothelial dysfunction, oxidative stress, the release of cytokines and inflammatory cell activation, fibrosis, and thrombosis. All these will lead to vascular and myocardial hypertrophy, left ventricular remodelling, atherosclerosis, and glomerulosclerosis. The resultant structural changes can ultimately result in MI, stroke, and end-stage renal disease. Based on the pivotal role the system plays in the development and progression of HTN and HTN- related end organ damage, deactivation of the system is crucial in treating HTN and related end organ damage (Gradman, 2009).

9.1.1. PRO-RENIN AND RENIN

The synthesis of ang I and ang II is impossible in the absence of renin (or pro-renin) (figure 2.3). Renin cleaves the decapeptide ang I from the amino terminus of angiotensinogen (Fisher & Hollenberg, 2001). Renin is produced through the activation of pro-renin. Pro-renin is synthesized as a pre-prohormone (Gradman & Kad, 2008). Renin secretion is mainly regulated by the pressure in the afferent arteriole of the kidney, sympathetic nerve stimulation of the β_1 adrenergic receptors in the juxta-glomerular apparatus (JGA), sodium ions (Na^+) at the macula densa and negative feedback by Ang II (Brown, 2006).

Prorenin, initially believed to be inactive, becomes enzymatically active when bound to the (pro) renin receptors (localized to the VSMCs; human heart, kidney, and brain; mesangial cells; and cells in the distal and collecting tubules of the renal parenchyma) and can also produce ang I. This pathophysiologic role seen in vascular and renal disease is a clinical index of small vessel disease in patients with diabetes. Prorenin binds with greater affinity to this receptor than renin and the rate of ang I production is increased by 4 to 5 times with receptor-bound renin when compared to free renin (Nguyen *et al.*, 2002; Gradman & Kad, 2008; Gradman, 2009). Aliskiren is a potent orally active competitive inhibitor of renin in animals and humans and binds directly unto renin (Gradman & Kad, 2008; Riccioni *et al.*, 2010).

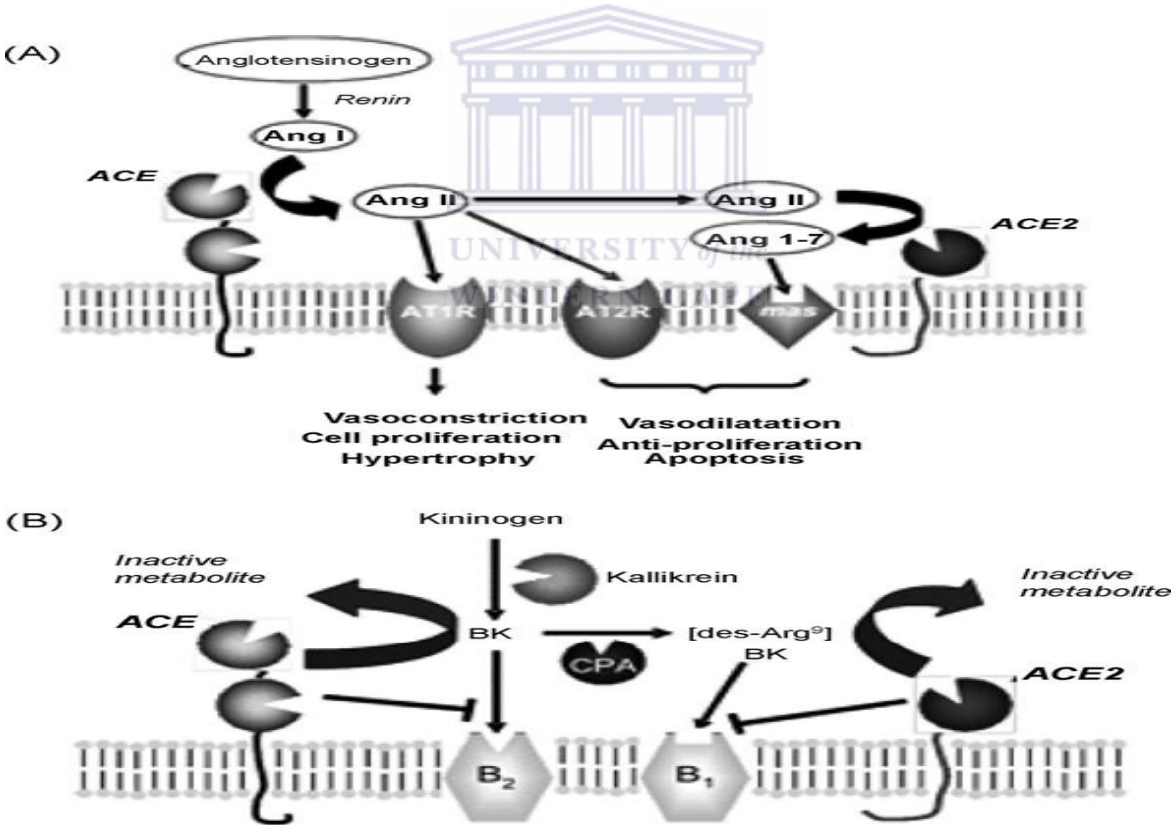

Figure 2. 3: Schematics illustrating the involvement of ACE and ACE 2 in the regulation of the functions of the RAAS (A), and the kinin-kininogen system (B), carboxypeptidase A, CPA (Lambert *et al.*, 2008).

9.1.2. ANGIOTENSIN-I CONVERTING ENZYME (ACE)

Angiontensin I (ang I) is rapidly converted to ang II by the ACE located on endothelial cells in vascular beds and on the membranes of many other cells including the brush border membranes of the renal proximal tubules (Mezzano *et al*., 2003). The ACE cleaves the C-terminal dipeptide from Ang I and bradykinin, i.e., it interacts with both the RAAS and the kallikrein–kinin system (Hanif *et al.*, 2010). Approximately, 10% of ACE circulates in the plasma, while the rest is found in the tissues, heart, brain, kidney, and arteries. The circulating RAAS that controls acute hemodynamic modulation is an ACE-dependent system. Angiotensin II acts on both the ang II type 1 (AT_1) and the angtiotensin II type 2 (AT_2) receptors (figure 2.3) (Gradman, 2009). The ACE 2 shares 42% identity with the catalytic domain of ACE (Towler *et al*., 2004) and is able to metabolize ang I into the nonapeptide Ang-(1–9). Ang II itself is also a substrate for ACE 2, and is the preferred pathway for ACE 2 with an almost 500- fold greater efficiency than the cleavage of ang I. (figure 2.3) (Luhder *et al.*, 2009).

Inhibitors of ACE (ACE-Is) arose from early studies performed in the 1960s showing that components of venom from the Brazilian arrowhead viper (*Bothrops jararaca*) inhibited kinase II, an enzyme involved in the degradation of bradykinin and later found to be identical to ACE. Analogues of the nonpeptide fraction of the snake venom (teprotide) which inhibited ACE were found to lower BP in hypertensive patients and produce beneficial effects in heart failure patients. Subsequent research revealed that ACE inhibition could be achieved by succinyl amino acids (e.g., carboxyalkanoyl and mercaptoalkanoyl derivatives), a finding which ultimately led to the discovery of captopril. Since that time, a plethora of ACE-Is having been developed (enalapril, quinapril, ramipril, spirapril, e.t.c.) which share a

common structural moiety that interacts with the zinc ion in the ACE active site. Initially introduced as antihypertensive agents, ACE-Is have demonstrated clinical efficacy in the treatment of a wide range of CV and renal diseases, including heart failure, MI, chronic renal failure, and sclerodermal renal crisis (De Leo *et al.*, 2009; Bakris, 2010; Hanif *et al.*, 2010). These agents also inhibit the enzyme kininase II which catalyzes the breakdown of bradykinin and related peptides; therefore the reduction in BP is a result of both a decrease in the levels of ang II as well as an increase in the levels of bradykinin. They also stimulate whole body glucose dispersal and uptake of glucose by skeletal muscle by increasing insulin sensitivity and upregulation of the cell surface glucose transport protein glucose transporter, (GLUT)–4, in insulin resistance states. However, ang II can be formed via non-ACE dependent pathways, that are not affected by ACE inhibitors; hence, circulating concentrations can return to normal in some patients, despite continuing treatment (a phenomenon known as "angiotensin escape") (Unger & Stoppelhaar, 2007).

9.1.3. ANGIOTENSIN II

Angiotensin II (ang II) is the most powerful biologically active product of the RAAS (figures 2.3.). The ACE removes the carboxy-terminal dipeptide of ang I to produce the octapeptide ang II (Toda *et al.*, 2007; Skrbic & Igic, 2009). Angiotensin II directly constricts VSMCs, enhances myocardial contractility, stimulates aldosterone production, blunts the baroreflex, stimulates the release of catecholamines from the adrenal medulla and sympathetic nerve endings, increases sympathetic nervous system activity, and stimulates thirst and salt appetite. Locally produced ang II induces inflammation, cell growth, mitogenesis, apoptosis, migration, and differentiation, regulates the gene expression of bioactive substances, and activates multiple intracellular signalling pathways (Kobori *et al.*, 2007; Carlson & Wyss,

2008). Angiotensin II also plays an important role in atherosclerosis. Most of its hypertensinogenic actions are mediated through the AT_1 receptor; even though an angiotensin II type 2 (AT_2) receptor, has also been identified (Gradman, 2009).

The ang II receptor blockers (ARBs) have proven particularly useful in identifying the pathophysiological role of ang II in CVDs. These agents specifically block ang II, formed from both ACE and non-ACE sources, and do not impede the additional beneficial haemodynamic and metabolic actions of other biologically active peptides such as bradykinin and angiotensin-(1-7) [Ang-(1-7)] (figure 2.3.). Losartan is the the first orally active, selective, and potent nonpeptide ARB (Ferrario, 2006). The ARBs do produce more specific blockade of pro-inflammatory mediators than ACE inhibitors (Unger & Stoppelhaar, 2007), and reduce the incidence of new-onset type 2 diabetes by approximately 20%–25% (Picard & Auwerx, 2002). The ARB, telmisartan, displays a structural resemblance to pioglitazone, a thiazolidinedione peroxisome proliferator-activated receptor gamma (PPAR-γ) ligand, that is used in the treatment of type 2 diabetes (Pershadsingh, 2004). However, not all ARBs are capable of activating PPAR-γ (Unger & Stoppelhaar, 2007).

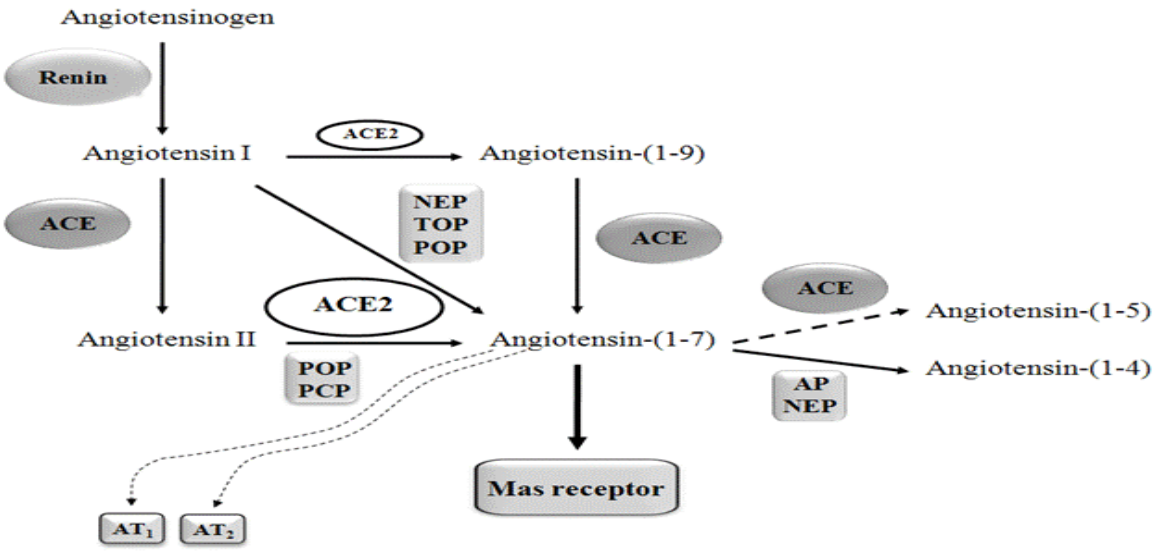

Figure 2. 4: Pathways for the formation and degradation of ang-(1-7) in the kidney (Dilauro & Burns, 2009).

Several angiotensinases and peptidases are able to metabolize ang II further to form biologically active smaller peptides such as ang III, ang IV, and ang 1-7 (Kobori *et al*., 2007). The heptapeptide ang-(1-7) produces its biological effects such as vasodilatation, natriuresis, diuresis, and anti-trophic effects by interacting with a non AT_1/AT_2 receptor. Ang-(1-7) is the endogenous ligand for the G-protein-coupled receptor *mas*, the stimulation of which leads to production of NO and prostacyclin (PGI_2). Ang-(1-7) (endogenous and exogenous) enhances the baroreflex (Diz *et al*., 2008). Ang-(1-7) may contribute to the antihypertensive effects of both ACE-Is and ARBs. By preventing ACE-mediated peptide degradation and increasing ang I availability, ACE inhibition elevates ang-(1-7). The ARBs also increase ang-(1-7) by increasing the availability of ang I, by blocking the AT_1-mediated negative feedback on renin, and promoting conversion of ang II to ang-(1-7) via increased ACE2 expression (figure 2.4) (Ferrario, 2006; Dilauro & Burns, 2009).

9.1.4. MULTIPLE ANTAGONISMS OF THE RENIN ANGIOTENSIN ALDOSTERONE SYSTEM

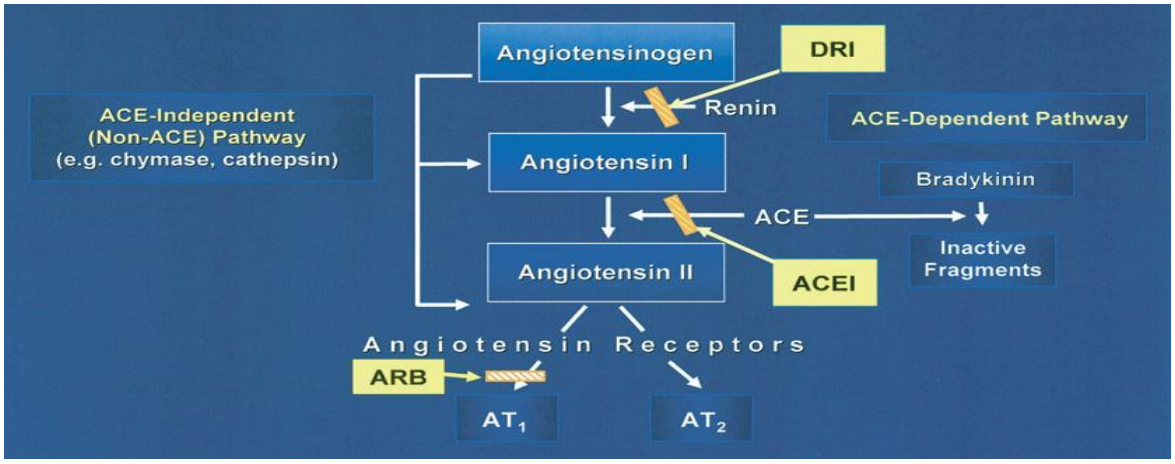

Figure 2. 5: The renin angiotensin cascade and the three available approaches towards pharmacological inhibition of the production or action of ang II. Direct renin inhibitors (DRI), angiotensin-converting enzyme inhibitors (ACEI), and angiotensin (AT) type 1 receptor blockers (ARB) (Gradman & Kad, 2008).

Monotherapy with ACE-Is, in doses commonly used in clinical practice, does not always result in complete RAAS blockade. Furthermore, there are alternative pathways for converting ang I to ang II; such as chymase, cathepsin D and chymotrypsin-like angiotensin generating enzyme, in the kidney, heart and blood vessels (figure 2.5). The use of an ACE-I reduces the short loop negative feedback of ang II on the JGA, resulting in a reactive increase in renin release and, thereby, continued ang I generation (Doulton & Macgregor, 2009). The use of aliskiren in combination with other inhibitors of the RAAS prevents the reactive rise in ang I and ang II which occurs with ACE-I and ARB combinations (figure 2.5) (Berl, 2009; Doulton & Macgregor, 2009).

9.1.5. ALDOSTERONE

Aldosterone is a mineralocorticoid synthesized in the zona glomerulosa of the adrenal gland in response to increased ang II, adrenocorticotropin and potassium levels in plasma. It regulates electrolyte, fluid balance and BP homoeostasis (Connell & Davies, 2005). It also mediates maladaptive tissue remodelling throughout the CV and central nervous system. Primary hyperaldosteronism leads to a greater frequency of resistant HTN (a state in which the BP remains high despite the use of three antihypertensive agents, one of which is ideally a diuretic), as well as CVD and chronic kidney disease (CKD) morbidity and mortality, compared with essential HTN (Aoki *et al.*, 2010; Briet & Schiffrin, 2010; De-An *et al.*, 2010; Whaley-Connell *et al.*, 2010; Lymperopoulos *et al.*, 2011). Spironolactone and eplerenone both antagonise the effects of aldosterone at its receptor (Ferrario, 2006).

9.1.6. THE BRADYKININ SYSTEM

The BP lowering property of the bradykinin system has been documented for more than 80 years. Kallikreins are a distinct group of serine proteases that can generate vasoactive kinins. The CVS actions of bradykinin, the most important endogenous kinin involved in the regulation of arterial BP includes reducing TPR by producing vasodilatation in most areas of the circulation, and the regulation of sodium excretion from the kidney. The bradykinin system regulates sodium water balance, renal and cardiac haemodynamics, and BP by mediating and modulating a) the vasoconstricting RAAS; and b) the vasodilating prostaglandin, prostacyclin, adrenomedullin and NO. Reduced activity of the system has been reported in various hypertensive situations in both clinical and experimental models of HTN

(Lessa *et al.*, 2008; De Leo *et al.*, 2009; Günthner *et al.*, 2009; Sharma, 2009; Bakris, 2010; Hanif *et al.*, 2010).

9.2. PERIPHERAL VASCULAR RESISTANCE

Another method of controlling high BP is by reducing the TPR with drugs, such as prazosin and nifedipine (Levick, 2003). The main haemodynamic abnormality in established severe HTN is an elevation of TPR with normal CO. However, in mild early HTN, there is a high CO with normal TPR often accompanied by an elevated HR. Elevated TPR is usually associated with higher plasma noradrenaline levels. Vasoconstrictor α_1-adrenergic fibres are the dominant nerve supply to most arteries, although some β_1-adrenergic and some cholinergic vasodilatator nerves may be present. Sympathetic nerves (acting through the β_1-receptors) and parasympathetic nerves (acting through muscarinic receptors) alter the rate and force of contraction of the heart muscle. Sympathetic activation of β_1-adrenoceptors also stimulates the release of renin into the circulation from the kidneys (Grassi, 2009; Paton & Waki, 2009; Weir, 2009).

9.2.1. CALCIUM CHANNEL BLOCKERS

Calcium channel blockers (CCB) block the entry of calcium ions into VSMCs, thereby, reducing the active tone of VSMs and leading to vasodilatation. They include (a) the phenylalkylamines (e.g. verapamil), (b) the benzothiazepines (e.g. diltiazem), (c) the dihydropyridines (e.g. nifedipine) and (d) the diphenylpiperazine. The agents in groups (a), (b), and (c) are used in the management of HTN; while the last group is used in treating

neurological disorders such as migraines, neuropathic pain, and dementia. A major feature of the long-term action of CCBs is the prevention of the structural changes induced by HTN in heart and arteries (Mason, 2002; Godfraind, 2005).

9.2.2. DRUGS THAT ALTER SYMPATHETIC NERVOUS SYSTEM FUNCTION

These agents are able to reduce one or more of the following; venous tone, HR, cardiac contractility, CO, and TPR; by interfering with the sympathetic control of the CVS by (a) reducing sympathetic outflow from VMC in the brainstem but allowing these centres to retain or even increase sensitivity to baroreceptor control, e.g. methyldopa, clonidine, guanabenz, and guanfacine, (b) blocking acetylcholine from stimulating the postganglionic autonomic neurons, (c) blocking the normal physiologic release of noradrenaline from postganglionic sympathetic neurons, e.g. guanethidine and reserpine, (d) selectively blocking the α_1 adrenergic receptors in arterioles and venules, while allowing noradrenaline to exert unopposed negative feedback on its own release, consequently, dilating both resistance and capacitance vessels, and producing vasodilation as well as venodilation. Salt and water retention occurs when these agents are used without diuretics, and are more effective when used in combination with β blockers and diuretics, e.g., prazosin, terazosin, doxazosin, (e) reducing the BP secondary to reducing CO, and are very useful in lowering BP in mild to moderate HTN, and prevent reflex tachycardia associated with treatment with direct vasodilators in severe HTN. They prevent catecholamines from binding to β_1, β_2, and α_1 adrenoceptors. Traditional beta-blockers (eg, atenolol, metoprolol and propranolol) affect only the β adrenergic receptors, while carvedilol and labetalol mediate vasodilation through blockade of the α_1 adrenoceptor. Other drugs like nebivolol may induce vasodilation by

stimulating the release of NO (Cruickshank, 2007; Cheng, 2009; Weir, 2009; Benowitz, 2011; Trevor *et al.*, 2011).

9.3. EXTRACELLULAR FLUID VOLUME

Diuretics can lower BP up to 10 -15 mmHg by increasing sodium and water excretion in urine, thereby, reducing the ECF volume, blood volume and CO; the TPR may, however, increase. After six to eight weeks, CO returns to normal while TPR falls. Sodium may increase vascular resistance directly by increasing vessel stiffness and neural activity. These drugs are adequate for mild to moderate essential HTN, and induce diuresis by (a) acting on specific membrane transport proteins in renal tubular epithelial cells (loop diuretics, thiazides, amiloride, and triamterene), (b) exert osmotic effects that prevent water reabsorption (mannitol), (c) inhibit enzymes (acetazolamide), or (d) interfere with hormone receptors in renal epithelial cells (spironolactone) (Levick, 2003; Katzung, 2004).

9.4. VASCULAR SMOOTH MUSCLE

Vasodilators can relax the VSMs of arterioles, thereby, reducing TPR. They do not induce orthostatic hypotension or sexual dysfunction since the sympathetic reflexes are intact. They work best in combination with other antihypertensives to oppose the compensatory CV responses to decreased arterial resistance and MAP such as sympathetic activation; and comprise of (a) the oral vasodilators (hydralazine and minoxidil, used for long term management of HTN); (b) the parenteral vasodilators (nitroprusside, diazoxide, and fenoldopam, used in hypertensive emergencies); and (c) the CCBs (discussed above, which are used in both situations) (Katzung, 2004).

Gradually the pattern of use of antihypertensive agents has changed, from prime use of diuretics and β blockers, to preference for the inhibitors of the RAAS as well as the CCBs (Opie, 2009).

10. THE RESEARCH PROBLEM

T. violacea has been observed to reduce BP in normotensive rats by inhibiting the ACE (Ramesar *et al*, 2008) and also in the DSS rats, by decreasing the renal AT_1 receptor gene (Mackraj *et al*, 2008). The present study aims to confirm these anti-hypertensive effects and furthermore, investigate other mechanisms (beside ACE inhibition and reduction in renal AT_1 receptor gene expression) by which the *T. violacea* may bring about its hypotensive effect in SHR.

11. THE RESEARCH HYPOTHESIS

In SHR, *T. violacea*;

(i) reduces BP and HR

(ii) inhibits ACE

(iii) blocks angiotensin II receptors

(iv) blocks α_1 and β_1 adrenoceptors

(v) stimulates cholinergic receptors

(vi) reduces serum aldosterone levels

12. AIMS

To determine the effect of crude methanol leaf extracts of *T. violacea* on the BP and HR of male SHR.

To propose mechanisms of action by which *T. violacea* brings about its effects on the BP and HR in SHR.

To compare the effects of known drugs with that of *T. violacea.*

To find out the effect of the plant extract on serum aldosterone levels.

13. OBJECTIVES

1. To investigate if *T. violacea* decreases BP and HR in the SHR.
2. To investigate if *T. violacea* acts by blocking ACE in the SHR.
3. To investigate if *T. violacea* acts by blocking angiotensin II receptors in the SHR.
4. To investigate if *T. violacea* works by blocking α_1 and β_1 adrenoceptors in the SHR.
5. To investigate if *T. violacea* works by stimulating the cholinergic receptors in the SHR.
6. To investigate if *T. violacea* works by reducing plasma aldosterone levels.
7. To compare the effects of T. violacea with that of known antihypertensive drugs.

CHAPTER THREE

METHODS AND MATERIALS

Chapter 3 describes the different methods employed in the extraction of the plant, the *in vivo* assessment of the crude extract, the analysis of the blood gas, and the radio-immunoassay quantification of aldosterone levels in serum. The equipment and chemicals used in the study are also listed in this chapter.

1. METHODS USED IN EXTRACTION

Fresh plants were purchased from the New Plant Nursery, George, Western Cape, SA, in August and September, 2008. The plant was identified by the taxonomist at the Department of Biodiversity and Conservation Biology of the University of the Western Cape (UWC), Bellville, SA; and deposited at the herbarium with voucher numbers 6955 and 6956. The remaining leaves were separated from the bulb, and weighed 2.378 kg before they were washed with normal water and afterwards, with distilled water, and then dried at 30°C in a ventilated oven for 72 hours, before being ground into a fine powder. The soxhlet apparatus was used to extract the ground leaves in methanol over a period of 24 hours and the resulting extract was filtered through a filter paper to remove small solid particles. The rotavapor was used to remove excess solvent *in vacuo* to afford a thick black paste. 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 DMSO and the required concentration made up with 0.9% NS and filtered before being infused into each rat to prevent the formation of emboli.

1.1. MATERIALS USED IN EXTRACTION

The equipments used in the extraction process included: a) the Soxhlet apparatus, b) heating mantles, c) grinder, d) Rotovapor (Bibbly Sterilin, England), e) freeze-drier (Labconco, Missouri), f) 0.45 µm filter papers (Schleicher & Schuell MicroScience). The chemicals used were methanol (Sigma-Aldrich, Steinheim, Germany) and b) distilled water

2. THE ANAESTHETIZED SPONTANEOUSLY HYPERTENSIVE RAT MODEL

Healthy male SHR within the weight range of 300 g and 350 g were used for the *in vivo* study of the compound. The animals were weighed and anaesthetized with sodium pentobarbitone (40 mg/kg) injected by the intra-peritoneal route. Anaesthetized animals were then transferred to a small animal operating table and restrained. A temperature probe was inserted into the rectum to monitor the animal's temperature throughout the experiment. Adjustments to body temperature were done by either increasing or decreasing the heating on the small animal operating table to keep rectal temperature at 37.3 ± 0.5 ^{o}C. A midline incision was made in the throat region to access the trachea, and an incision made into the trachea. A lubricated hollow tracheal tube was carefully inserted into the trachea through the incision and secured in place with knot. The tracheal tube was cleared of fluids, and an oxygen mask placed over the (head and) throat region to facilitate breathing. The external jugular vein was then exposed and excess tissue carefully cleaned off it, before it was clamped at the end proximal to the heart with a bulldog clamp to prevent blood loss. The exposed section was then filled with blood by gently massaging the head, and tied off at the end proximal to the head. An incision was made into the vein, and a catheter lubricated with K-Y jelly and filled with a

10% heparin solution was carefully inserted into the vein and secured in place. The femoral region was afterwards opened and the femoral artery carefully separated from the accompanying vein, nerve as well as surrounding tissue. The artery was tied off at the distal end and clamped with the bulldog at the proximal end. An incision was made into the artery between the tied end and the clamp, and a lubricated catheter filled with a 10% heparin solution carefully inserted and secured with knot. The bulldog clamp was then carefully removed to allow for blood flow into the catheter. Incisions were cleaned, covered with gauze and kept moist for the duration of the experiment. After surgery, animals were allowed a 30 minute recovery period before the commencement of experiments. During the recovery period, BP calibrations were done on the Chart 5 software and recording of parameters started (Obikeze, 2009). After the 30 minute stabilization period, animals whose BP and HR readings were below the expected values for HTN in the SHR (systolic BP (SBP) less than 150 mmHg and HR below 300 bpm) were excluded from the experiments. SHR normally have higher HR rates compared to their normotensive counterparts (Dickhout & Lee, 1998; Valenti *et al.*, 2009; Williams, 2010). A syringe pump was used to infuse drugs at a constant rate (0.3 ml/min) through the venous cannula. A sufficient recovery period of 30 minutes was allowed between individual doses so that the BP and HR can return to baseline values. In the experiments in which normotensive animals were used as control for the effect of *T. violacea on* BP and HR, the age of the animals were kept below 5 months, and animals which had BP and HR readings that were beyond the normal ranges (SBP 116 mmHg – 145 mmHg; diastolic BP 76 mmHg – 97 mmHg; mean arterial BP 103 mmHg – 129 mmHg; and HR 296 – 388 bpm) were excluded from the experiment (Krinke, 2000).

2.1. ANIMALS

Male spontaneously hypertensive Wistar rats (SHR) aged less than 5 months, were used to investigate the BP and/ or HR effects of the crude methanol leaf extract (MLE) of *T. violacea*. The SHR was used in this study as it (i) mimics human HTN remarkably well (ii) allows studies in chronic, stable disease, (iii) produces symptoms which are predictable and controllable, (iv) satisfied economical, technical and animal welfare considerations, and (v) allows measurement of relevant cardiac, biochemical and haemodynamic parameters (Doggrell & Brown, 1998; Nakao *et al.*, 2009). Male animals were preferred to female animals due to the higher prevalence of HTN in male animals compared to females (Iams & Wexler, 1979; Bachmann *et al.*, 1991; Reckelhoff, 2001; Ostadal *et al.*, 2009; Valenti *et al.*, 2009). Young adult rats (aged < 5 months) were also used as haemodynamic properties such as HR, CO, BP and related vascular parameters, have been found to change during the lifetime of rats (Roberts & Goldberg, 1976). To serve as control, some male normotensive Wistar rats aged less than 5 months were used (Doggrell & Brown, 1998).

2.2. MATERIALS USED IN THE ANAESTHETIZED HYPERTENSIVE RAT MODEL

The equipments used includes a) a small animal operating table (BioScience, Cape Town, SA), b) syringe pump (Ascor AP22, CA, USA), c) oxygen mask; and d) the BP transducer, Power Lab 4/20T unit, Chart 5 for windows software, and BP amplifier were all obtained from AD instruments, Bella Vista, Australia. The drugs and chemicals used include a) angiotensin I (ang I), b) angiotensin II (ang II), c) captopril, d) losartan, e) phenylephrine, f) prazosin, g) dobutamine, h) propranolol, i) muscarine, and j) atropine. Except when stated otherwise, all drugs were sourced from Sigma-Aldrich, Steinheim, Germany. Other

drugs/chemicals used include i) sodium pentobarbitone (Kyron Laboratories, Johannesburg, SA), ii) heparin (Intramed, Porth Elizabeth, SA), iii) normal saline (NS) (Adcock Ingram, SA), iv) dimethylsulfoxide (DMSO) (Merck Chemicals, SA), v) oxygen (Afrox, SA) and vi) K-Y jelly (Johnson & Johnson, Midrand, SA).

The doses of the crude extract as well as that of the standard drugs used in this study were obtained using doses previously used in literature as a guide for the possible dose ranges that will be effective in the SHR. Dilutions below and above the doses used in literature were tested to obtain the lowest dose that produced an effect on BP and /or HR, as well as the maximum effect, which was not lethal in the SHR used in the study. The animals were divided into groups containing 8 rats each. The drugs and their respective doses infused were a) crude methanol leaf extract (MLE) of *Tulbaghia violacea* (5- 360 mg/kg), b) ang I (3.125 - 400 µg/kg, c) ang II) (3.125 - 400 µg/kg), d) captopril (0.03125 - 20 mg/kg), d) phenylephrine (0.01 - 5.12 mg/kg), e) dobutamine (0.01 - 0.32 mg/kg), f) propranolol (0.1 - 12.8 mg/kg), g) muscarine (0.16 -10 µg/kg), and h) atropine (0.02 - 20.48 mg/kg)

The doses of losartan (30 mg/kg) (Wong *et al.*, 1990; 1995; Choi *et al.*, 2009a; Susic *et al.*, 2009b), and prazosin (1 mg/kg) (Nagai *et al.*, 2003; Dabire, 2004; Antunes *et al.*, 2006; Wang *et al.*, 2006; Braga *et al.*, 2008; PintÉRovÁ *et al.*, 2009) were obtained from literature. These agents, along with captopril do not permit repetitive dosing in the same animal, i.e., the administration of higher or lower doses after the previous dose fails/failed to produce a dose-dependent response in the same animal. A large number of rats (approximately 50) would have been needed for each drug. This observation is supported by the previous work by Nagai *et al*. (2003).

2.3. EXPERIMENTAL PROTOCOL

To reveal the mechanisms through which *Tulbaghia violacea* produced its effect, the following protocols were used to co-infuse the standard drug with the crude MLE of *T. violacea*.

a) Group I: ang I (3.1 - 100 µg/kg) was co-infused with *T. violacea* (60 mg/kg) to find out if the MLE acts through an inhibitory action on the ACE. For each dose of ang I co-infused with the MLE; (i) a baseline effect of the compound was first determined and compared to the effect of the infusion of the DMSO + NS alone, (ii) ang I (3.1 - 100 µg/kg) was infused, (iii) after the BP and HR returned to baseline before (iv) ang I (3.1 - 100 µg/kg) was co-infused with *T. violacea* (60 mg/kg).

b) Group II: a dose-response experiment (DRE) of ang I (3.1 – 100.0 µg/kg) was repeated in 8 rats that were pre-treated with captopril (10 mg/kg) to ascertain that the exogenous ang I used in the study is converted to ang II, and this conversion can be blocked by a known antagonist of the ACE.

c) Group III: ang II (3.1 - 50 µg/kg) was co-administered with *T. violacea* (60 mg/kg) to investigate if the MLE act by blocking the ang II receptors. For each dose of ang II co-infused with the MLE; (i) a baseline effect of the compound was first determined and compared to the effect of the infusion of the DMSO + NS alone, (ii) ang II (3.1 - 50 µg/kg) was infused, (iii) after the BP and HR returned to baseline before (iv) ang II (3.1 - 50 µg/kg) was co-infused with *T. violacea* (60 mg/kg).

d) Group IV: a dose-response experiment (DRE) of ang II (3.1 – 50.0 µg/kg) was repeated in 8 rats that were pre-treated with losartan (30 mg/kg), to ascertain that the exogenous ang II used in the study acts through the ang II receptors, and its action can be blocked by a known blocker of the ang II receptors.

e) Group V: ang II (0.39 mg/kg/hr) was infused into 8 animals, followed by the infusion of *T. violacea* (60 mg/kg) SHR, after the BP and HR returned to the values observed prior to the infusion of the MLE, losartan (30 mg/kg) was thereafter, infused into the same animal. This protocol was carried out to compare the efficacy of the MLE of *T. violacea* with that of losartan

f) Group VI: phenylephrine (0.01 – 0.16 mg/kg) was co-administered with *T. violacea* (60 mg/kg) to investigate if the MLE acts via the α_1 receptors. For each dose of phenylephrine co-infused with the MLE; (i) a baseline effect of the compound was first determined and compared to the effect of the infusion of the DMSO + NS alone, (ii) phenylephrine (0.01 – 0.16 mg/kg) was then infused, (iii) after the BP and HR returned to baseline before (iv) phenylephrine (0.01 – 0.16 mg/kg) was co-infused with *T. violacea* (60 mg/kg).

g) Group VII: the protocol in (f) was repeated in some other rats that were pre-treated with prazosin (1 mg/kg) to further investigate a possible contribution of the α_1 receptors in the effect of the MLE.

h) Group VIII: dobutamine (0.2 – 10.0 µg/kg) was co-administered with *T. violacea* (60 mg/kg) to investigate if the MLE act through the β_1 receptors. For each dose of dobutamine co-infused with the MLE; (i) a baseline effect of the compound was first determined and compared to the effect of the infusion of the DMSO + NS alone, (ii) dobutamine (0.2 – 10.0 µg/kg) was infused, (iii) after the BP and HR returned to baseline before (iv) dobutamine (0.2 – 10.0 µg/kg) was co-infused with *T. violacea* (60 mg/kg).

i) Group IX: dobutamine (2.3 mg/kg/hr) was infused into 8 animals, followed by the infusion of *T. violacea* (60 mg/kg) SHR, after the BP and HR returned to the values observed prior to the infusion of the MLE, propranolol (1.6 mg/kg) was thereafter infused into the

same animal. This protocol was carried out to compare the efficacy of the MLE of *T. violacea* with that of propranolol

j) Group X: *T. violacea* (30, 60 and 120 mg/kg) was infused into 8 animals, that were pre-treated with atropine (5.12 mg/kg) to investigate if the MLE acts through the muscarinic receptors.

k) Group XI: This experiment was carried to compare the effect of muscarine with that of the MLE, in animals pre-treated with atropine. 8 animals were pre-treated with atropine (5.12 mg/kg), and then *T. violacea* (60 mg/kg) was infused into the animals. Muscarine (2.5 µg/kg) was then infused into the same animal, after the BP and HR returned to the level prior to the infusion of the MLE. The drugs were infused 20 minutes after atropine infusion (Thán *et al.*, 2000; Dimo *et al.*, 2003; Dabire, 2004; Adami *et al.*, 2006; Ajay *et al.*, 2007; D. Holopherne *et al.*, 2008; Khwanchuea *et al.*, 2008; Lessa *et al.*, 2008; Rattmann *et al.*, 2008; De Menezes *et al.*, 2010).

The effects of the MLE and the standard drugs on SBP, DBP, MAP and HR were evaluated.

2.4. ARTERIAL BLOOD GAS ANALYSIS

Arterial blood samples were collected from the arterial cannula at the beginning as well as at the end of some experiments, to ascertain that the animals received enough oxygen throughout the period of the experiment. The samples were collected into EDTA tubes, and kept on ice, before being sent to PathCare Laboratory (PathCare Park, Neels Bothma Street, N1 City, Good Wood, Cape Town, SA) for analysis.

3. THE EFFECT OF CHRONIC (21 DAYS) ADMINISTRATION OF *T. VIOLACEA* (60 MG/KG), CAPTOPRIL (10 MG/KG) OR THE VEHICLE ON BODY WEIGHT, BLOOD PRESSURE, HEART RATE AND PLASMA ALDOSTERONE LEVELS

Animals used in this protocol were put through 14 days of acclimatization to get them used to being in the restrainer while the BP and HR recordings are being obtained. The body weight, BP and HR of the rats used were measured on the first day of study, before rats were divided into three groups of eight animals each. The intraperitoneal injection given to the animals during the next 21 days were *T. violacea* (60 mg/kg), captopril (10 mg/kg) or 0.2 mls of the vehicle (DMSO + NS). The BP and HR of the animals were measured using the non-invasive tail cuff method during the intervention period; while the invasive measurement was used at the end of the study period. Blood was collected from the femoral artery of the SHR after the BP and HR values had stabilized. The blood was stored in ethylenediaminetetraacetic acid (EDTA) tubes and rapidly spun at 10 000 rpm, in a centrifuge to separate the plasma from the blood cells. The plasma obtained was then stored in another set of EDTA tubes and placed in a – 40 °C freezer. Plasma collected in EDTA bottles give 15% higher yields compared to that kept in heparinized tubes (SIEMENS MEDICAL SOLUTIONS DIAGNOSTICS; Prisant *et al.*, 2003). The plasma samples were sent to the Veterinary Hormone Laboratory, Faculty of Veterinary Science, Onderstepoort, University of Pretoria, for analysis of plasma aldosterone levels.

4. STATISTICAL ANALYSIS

The means ± standard errors of the means of the actual values for SBP, DBP, MAP and HR from the experiments are reported. The difference between the mean of the BP and/or HR obtained at baseline, or with the vehicle and the maximum response obtained with the infusion of *T. violacea* or the standard drug was calculated. The mean change, the mean of the individual percentage changes and the percentage difference (in mean) observed with each intervention was calculated and statistically analyzed using the Student's t test for significant difference ($p < 0.05$). The Microsoft Excel software was used for statistical analysis, and the Graph pad prism 5 software was used to illustrate the results as graphs.

5. ETHICAL CONSIDERATIONS

The animals were allowed free access to food and water before the commencement of the experiments. The methodology and ethics adhered to in this study were approved by the Ethics Committee of the University of the Western Cape, and the registration number obtained was 09/7/35.

CHAPTER FOUR

RESULTS

The presented results include the effect of time (minutes) on the stability of the blood pressure (BP), heart rate (HR) and blood constituents during the continuous infusion of a vehicle (normal saline, NS) in the SHR. Also presented in this chapter, are the results obtained from the dose-response experiments (DRE) of the methanol leaf extract (MLE) of *T. violacea* (alone) and the standard drugs (alone). The results of the effect of the standard drugs co-administered with *T. violacea* on BP and/or HR in the SHR are also presented here.

1. BASELINE BLOOD PRESSURE AND HEART RATE IN THE SPONTANEOUSLY HYPERTENSIVE RATS

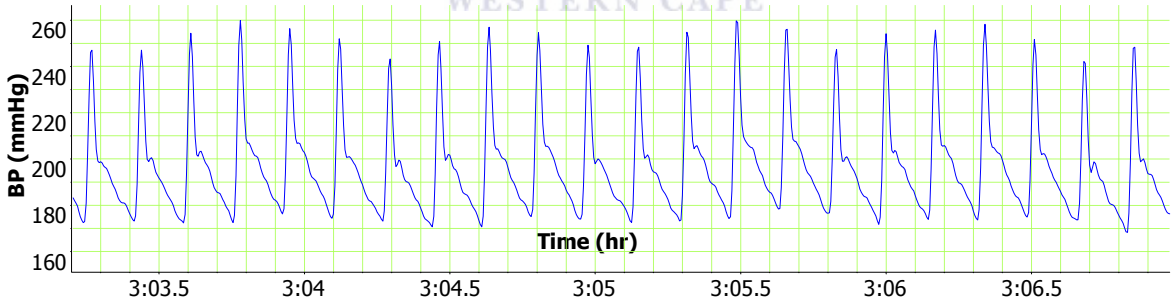

Figure 4. 1: Recording of the systolic BP (SBP) and diastolic BP (DBP) in a male SHR. Chart scaling 1:1.

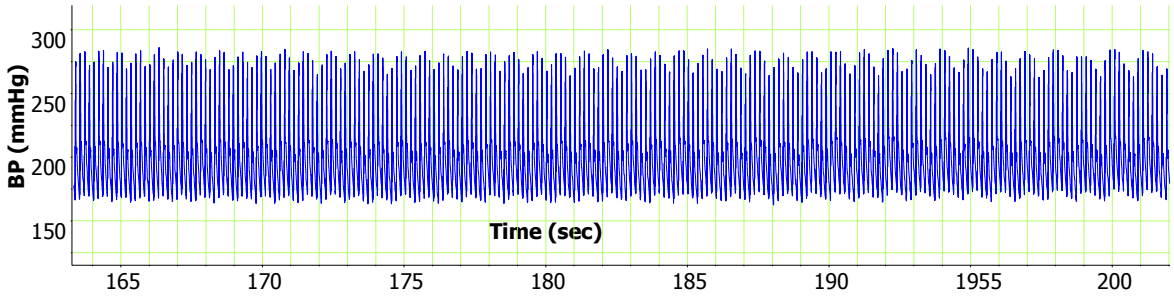

Figure 4. 2: Recording of the BP of the same animal above. Chart scaling 10:1.

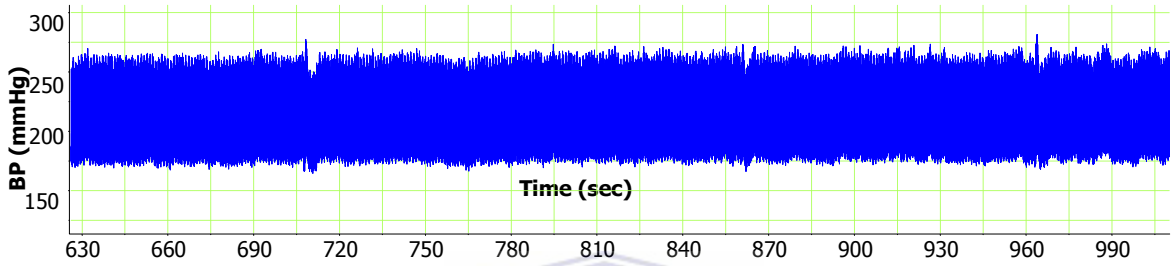

Figure 4. 3: Recording of the BP of the same animal above. Chart scaling 100:1.

This protocol was carried out to ascertain that the SHRs used in this study were indeed hypertensive in the absence of any (external) intervention. Figure 4.1 above shows a typical BP recording on the Chart 5 graph screen obtained from one of the animals used in the present study. It can be deduced that the anaesthetized SHR used in the above recording was hypertensive with an approximate baseline SBP of 260 mmHg and a DBP of 170 mmHg. Each 0.01 value seen on the chart at the scaling of 1:1 represents a second. Therefore, by counting the number of BP 'spikes' in each of such boxes and multiplying this by 60, the approximate value of the HR of the animal above can be obtained. Using this equation, the HR of the rat above is 360 beats per minute (bpm) which is within the normal range for a SHR (figure 4.1). However, it should be noted that the values recorded in this study were those obtained by clicking on the 'Data Pad' icon at the top of the Chart 5 screen which

should be more accurate and eliminate the possibility of human error or/ and bias. It is advantageous to compress the recording chart, as this permits sampling of a larger number of heart beats (spikes) and thus, a more accurate mean value of BP and HR can be obtained. It also makes it easier to appreciate the effect of agents that have appreciable effects on BP (figures 4.2 and 4.3).

2. THE STABILITY OF BLOOD PRESSURE AND HEART RATE

This experiment was performed to ascertain that the BP and HR of the rats used in this study were steady. The vehicle (drops of DMSO mixed with NS) was infused at a rate of 0.3 ml/hr into anaesthetized healthy male SHRs. The ratio of DMSO to NS used herein, and subsequently used as vehicle in experiments requiring the dissolution of a drug in DMSO in this study was 3:100. Care was taken to use the least amount of DMSO needed to make a solution. After 180 minutes, the maximum duration of the *in-vivo* experiments, it was observed that the SBP, DBP, MAP and HR did not significantly deviate from the baseline values at time zero (0); of 197.3 ± 1.5 mmHg, 137.3 ± 1.3 mmHg, 157.3 ± 1.6 mmHg and 382.4 ± 14.3 bpm respectively; and the vehicle did not have any significant effect on either the BP or HR (table 4.1).

Table 4. 1: The effect of a mixture of dimethyl sulfoxide and normal saline (0.3 ml/hr) on BP and HR in SHR. n=8

TIME	SBP	DBP	MAP	HR
minutes	(mmHg) ± SEM			(bpm) ± SEM
0	197.3 ± 1.5	137.3 ± 1.3	157.3 ± 1.6	382.4 ± 14.3
20	195.0 ± 5.0	133.0 ± 4.4	153.7 ± 4.5	382.4 ± 8.1
40	188.0 ± 1.0	126.0 ± 1.0	146.7 ± 0.6	385.1 ± 6.4
60	195.3 ± 0.3	132.0 ± 3.0	153.1 ± 2.1	382.7 ± 5.4
80	199.0 ± 7.4	134.3 ± 5.8	155.9 ± 6.3	385.7 ± 7.8
100	198.3 ± 7.3	137.0 ± 7.0	157.4 ± 7.0	378.4 ± 5.7
120	199.3 ± 8.1	132.7 ± 8.7	154.9 ± 8.5	379.7 ± 4.1
140	187.3 ± 1.8	127.3 ± 4.3	147.3 ± 2.8	386.7 ± 8.9
160	194.3 ± 0.7	125.7 ± 5.6	148.6 ± 3.7	387.3 ± 6.1
180	191.0 ± 3.2	132.0 ± 2.1	151.7 ± 1.9	384.0 ± 9.3

3. EFFECT OF *T. VIOLACEA* ON BLOOD PRESSURE AND HEART RATE IN THE SPONTANEOUSLY HYPERTENSIVE RATS

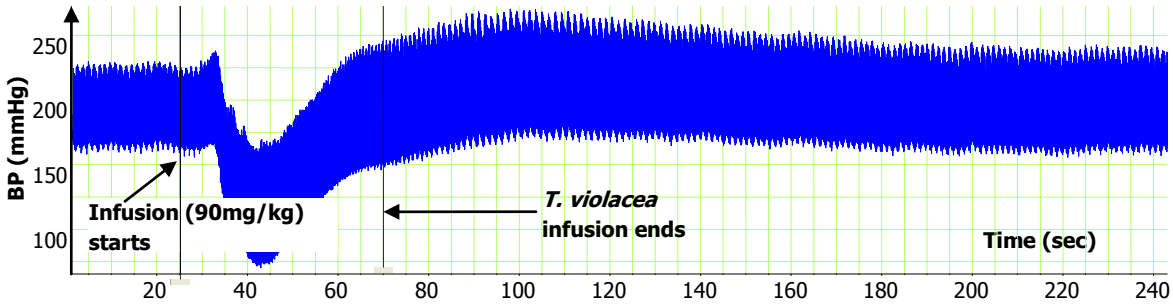

Figure 4. 4: Effect of *T. violacea* (90 mg/kg) on BP. Chart scaling 100:1.

This experiment was performed to investigate if *T. violacea* had any effect on the BP and/or HR in the SHR. The dose at which 80% of the maximum effect on BP, of the MLE was achieved was also sought. The doses of the crude MLE of *T. violacea* used were 5 mg, 10 mg, 20 mg, 30 mg, 60 mg, 90 mg, 120 mg and 150 mg/kg. In a dose dependent fashion, *T. violacea* reduced (a) the SBP from 212.8 ± 6.4 mmHg at baseline, to 209.2 ± 6.4 mmHg (maximum effect of the lowest dose), and to 128.7 ± 8.3 mmHg (maximum effect of the highest dose) (figure 4.5 a; table 4.2); (b) the DBP from 156.1 ± 5.0 mmHg at baseline, to 152.0 ± 5.0 mmHg (maximum effect of the lowest dose), and to 75.3 ± 5.9 mmHg (maximum effect of the highest dose) (figure 4.5 b; table 4.2); (c) the MAP from 175.1 ± 5.3 mmHg at baseline, to 171.0 ± 5.3 mmHg (maximum effect of the lowest dose), and to 93.3 ± 6.2 mmHg (maximum effect of the highest dose) (figure 4.5 c; table 4.2); (d) the HR from 398.8 ± 17.4 bpm at baseline, to 393.5 ± 17.0 bpm (maximum effect of the lowest dose), and to 316.8 ± 18.4 bpm (maximum effect of the highest dose) (figure 4.5 d; table 4.2). These results equate to increasing statistically significant ($p < 0.05$) reductions at doses above i) 20 mg/kg (for the SBP), ii) 10 mg/kg (for the DBP), iii) 10 mg/kg (for the MAP) and iv) 60 mg/kg for the HR (figure 4.5; table 4.2). The absolute reductions in the BP and HR values were similar at all doses except at the doses of 30 mg/kg and 60 mg/kg, where the reduction in HR recorded was significantly ($p < 0.05$) less than those of the other parameters (SBP, DBP and MAP) (figure 4.5; table 4.2). Doses of *T. violacea* above 150 mg/kg produced further reductions in BP and HR parameters, but were lethal in some animals, and were consequently not included.

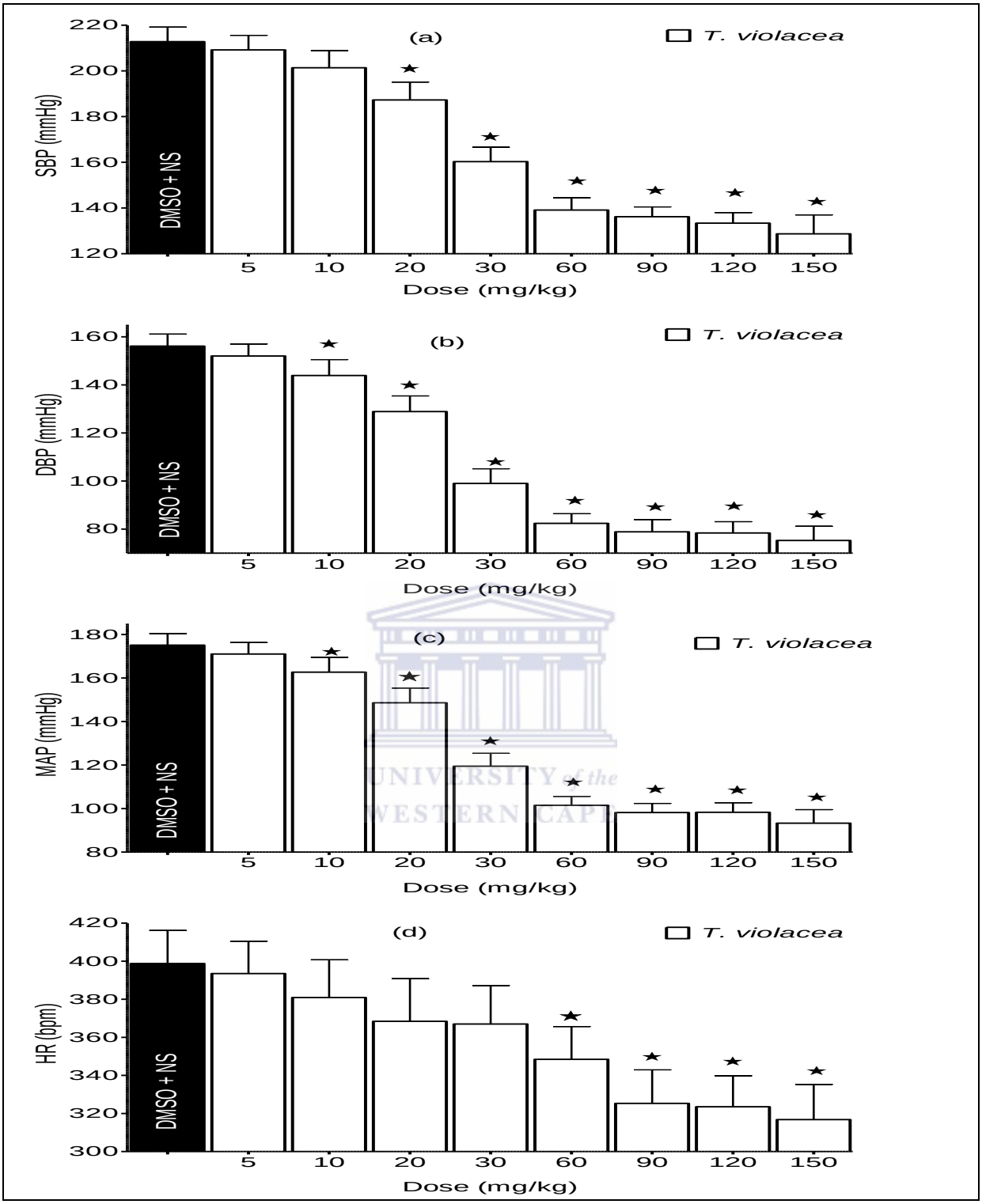

Figure 4. 5: Effect of *T. violacea* (5 - 150 mg/kg) on SBP (a), DBP (b), MAP (c), and HR (d). Values are presented as mean ± SEM. * indicates statistical significance.

Table 4. 2: Effect of *T. violacea* on BP and HR.

DOSE (mg/Kg)	MEAN SBP (mmHg) ± SEM	MEAN DBP (mmHg) ± SEM	MEAN MAP (mmHg) ± SEM	MEAN HR (bpm) ± SEM
DMSO	212.8 ± 6.4	156.1 ± 5.0	175.1 ± 5.3	398.8 ± 17.4
5.0	209.2 ± 6.4	152.0 ± 5.0	171.0 ± 5.3	393.5 ± 17.0
10.0	201.4 ± 7.5	143.9 ± 6.6 *	162.7 ± 6.8 *	381.0 ± 19.8
20.0	187.3 ± 7.8 *	128.9 ± 6.5 *	148.6 ± 6.8 *	368.5 ± 22.4
30.0	160.3 ± 6.3 *	99.0 ± 6.1 *	119.5 ± 5.9 *	367.0 ± 20.2
60.0	139.1 ± 5.4 *	82.4 ± 4.0 *	101.5 ± 4.1 *	348.5 ± 17.2 *
90.0	136.2 ± 4.3 *	78.9 ± 5.1 *	98.2 ± 4.2 *	325.3 ± 17.6 *
120.0	133.4 ± 4.6 *	78.4 ± 4.7 *	98.3 ± 4.4 *	323.5 ± 16.3 *
150.0	128.7 ± 8.3 *	75.3 ± 5.9 *	93.3 ± 6.2 *	316.8 ± 18.4 *

n=8. * indicates statistical significance.

4. DOES *T. VIOLACEA* ACT BY INHIBITING THE ANGIOTENSIN I CONVERTING ENZYME?

4.1. Angiotensin I dose response curve

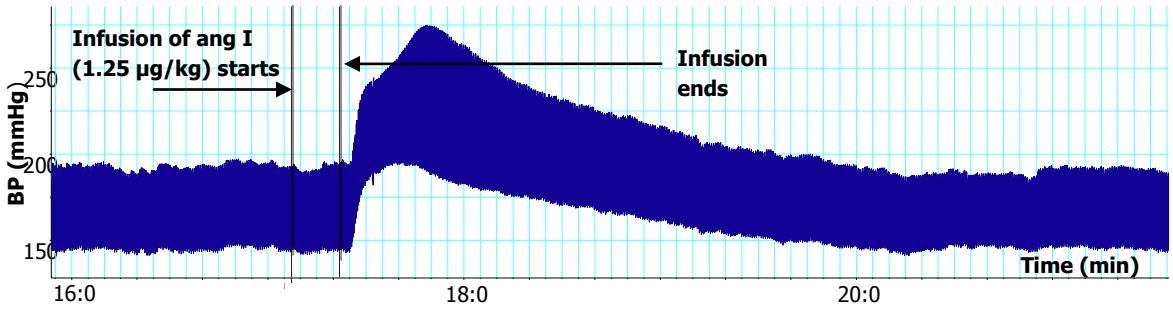

Figure 4. 6: Effect of ang I (12.5 µg/kg) on BP. Chart scaling 100:1.

This experiment was performed to obtain a dose response curve (DRC) for ang I, which will be used in the ensuing experiment, whose aim was to investigate whether *T. violacea* decreases the BP by inhibiting the angiotenisn I converting enzyme (ACE). In a dose dependent fashion ang I (3.1 - 100.0 µg/kg) increased; (a) significantly ($p < 0.05$) the SBP from 181.6 ± 7.2 mmHg at baseline, to 195.9 ± 5.8 mmHg, i.e., by 9.5 ± 2.0% (maximum effect of the lowest dose), and to 294.9 ± 9.3 mmHg, i.e., by 58.7 ± 3.3% (maximum effect of the highest dose); (b) the DBP from 138.6 ± 6.9 mmHg at baseline, to 148.9 ± 5.6 mmHg, i.e., by 11.9 ± 2.5% (maximum effect of the lowest dose), and to 187.8 ± 7.5 mHg, i.e., by 41.4 ± 2.6% (maximum effect of the highest dose); and (c) the MAP from 152.9 ± 6.6 mmHg at baseline, to 164.6 ± 6.3 mmHg, i.e., by 11.0 ± 2.3% (maximum effect of the lowest dose), and to 223.5 ± 7.3 mmHg, i.e., by 48.2 ± 2.4% (maximum effect of the highest dose). The increases in DBP and MAP were only significant ($p < 0.05$) at all doses above the least dose (3.1 µg/kg) given (figure 4. 7 a). The infusion of ang I doses above 100.0 µg/kg did not lead to any further increase in BP, prior to the lethal dose (s) being achieved. The effect of ang I (3.1 – 100.0 µg/kg) on HR was not dose-dependent, as both increases as well decreases were observed. Significant ($p < 0.05$) changes in the HR were only observed at the doses of 25.0 µg/kg and 50.0 µg/kg at which the HR was increased by 5.7 ± 1.3% (from 387.3 ± 4.5 bpm to 404.6 ± 6.3 bpm) and 4.5 ± 1.3% (from 387.3 ± 4.5 bpm to 400.6 ± 7.5 bpm) respectively (figure 4. 7 b).

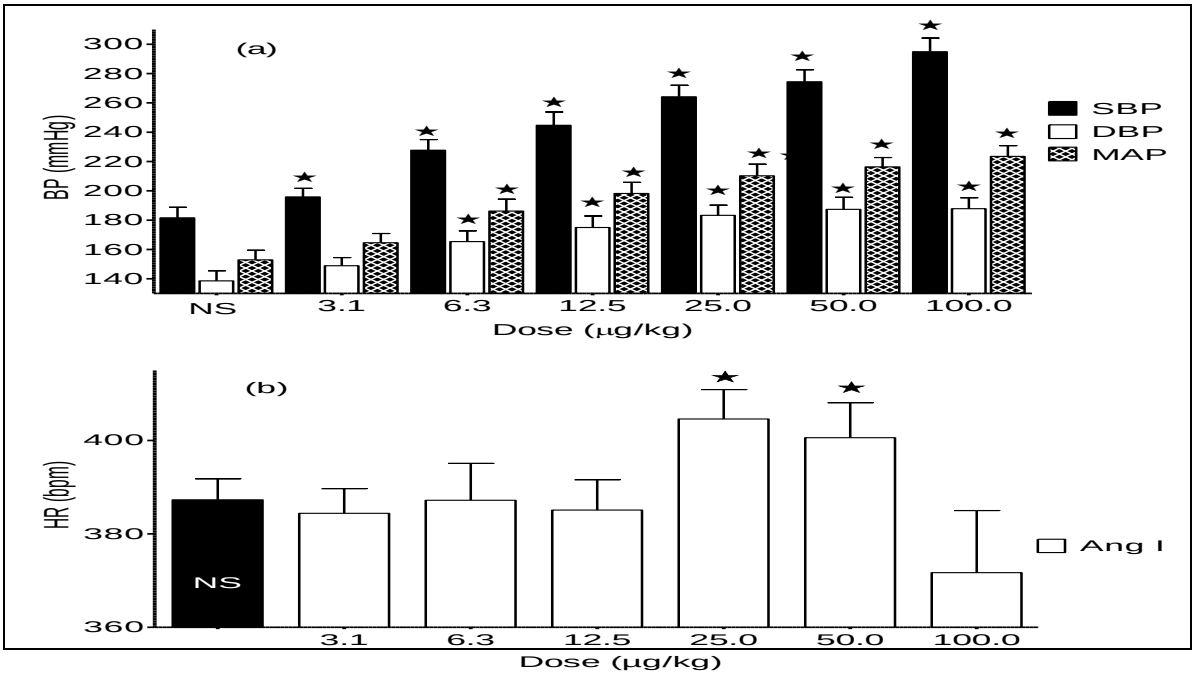

Figure 4. 7: Effect of angiotensin I (3.1 – 100.0 µg/kg) on BP (a) and HR (b). Values are presented as mean ± SEM. * indicates statistical significance.

4.1.2. Effect of angiotensin I co-infused with T. violacea on the BP and HR

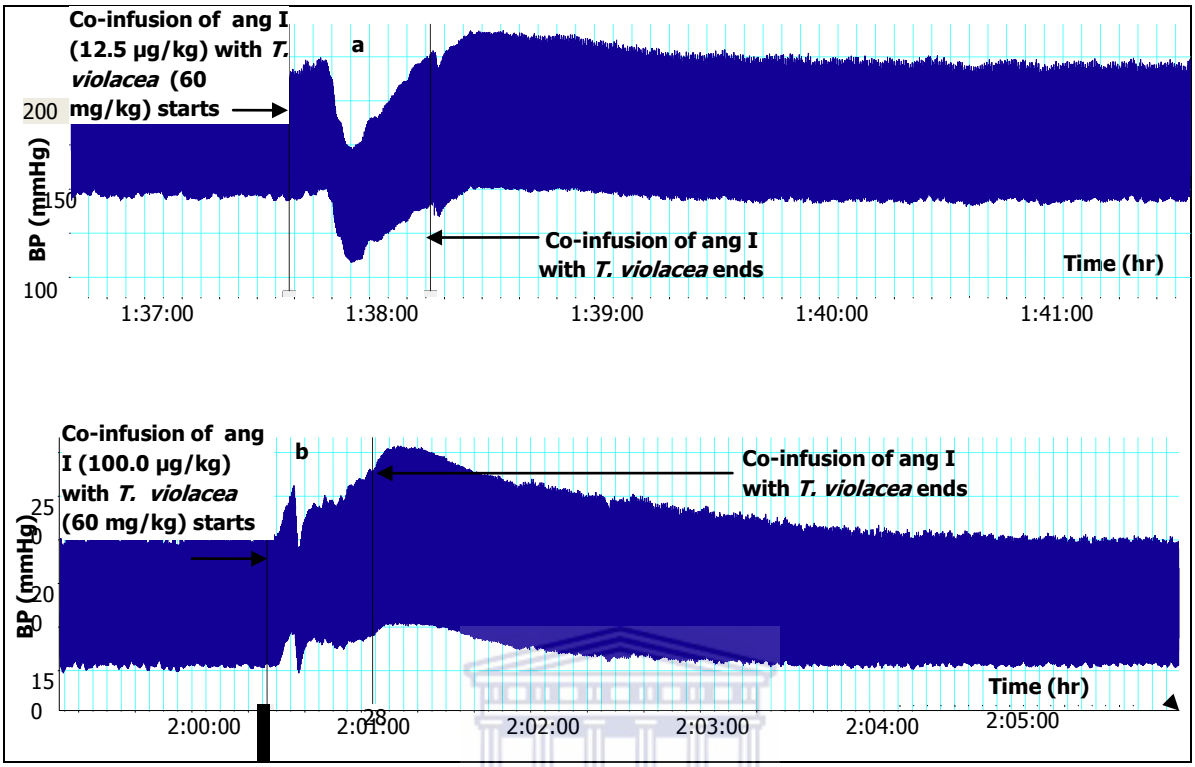

Figure 4. 8: Effects of ang I (a = 12.5 µg/kg, or b = 100.0 µg/kg); co-infused with *T. violacea* (60 mg/kg) on BP. Chart scaling 100:1.

This protocol was carried out to investigate if *T. violacea* acts by inhibiting the ACE in the SHR. Relative to the respective values at baseline, *T. violacea* (60 mg/kg) infused alone, significantly ($p < 0.05$) reduced the SBP (from 189.5 ± 5.7 mmHg to 112.0 ± 5.9 mmHg) (figure 4.9 a), DBP (from 144.6 ± 6.1 mmHg to 66.3 ± 6.4 mmHg) (figure 4.9 b), MAP (from 159.5 ± 6.1 mmHg to 81.5 ± 5.9 mmHg) (figure 4. 9 c) and HR (from 365.5 ± 5.7 bpm to 320.4 ± 10.5 bpm) (figure 4.9 d).

The infusion of ang I (3.1 – 100.0 µg/kg) as well as the co-infusion of ang I (3.1 – 100.0 µg/kg) with *T. violacea* (60 mg/kg) significantly ($p < 0.05$) increased the mean of the maximum SBP, DBP and MAP values attained when compared to the respective values at baseline (figure 4.9). The only exception was at the lowest dose (3.1µg/kg) of ang I, a dose at which the infusion of ang I alone did not produce a significant increase in SBP (figure 4.9 a). The infusion of ang I (3.1 – 100.0 µg/kg) alone had variable effect on the HR values when compared to the values at baseline; with (a) similar HR values observed at the doses of 3.1 µg/kg, 6.3 µg/kg and 12.5 µg/kg; (b) significant ($p < 0.05$) increases in HR values observed at the doses of 25 µg/kg and 50 µg/kg; and (c) a significant ($p < 0.05$) decrease in the HR observed at 100 µg/kg. Meanwhile, the co-infusion of ang I (3.1 – 100.0 µg/kg) with *T. violacea* (60 mg/kg) did not produce any significant change in HR, except at the dose of 6.3 µg/kg, at which there was a significant ($p < 0.05$) fall in HR (figure 4.9 c).

Consequently the co-infusion of ang I (6.3 – 100.0 µg/kg) with *T. violacea* (60 mg/kg) produced reductions in the final value of SBP, DBP, and MAP obtained when compared to the values obtained with the corresponding dose of the ang I (6.3 – 100.0 µg/kg) infused alone (figures 4.8 and 4.9). The reductions in (a) the SBP were significant ($p < 0.05$) at the ang I doses of 6.3 µg/kg, 12.5 µg/kg, 25.0 µg/kg and 50.0 µg/kg, and equated to 5.0% (from 236.7 ± 6.1 mmHg to 225.0 ± 5.3 mmHg), 7.6% (from 251.1 ± 5.6 mmHg to 232.0 ± 7.0 mmHg), 7.1% (from 270.3 ± 6.3 mmHg to 251.0 ± 8.9 mmHg) and 6.3% (from 280.8 ± 7.4 mmHg to 263.2 ± 6.5 mmHg) reductions (figure 4.9 a) respectively; (b) the DBP were only significant ($p < 0.05$) at the ang I doses of 6.3 µg/kg (from 179.9 ± 8.3 mmHg to 168.5 ± 5.1 mmHg; i.e., a 6.3% reduction) and 12.5 µg/kg (189.2 ± 6.4 mmHg to 173.7 ± 7.0 mmHg; i.e., a 8.2% reduction) (figure 4.9 b); (c) the MAP were significant ($p < 0.05$) at the ang I doses of

12.5 µg/kg (from 209.8 ± 7.2 mmHg to 192.8 ± 5.6 mmHg; i.e., a 8.1% reduction) and 25.0 µg/kg (from 222.8 ± 6.0 mmHg to 208.7 ± 7.3 mmHg; i.e., a 6.3% reduction) (figure 4.9 c).

The infusion of ang I alone, as well as the co-infusion of ang I (3.1 – 100.0 µg/kg) with *T. violacea* (60 mg/kg) produced variable effects. Therefore, the co-infusion of ang I with *T. violacea* (60 mg/kg) led to variable changes in the mean HR values obtained when compared to the infusion of ang I alone; with (a) similar HR observed at the doses of 3.1 µg/kg, 12.5 µg/kg and 50.0 µg/kg, (b) significant ($p < 0.05$) reductions HR values obtained at the doses of 6.3 µg/kg and 25.0 µg/kg, and (c) a significant ($p < 0.05$) increase in HR observed at the dose of 100 µg/kg (figure 4.9 d).

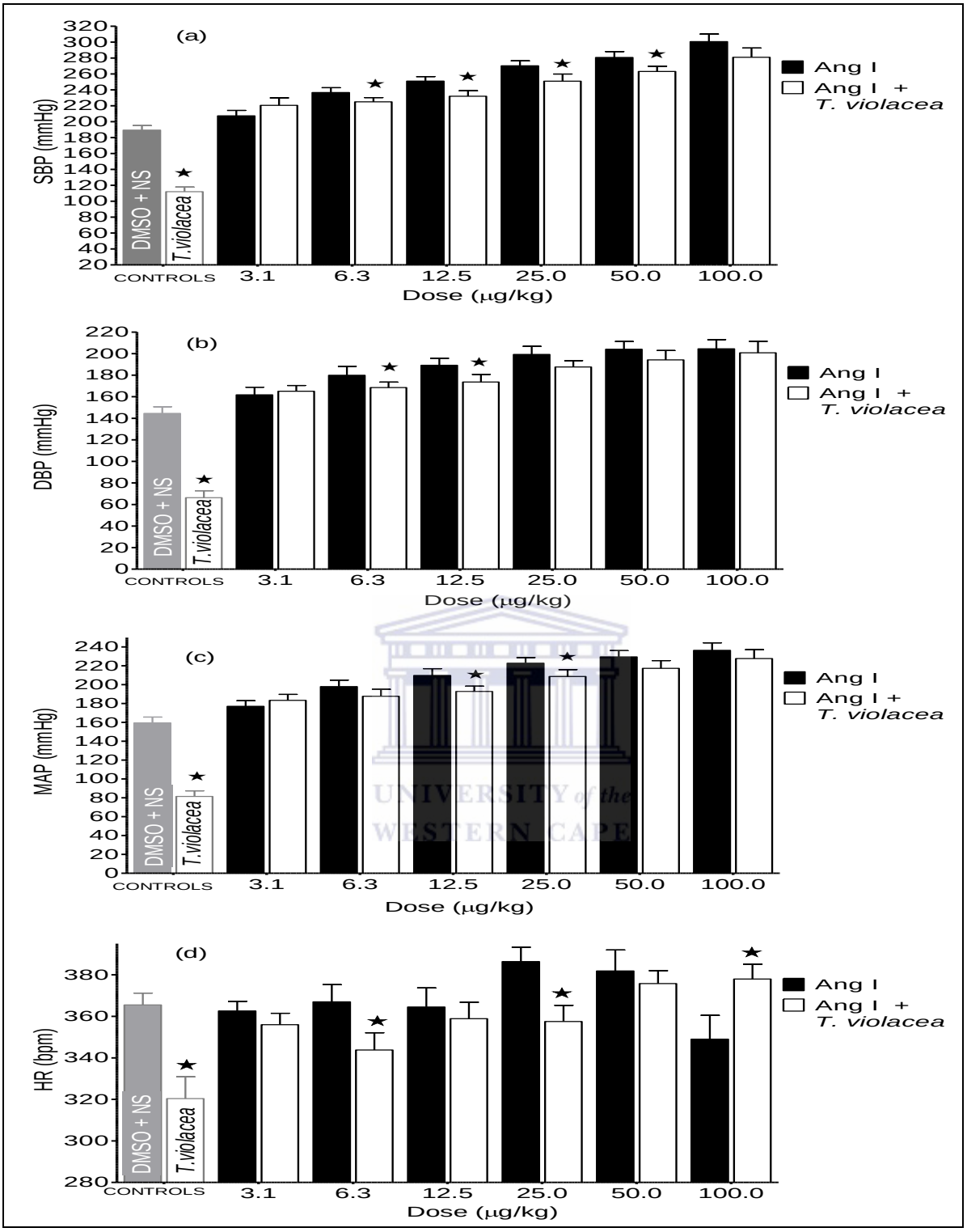

Figure 4. 9: Effect of ang I (3.1 - 100 µg/kg) co-infused with *T. violacea* (60 mg/kg) on the SBP (a), DBP (b), MAP (c), and HR (d). Values are presented as mean ± SEM. * indicates statistical significance.

4.1.3. Captopril dose response curve

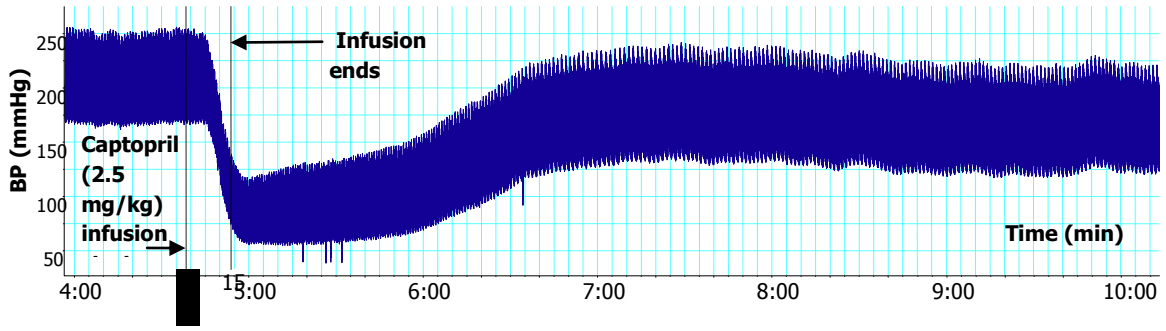

Figure 4. 10: Effect of captopril (2.5 mg/kg) on BP. Chart scaling 100:1.

Captopril is a ACE inhibitor (Smith & Vane, 2003), and was used here to act as a positive control for the possible effect of *T. violacea* on the ACE. The DRE of captopril was carried out to obtain the dose at which 80% of the maximum effect of captopril occurs. In this study, captopril did not produce any effect on the BP or HR at doses lower than those displayed here, when compared to baseline values. All doses (1.3 – 20 mg/kg) presented here, produced significant ($p < 0.05$) reductions in the BP. A dose dependent reduction in BP was only observed between the doses of 1.3 mg/kg to 2.5 mg/kg. Captopril reduced (a) the SBP from 191.2 ± 4.0 mmHg at baseline, to 167.0 ± 9.4 mmHg, i.e., by 12.6 ± 2.4% (maximum effect of the lowest dose), and to 101.8 ± 14.1 mmHg, i.e., by 46.7 ± 6.1% reduction (maximum effect of the highest dose); (b) the DBP from 141.9 ± 3.7 mmHg at baseline, to 110.0 ± 10.0 mmHg, i.e., by 22.5 ± 4.3% (maximum effect of the lowest dose), and to 53.6 ± 8.9 mmHg, i.e., by 62.2 ± 5.3% (maximum effect of the highest dose); and (c) the MAP from 1158.4 ± 3.7 mmHg at baseline, to 129.3 ± 9.7 mmHg, i.e., by 18.4 ± 3.5% (maximum effect of the lowest dose), and to 69.4 ± 10.7 mmHg, i.e., by 56.2 ± 5.7% (maximum effect of the highest dose). Further increases in the dose of captopril did not produce any further reduction in BP, before the lethal dose was reached, which was at doses above 20 mg/kg (figure 4.11 a).

Captopril only produced a significant ($p < 0.05$) change in the HR value when compared to the value at baseline, at the dose of 2.5 mg/kg; which was a 6.1 ± 2.4% reduction (from 372.2 ± 7.4 bpm to 349.6 ± 10.8 bpm) (figure 4.11 b).

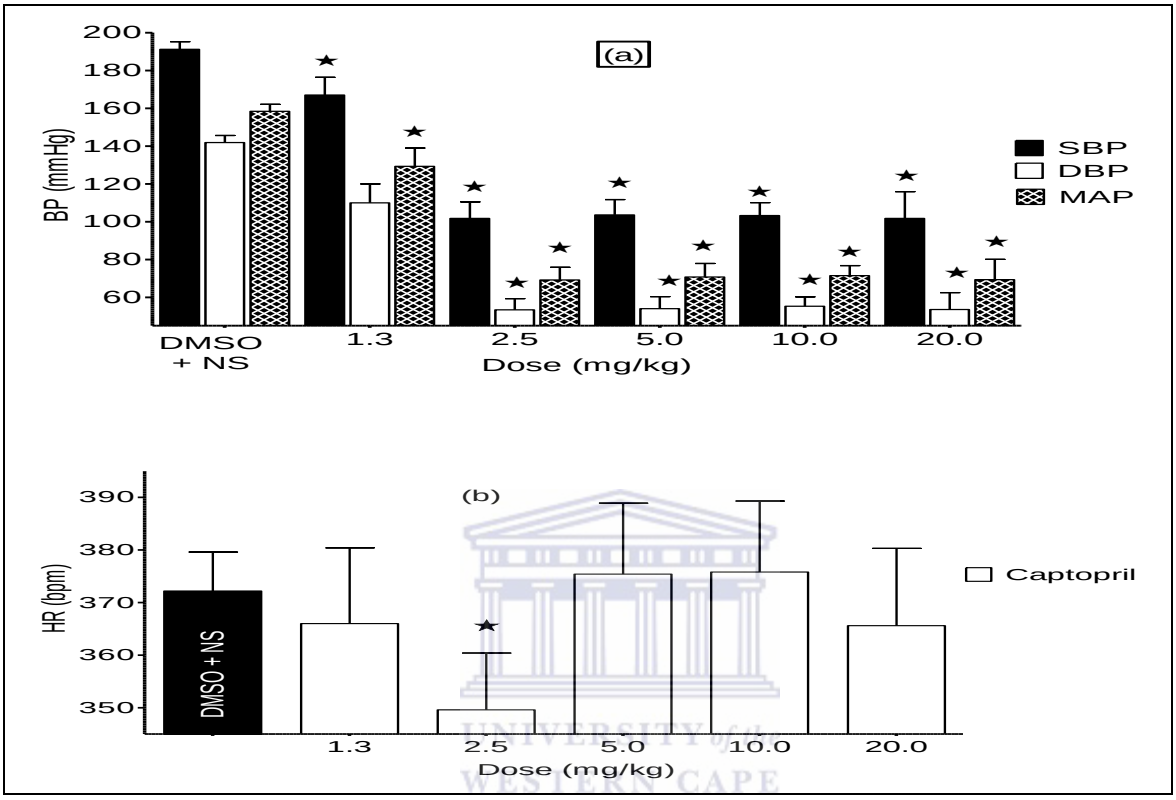

Figure 4. 11: Effect of captopril (1.3 – 20 mg/kg) on BP (a) and HR (b). Values are presented as mean ± SEM. * indicates statistical significance.

4.1.4. Angiotensin I dose response curve in rats pre-treated with captopril

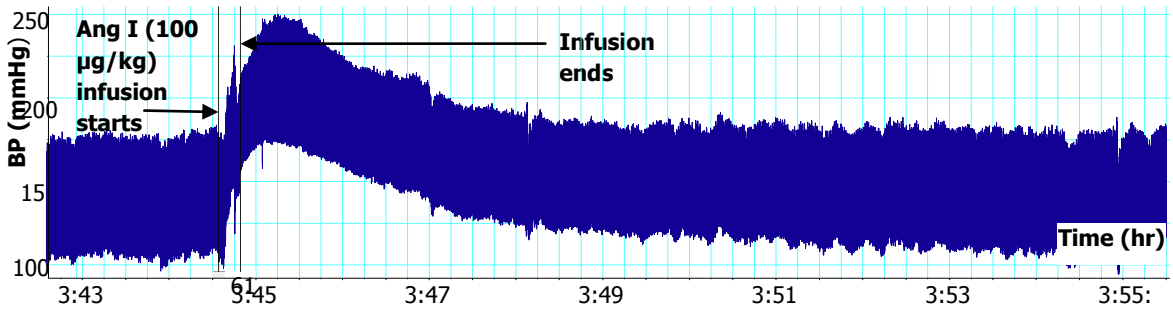

Figure 4. 12: Effect of angiotensin I (100.0 µg/kg) on BP after the pre-treatment with captopril (10.0 mg/kg). Chart scaling 100:1

Compared to the hypertensive effect of ang I observed in the absence of captopril (10 mg/kg) (figures 4.6 and 4.7); the hypertensive effect observed with ang I (figures 4.12 and 4.13) after the pre-treatment of animals with captopril were significantly ($p < 0.05$) attenuated; and the increases in BP were only significant at doses above 3.1 µg/kg. In a dose dependent fashion, ang I (3.1 – 100.0 µg/kg) increased (a) the SBP from 151.8 ± 5.2 mmHg observed with the infusion of the vehicle, to 157.3 ± 7.5 mmHg, i.e., by 3.6 ± 1.1% (maximum effect of the lowest dose), and to 223.1 ± 5.5 mmHg, i.e., by 47.0 ± 4.2% (maximum effect of the highest dose); (b) the DBP from 94.4 ± 4.6 mmHg observed with the infusion of the vehicle, to 102.0 ± 6.2 mmHg, i.e., by 8.1 ± 2.0% (maximum effect of the lowest dose), and to 155.8 ± 5.5 mmHg, i.e., by 65.0 ± 6.5% (maximum effect of the highest dose); (c) the MAP from 113.5 ± 4.6 mmHg observed with the infusion of the vehicle, to 120.3 ± 6.5 mmHg, i.e., by 6.0 ± 1.6% (maximum effect of the lowest dose), and to 178.1 ± 5.2 mmHg, i.e., by 57.0 ± 5.2% (maximum effect of the highest dose). After pre-treatment with captopril, dose-dependent increases in BP, as well as dose-dependent reductions in HR were observed. The reduction in the HR were from 363.2 ± 8.0 bpm observed with the infusion of the vehicle, to

358.9 ± 9.9 bpm, i.e., by 1.2 ± 0.4% (maximum effect of the lowest dose), and to 334.7 ± 8.3 bpm, i.e., by 7.8 ± 1.3% (maximum effect of the dose prior to the highest dose) (figure 4.13 b).

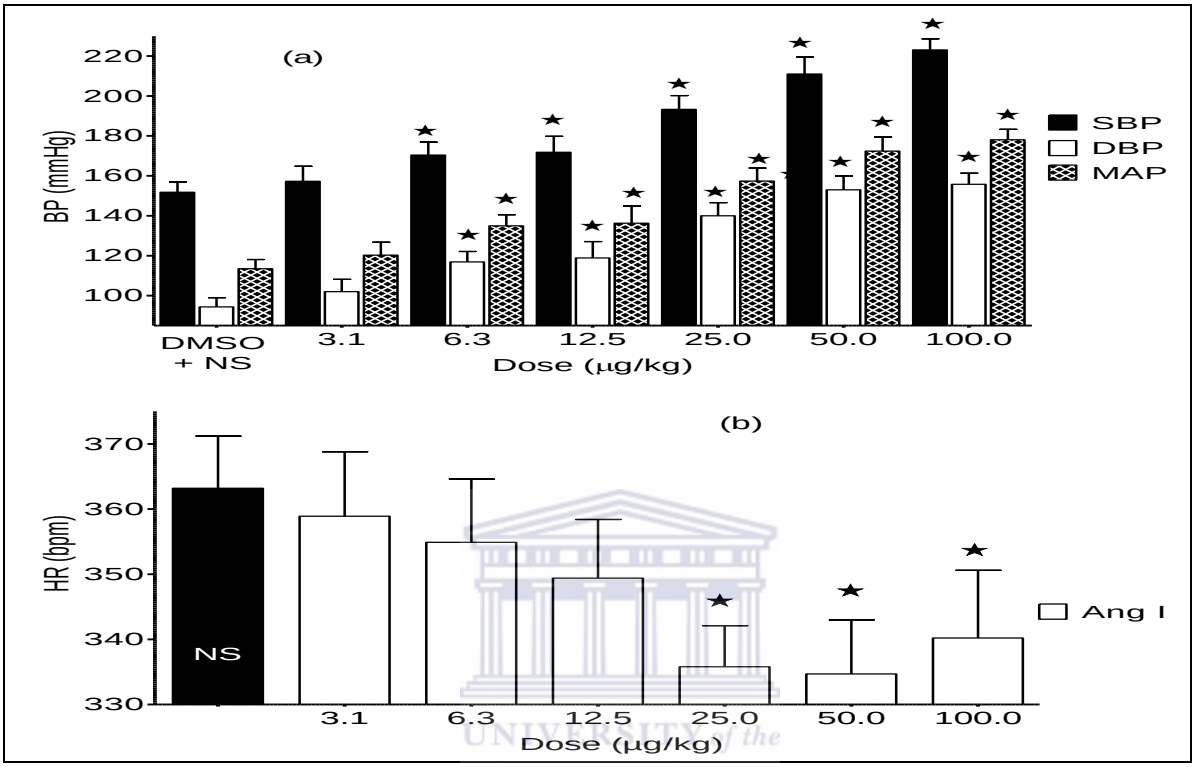

Figure 4. 13: Effect of ang I (3.1 – 100.0 µg/kg) on BP (a) and HR (b) after pre-treatment with captopril. Values are presented as mean ± SEM. * indicates statistical significance.

5. DOES *T. VIOLACEA* ACT BY BLOCKING THE ANGIOTENSIN II RECEPTORS?

5.1 Angiotensin II dose response curve

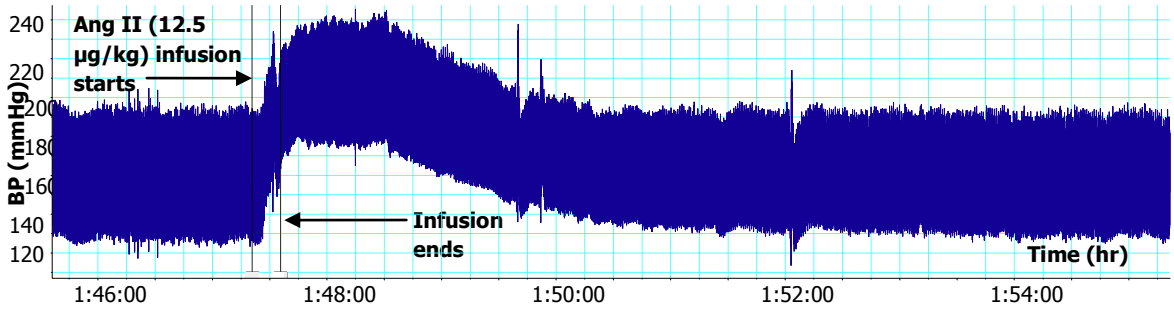

Figure 4. 14: Effect of ang II (12.5 µg/kg) on BP. Chart scaling 100:1

This experiment was performed to obtain DRC for ang II, which will be used in the experiment to investigate if the MLE reduces BP by blocking the ang II receptors *in-vivo*. Angiotensin II (ang II) (3.1 – 50.0 µg/kg) produced significant ($p < 0.05$) dose-dependent increases in BP (figure 4.15 a), which were not associated with any significant change in HR at all doses used (figure 4.15 b). Angiotensin II increased (a) the SBP from 182.6 ± 6.2 mmHg observed at baseline, to 205.4 ± 6.5 mmHg, i.e., by 12.5 ± 6.4% (maximum effect of the lowest dose), and to 296.0 ± 12.3 mmHg, i.e., by 62.1 ± 7.7% (maximum effect of the highest dose); (b) the DBP from 143.9 ± 5.7 mmHg observed at baseline, to 167.8 ± 3.9 mmHg, i.e., by 16.6 ± 5.3% (maximum effect of the lowest dose), and to 231.0 ± 8.5 mmHg, i.e., by 60.5 ± 7.9% (maximum effect of the highest dose); and (c) the MAP from 156.8 ± 5.1 mmHg observed at baseline, to 180.3 ± 7.3 mmHg, i.e., by 15.0 ± 6.7% (maximum effect of the lowest dose), and to 252.6 ± 9.7 mmHg, i.e., by 61.1 ± 9.2% (maximum effect of the highest dose) (figure 4.15 a). No further increases in BP were observed with the infusion of ang II doses above 50.0 µg/kg, prior to the lethal dose being achieved.

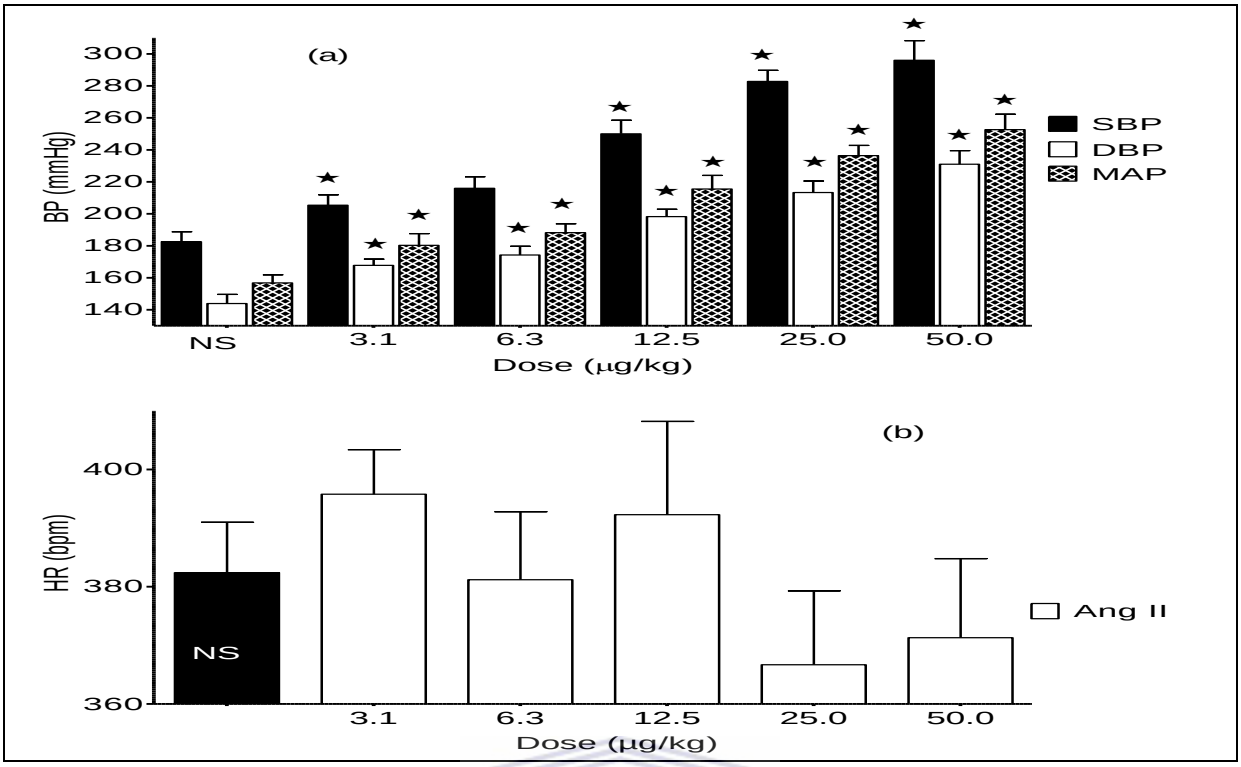

Figure 4. 15: Dose-response graph of the effect of angiotensin II (3.1 – 50.0 µg/kg) on BP (a) and HR (b). Values are presented as mean ± SEM. * indicates statistical significance.

5.2. Effect of angiotensin II co-infused with T. violacea on the BP and HR

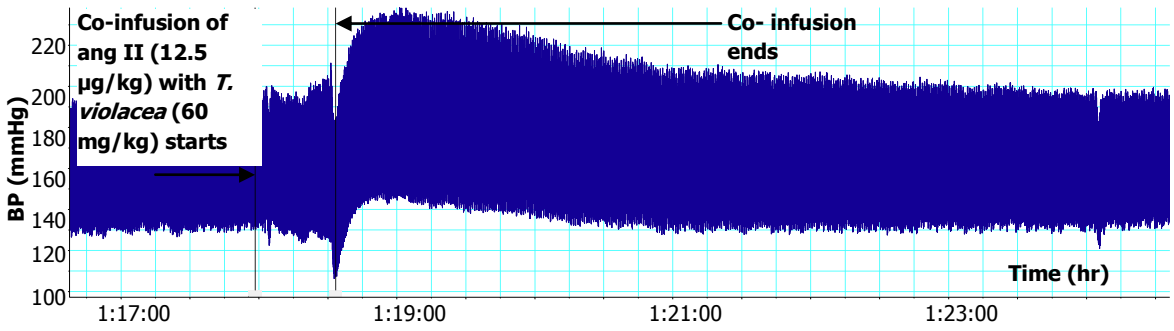

Figure 4. 16: Effect of ang II (12.5 µg/kg) co-infused with *T. violacea* (60 mg/kg) on BP in a SHR. Chart scaling 100:1

This experiment was performed to investigate whether *T. violacea* mediates its anti-hypertensive effect by blocking the ang II receptors. *T. violacea* (60 mg/kg) infused alone significantly ($p < 0.05$) reduced the SBP (from 175.2 ± 5.8 mmHg to 96.2 ± 9.1 mmHg), DBP (from 132.4 ± 4.5 mmHg to 51.7 ± 7.4 mmHg), MAP (from 146.7 ± 7.9 mmHg to 66.5 ± 7.8 mmHg) and HR (from 374.2 ± 11.6 bpm to 318.7 ± 13.7 bpm) from the values obtained with the infusion of the vehicle alone (figure 4.17).

Generally, the co-infusion of ang II with *T. violacea* (60 mg/kg) did not produce any significant change in the final BP and HR values observed when compared to the values obtained with the infusion of ang II alone (figure 4.17). However, significant ($p < 0.05$) (a) increases in the SBP were observed at the doses of 3.1 µg/kg (from 194.8 ± 7.4 mmHg to 214.4 ± 8.1 mmHg; i.e., a 10.1% increase) and 6.3 µg/kg (from 209.2 ± 10.5 mmHg to 234.1 ± 6.0 mmHg; i.e., a 11.9% increase), (b) decreases in the DBP were seen at the doses of 25.0 µg/kg (from 194.3 ± 6.3 mmHg to 171.8 ± 7.6 mmHg, i.e., a 11.6% reduction) and 50.0 µg/kg (from 207.2 ± 16.6 mmHg to 177.6 ± 9.0 mmHg, i.e., a 14.3% reduction), (c) decrease was observed in the MAP at the dose of 25.0 µg/kg (from 219.4 ± 7.9 mmHg to 200.7 ± 7.0 mmHg; i.e., a 8.5% reduction) and (d) increases in the HR at the doses of 25.0 µg/kg (from 341.4 ± 7.0 bpm to 403.4 ± 15.5 bpm; i.e., a 18.2% increase) and 50.0 µg/kg (from 356.7 ± 17.4 bpm to 395.0 ± 17.6 bpm; i.e., a 10.7% increase).

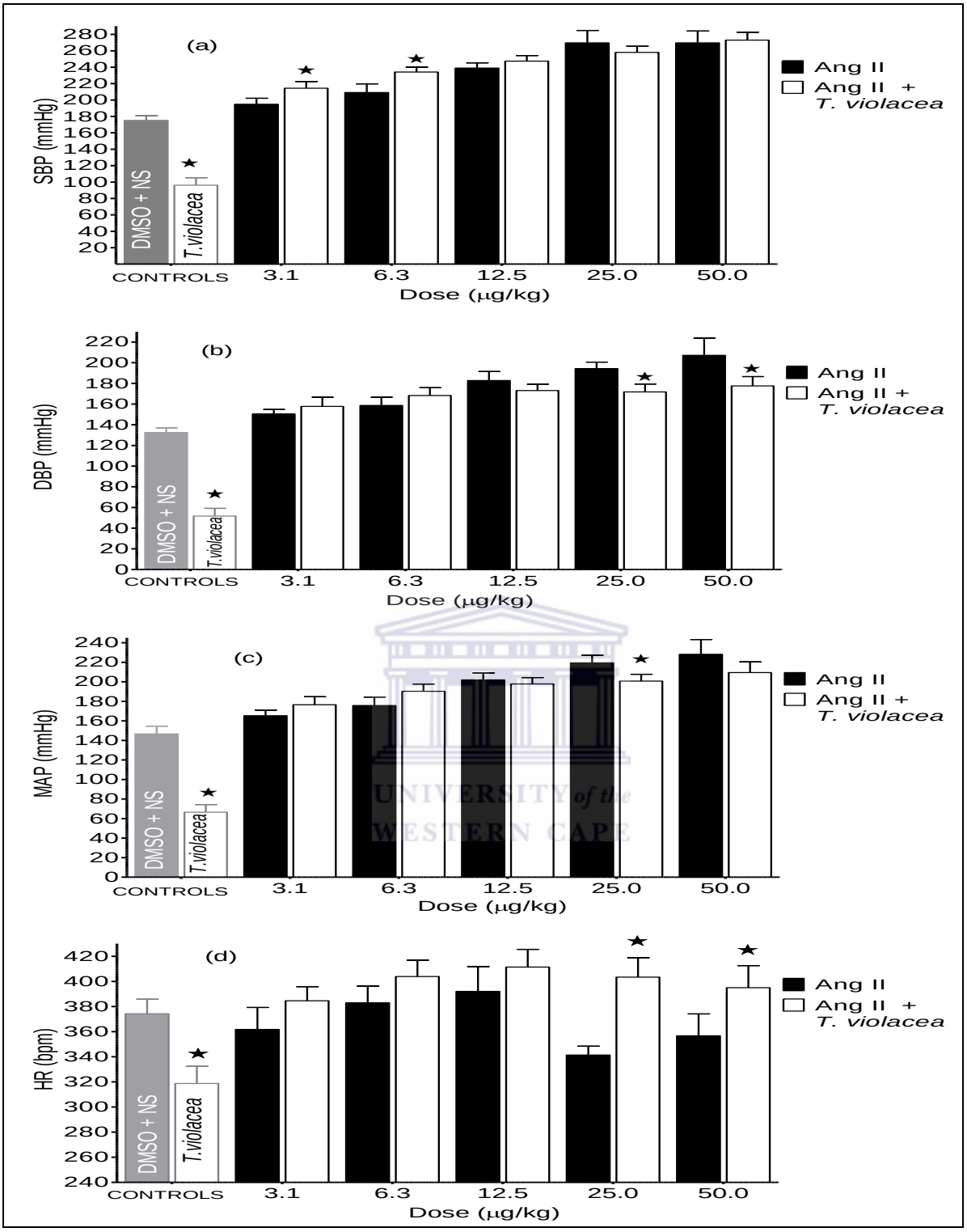

Figure 4. 17: Effect of ang II (3.1 - 50 µg/kg) co-administered with *T. violacea* (60 mg/kg) on the SBP (a), DBP (b), MAP (c), and HR (d). Values are presented as mean ± SEM. * indicates statistical significance.

5.3. Effect of losartan (30 mg/kg) on BP and HR

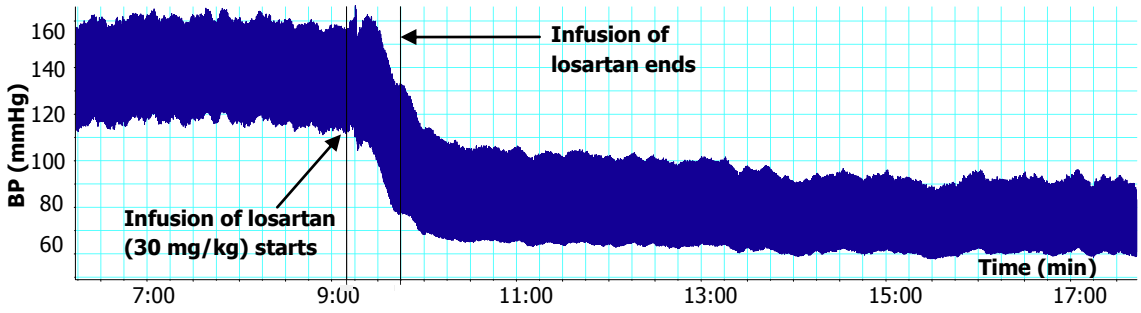

Figure 4. 18: The effect of losartan (30 mg/kg) on BP in a SHR. Chart scaling 100:1

Losartan (30 mg/kg) produced significant ($p < 0.05$) and sustained decreases in the SBP (from 186.8 ± 5.5 mmHg to 128.0 ± 5.6 mmHg; i.e., a 31.5 ± 4.2% reduction), DBP (from 136.5 ± 8.4 mmHg to 78.8 ± 7.6 mmHg; i.e., a 42.6 ± 5.5% reduction) and MAP (from 153.3 ± 7.1 mmHg to 95.2 ± 6.0 mmHg; i.e., a 38.0 ± 4.3% reduction); which were associated with little or no effect on HR, when compared with their respective baseline values (figure 4.19).

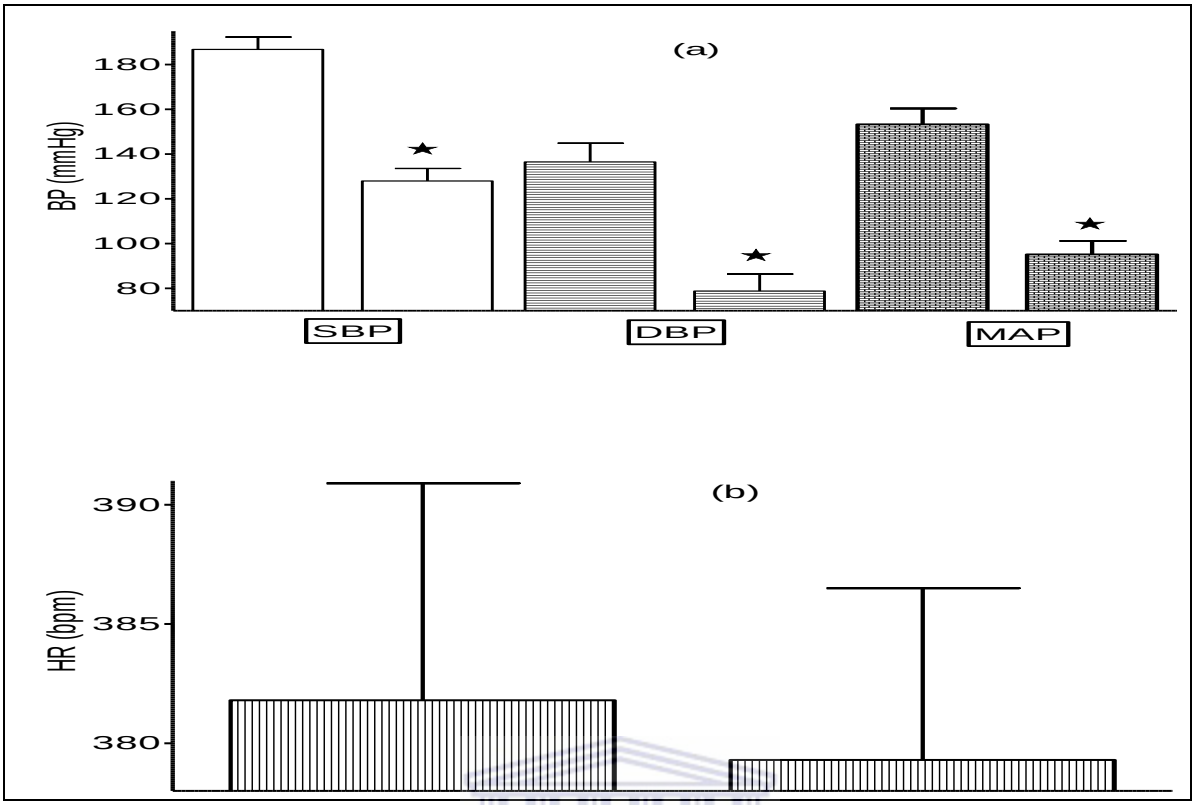

Figure 4. 19: Effect of losartan (30 mg/kg) on BP (a) and HR (b) in the SHR. Values are presented as mean ± SEM. * indicates statistical significance.

5.4. Angiotensin II dose response curve in SHR pre-treated with losartan (30 mg/kg)

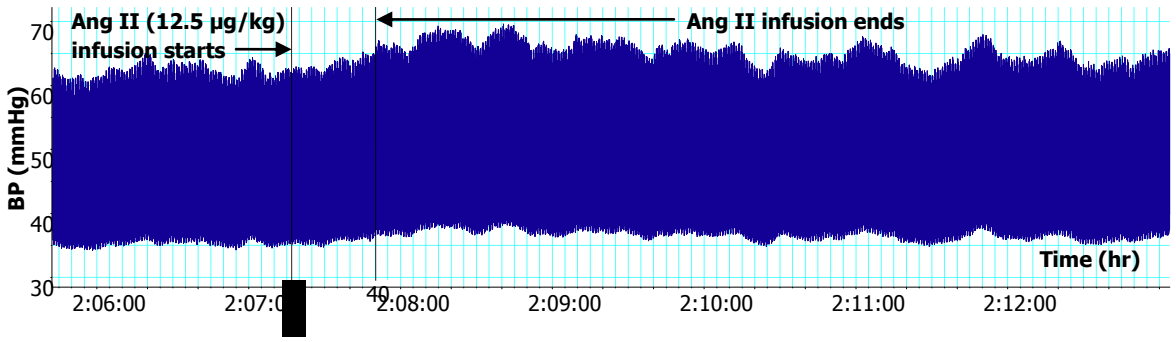

Figure 4. 20: Effect of angiotensin II (12.5 µg/kg) on BP after the pre-treatment of a SHR with losartan (30 mg/kg) Chart scaling 100:1

Losartan (30 mg/kg) was used as positive control to investigate if T. violacea acts via the ang II receptors in reducing BP. After pre-treatment of animals with losartan, the BP responses to the infusion of increasing doses of ang II (3.1 – 50.0 µg/kg) were significantly ($p < 0.05$) attenuated (figures 4.20 and 4.21 a) when compared to the values previously obtained in the absence of losartan (figures 4.14 and 4.15 a). These increases were only significantly ($p < 0.05$) higher than those observed with the infusion of the vehicle (alone) at the doses of 25 µg/kg and 50 µg/kg at which (a) the SBP was increased by 15.6 ± 3.5% (from 144.3 ± 5.1 mmHg to 166.7 ± 4.9 mmHg) and 24.7 ± 3.7% (from 144.3 ± 5.1 mmHg to 179.9 ± 9.5 mmHg) respectively; (b) the DBP was increased by 20.9 ± 6.6% (from 98.5 ± 4.7 mmHg to 119.0 ± 4.6 mmHg) and 30.4 ± 7.3% (from 98.5 ± 4.7 mmHg to 128.4 ± 10.9 mmHg) respectively; and (c) the MAP was increased by 18.8 ± 5.3% (from 113.6 ± 4.8 mmHg to 135.0 ± 4.5 mmHg) and 27.7 ± 5.8% (from 113.6 ± 4.8 mmHg to 145.1 ± 10.3 mmHg) respectively (figure 4.21 a). The effect of ang II on HR was not significant at any of the doses administered in the presence of losartan (figure 4.21 b), a finding that is similar to that previously observed in the absence of losartan (figure 4.15 b).

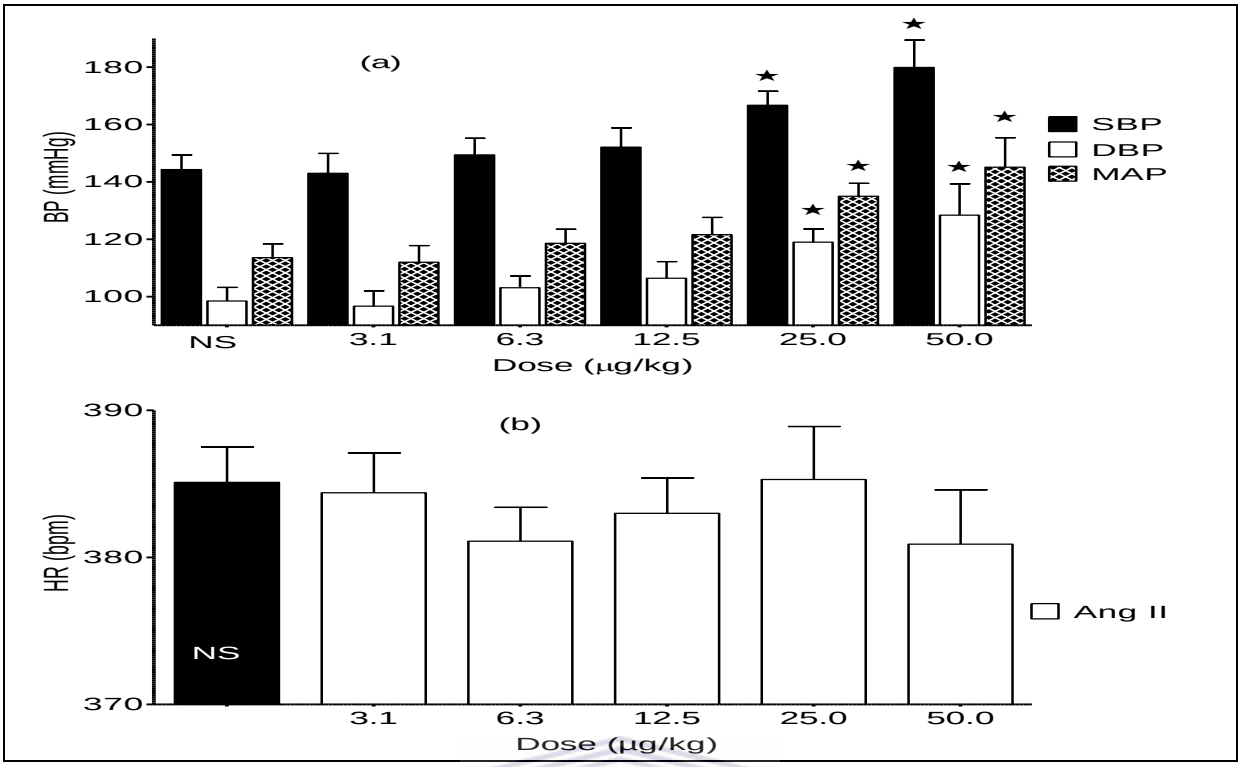

Figure 4. 21: Effect of ang II (3.1 – 50.0 µg/kg) on BP (a) and HR (b), after pre-treatment with losartan (30 mg/kg). Values are presented as mean ± SEM. * indicates statistical significance.

5.5. Experiment comparing the effect of losartan (30 mg/kg) with that of T. violacea (60 mg/kg) during continuous infusion of ang II (0.39 mg/kg/hr) in the SHR

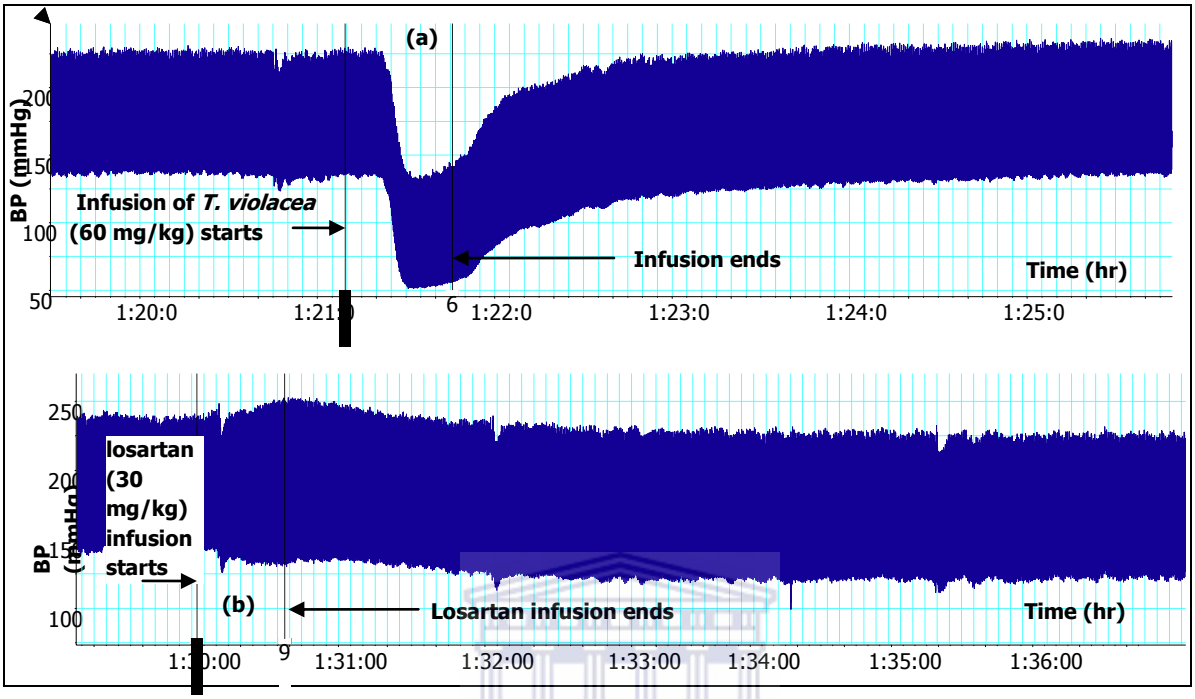

Figure 4. 22: Effect of *T. violacea* (60 mg/kg) (a) and losartan (30 mg/kg) (b) on BP during continuous infusion of ang II (0.39 mg/kg/hr) in a SHR. Chart scaling 100:1

The continuous infusion of ang II (0.39 mg/kg/hr) in the SHR significantly ($p < 0.05$) increased the SBP in the animals by about 30 mmHg. The initial baseline readings are not shown here. Effort was made to ensure that the new 'baseline' was relatively steady for proper comparison of the effect of the standard drug (losartan) with that of the test drug (*T. violacea*). During continuous infusion of ang II, *T. violacea* (60 mg/kg) infusion significantly ($p < 0.05$) reduced the SBP by 32.5 ± 2.1% (from 211.4 ± 3.6 mmHg to 142.6 ± 5.1 mmHg), the DBP by 47.2 ± 5.8% (from 160.6 ± 3.2 mmHg to 85.4 ± 10.5 mmHg), the MAP by 41.2 ± 4.2% (from 177.9 ± 2.7 mmHg to 104.9 ± 8.6 mmHg) and the HR by 18.0 ± 2.0% (from 380.8 ± 6.3 bpm to 312.2 ± 9.9 bpm) in the SHR. In a similar manner, losartan infusion

significantly ($p < 0.05$) reduced the SBP by 23.2 ± 2.4% (from 213.4 ± 3.4 mmHg to 164.0 ± 7.0 mmHg), the DBP by 32.1 ± 6.1% (from 156.6 ± 4.2 mmHg to 106.4 ± 9.7 mmHg), the MAP by 41.2 ± 4.2% (from 175.1 ± 3.8 mmHg to 125.3 ± 8.5 mmHg) and the HR by 4.2 ± 1.4% (from 383.0 ± 4.2 bpm to 367.0 ± 6.3 bpm. The reductions in SBP, MAP and HR were more significant ($p < 0.05$) when *T. violacea* was infused compared to when losartan was infused. However, the hypotensive effect of losartan lasted very much longer than that of *T. violacea* (which was momentary) (figures 4.22 and 4.23).

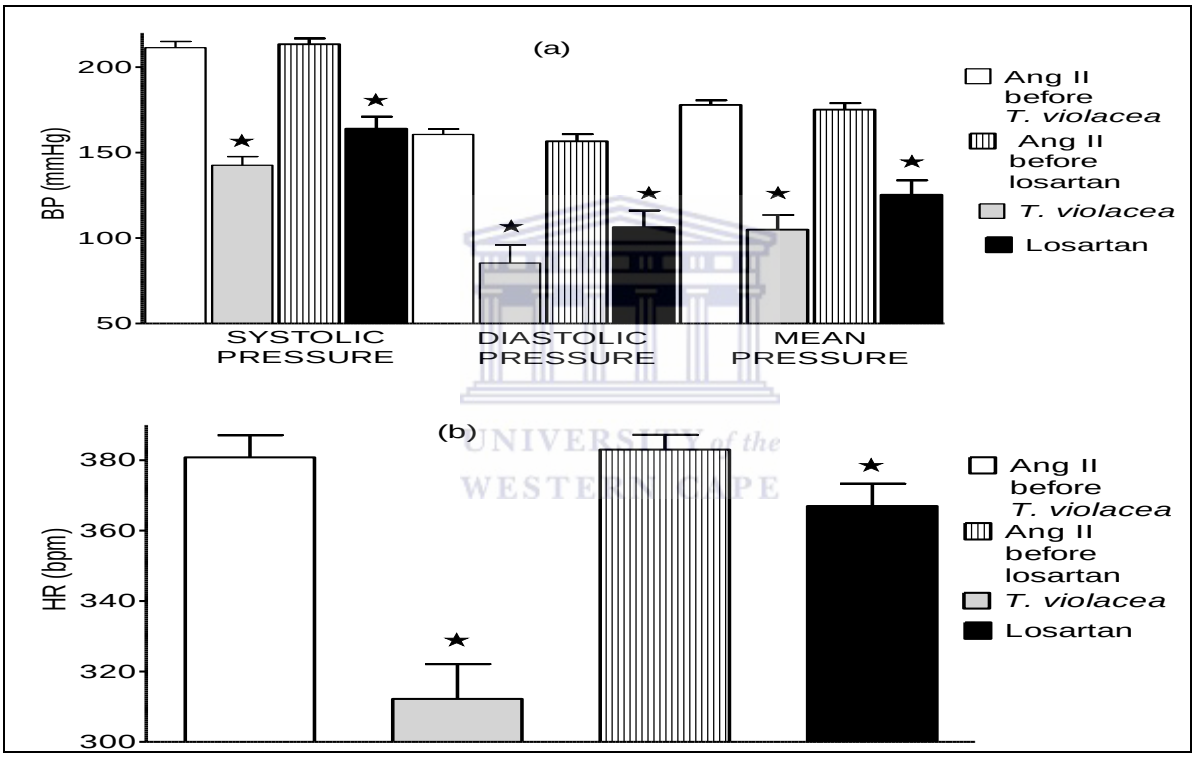

Figure 4. 23: Effects on BP (a) and HR (b) of losartan (30 mg/kg) or *T. violacea* (60 mg/kg) during continuous infusion of ang II (0.39 mg/kg/hr) in the SHR. Values are presented as mean ± SEM. * indicates statistical significance

6. DOES *T. VIOLACEA* ACT BY BLOCKING THE ALPHA I ADRENOCEPTORS?

6.1. Phenylephrine dose response curve

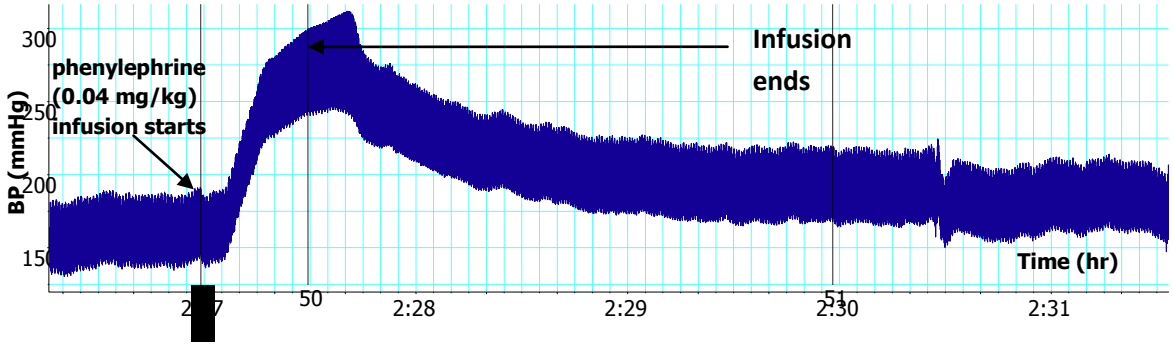

Figure 4. 24: Effect of phenylephrine (0.04 mg/kg) on BP in a SHR. Chart scaling 100:1

This experiment was performed to obtain a phenylephrine dose response curve. Phenylephrine, an alpha – 1 (α_1) receptor agonist (0.01 – 0.16 mg/kg) produced significant ($p < 0.05$) dose-dependent increases in BP, which were associated with dose-dependent reductions in HR at all doses when compared to the values at baseline (figure 4.25). Phenylephrine increased (a) the SBP from 202.3 ± 4.5 mmHg at baseline, to 226.2 ± 4.8 mmHg, i.e., by 11.8 ± 2.1% (maximum effect of the lowest dose), and to 345.9 ± 8.1 mmHg, i.e., by 71.0 ± 4.1% (maximum effect of the highest dose); (b) the DBP from 158.7 ± 3.7 mmHg at baseline, to 184.3 ± 4.1 mmHg, i.e., by 16.1 ± 3.5% (maximum effect of the lowest dose) and to 240.9 ± 6.6 mmHg, i.e., by 51.8 ± 7.3% (maximum effect of the highest dose); and (c) the MAP from 173.2 ± 2.6 mmHg at baseline, to 198.2 ± 5.3 mmHg, i.e., by 14.4 ± 2.6% (maximum effect of the lowest dose), and to 275.9 ± 7.1 mmHg, i.e., by 59.3 ± 5.2% (maximum effect of the highest dose (4.25 a). The associated reductions in HR were from 403.6 ± 9.0 bpm at baseline, to 401.6 ± 6.7 bpm, i.e., by 0.5 ± 1.5% (maximum effect of the

lowest dose), and to 350.7 1 ± 2.9 bpm, i.e., by 13.1 ± 0.6% (maximum effect of the highest dose), and were only significant ($p < 0.05$) at the three highest doses given (figure 4.25 b).

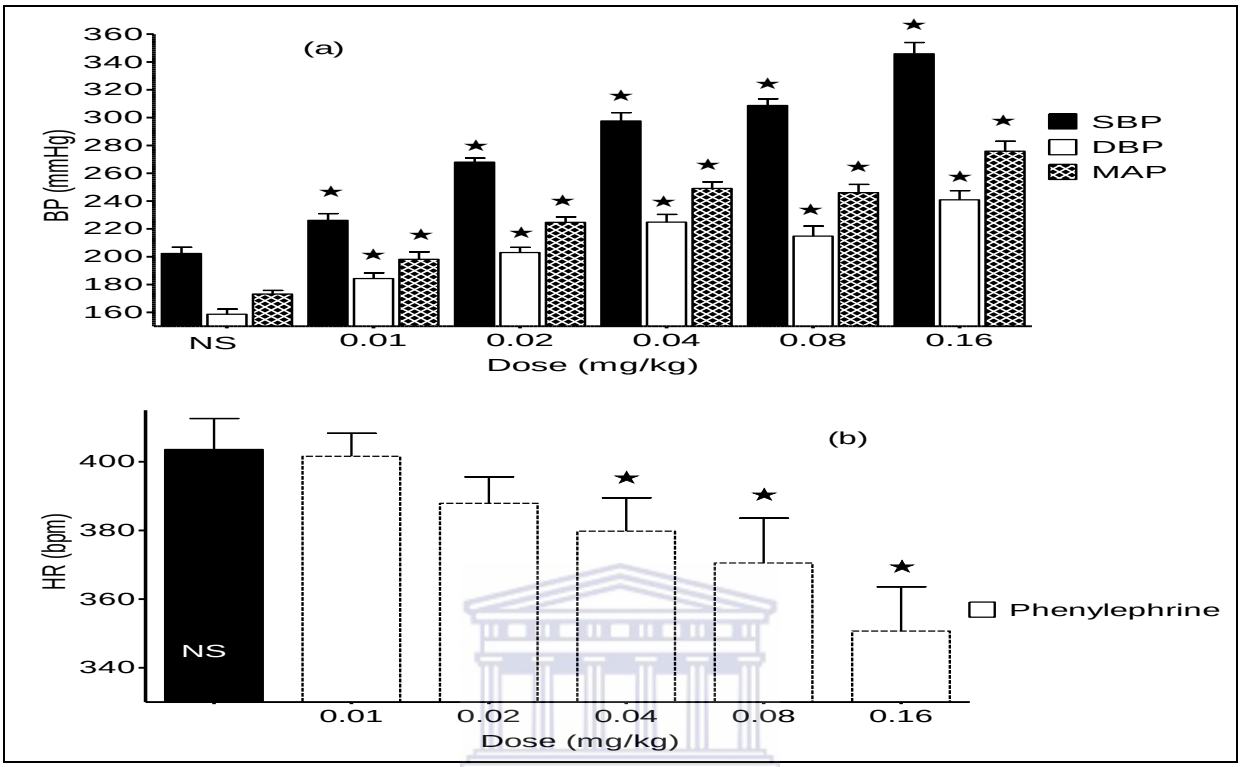

Figure 4. 25: Dose-response graph of the effect of phenylephrine (0.01 – 0.16 mg/kg) on BP (a) and HR (b). Values are presented as mean ± SEM. * indicates statistical significance.

6.2. Effect of phenylephrine (0.01 – 0.16 mg/kg) co-infused with T. violacea (60 mg/kg) on BP and HR

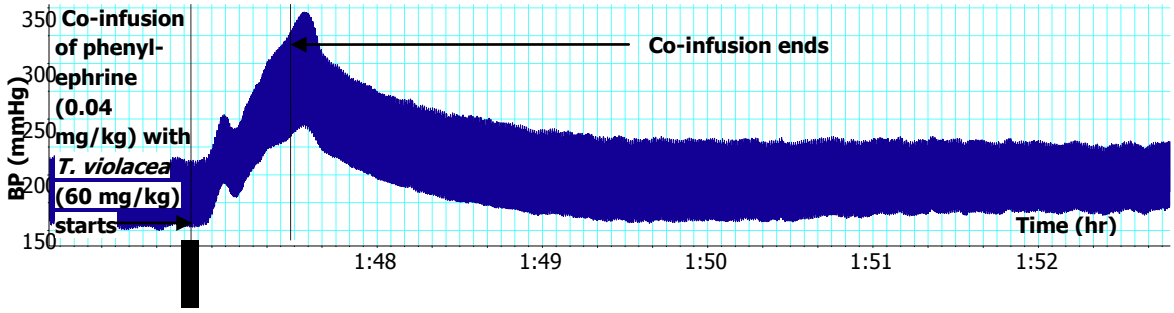

Figure 4. 26: Effect of phenylephrine (0.04 mg/kg) co-infused with *T. violacea* (60 mg/kg) on BP in a SHR. Chart scaling 100:1

This protocol was performed to assess if *T. violacea* reduces BP and HR by blocking the α_1 adrenoceptors. The infusion of *T. violacea* (60 mg/kg) alone, significantly ($p < 0.05$) reduced the SBP (from 195.0 ± 6.1 mmHg to 116.4 ± 6.3 mmHg), DBP (from 153.0 ± 4.6 mmHg to 72.2 ± 5.7 mmHg), MAP (from 167.0 ± 4.5 mmHg to 86.9 ± 5.2 mmHg) and HR (from 386.2 ± 11.9 bpm to 332.3 ± 18.4 bpm) when compared with values obtained with the vehicle alone (figure 4.27). Phenylephrine (0.01 – 0.16 mg/kg) produced significant ($p < 0.05$) increases in SBP, DBP, MAP; which were associated with dose-dependent decreases in HR when compared to the values obtained with the vehicle alone (figure 4.27). The co-infusion of phenylephrine (0.02– 0.08 mg/kg) with *T. violacea* (60 mg/kg) produced significant ($p < 0.05$) increases in SBP, DBP and MAP, which were associated with reductions in HR (that were not significant) when compared to the values obtained with the vehicle alone. The final mean values of the SBP, DBP, MAP and HR obtained with the co-infusion of the phenylephrine with *T. violacea* were similar to those obtained with the infusion of phenylephrine alone at all doses. Significant ($p < 0.05$) reductions were observed with the co-

infusion of the standard drug with the MLE at the dose of 0.01 mg/kg (for the DBP and MAP) and 0.16 mg/kg (for SBP, DBP and MAP) when compared to the values obtained with the infusion of the standard drug alone (figure 4.27).

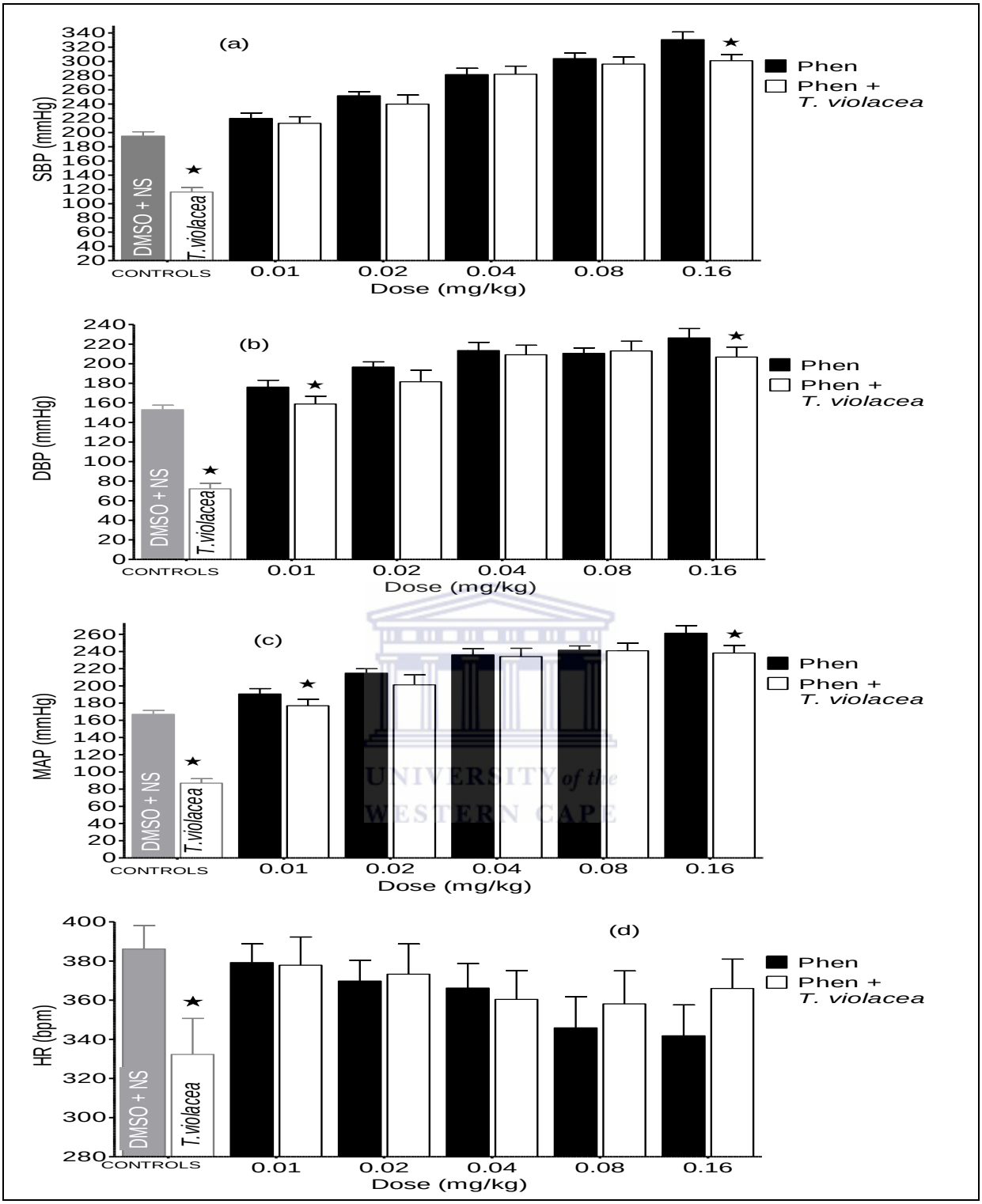

Figure 4. 27: Effect of phenylephrine (0.01 – 0.16 mg/kg) co-infused with *T. violacea* (60 mg/kg) on the SBP, DBP, MAP and HR in SHR. Values are presented as mean ± SEM. * indicates statistical significance.

6.3. Effect of prazosin (1 mg/kg) on the BP and HR

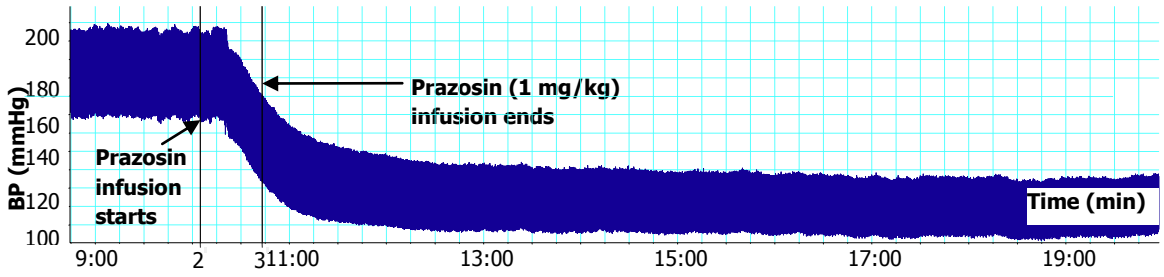

Figure 4. 28: Effect of prazosin (1 mg/kg) on BP in a SHR. Chart scaling 100:1

Prazosin is a specific antagonist of the α_1 adrenoceptor (Hui & Qiu, 1996; Dabire, 2004; Khwanchuea *et al.*, 2008), and was used as the positive control in assessing the effect of the MLE on the α_1 adrenoceptors. Prazosin (1 mg/kg) produced significant ($p < 0.05$) and sustained (a) 34.8 ± 2.2% decrease in the SBP (from 183.5 ± 5.1 mmHg to 119.9 ± 5.9 mmHg), (b) 37.7 ± 2.2% decrease in the DBP (from 141.3 ± 5.7 mmHg to 88.7 ± 6.0 mmHg), (c) 36.5 ± 2.1% decrease in the MAP (from 155.3 ± 5.2 mmHg to 99.1 ± 5.6 mmHg), and (d) 5.6 ± 0.9% decrease in HR (from 386.4 ± 4.2 bpm to 364.9 ± 5.3 bpm) (figures 4.28 and 4.29).

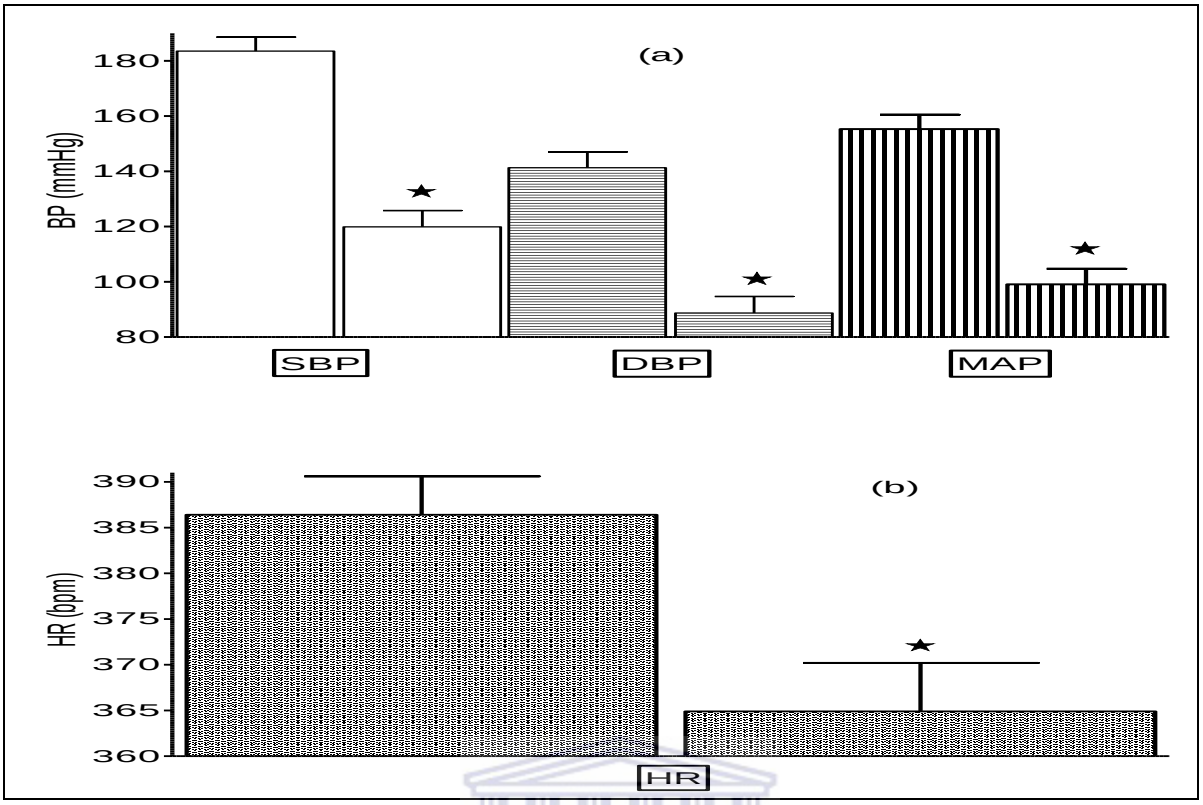

Figure 4. 29: Effect of prazosin (1 mg/kg) on BP (a) and HR (b) in the SHR. Values are presented as mean ± SEM. * indicates statistical significance.

6.4. Effect of phenylephrine (0.01 – 0.16 mg/kg) co-infused with T. violacea (60 mg/kg) on the BP and HR after pre-treatment of animals with prazosin (1 mg/kg).

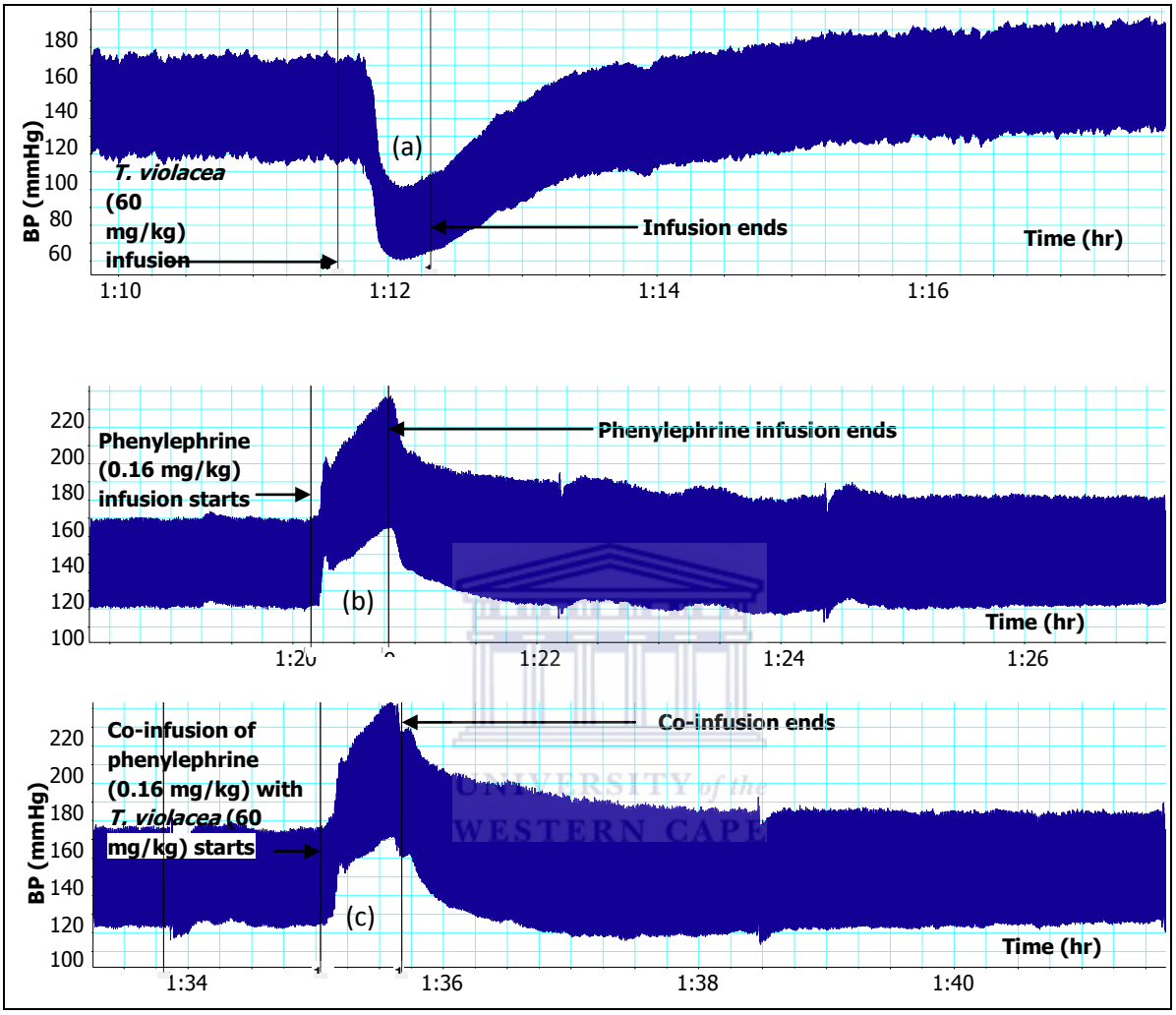

Figure 4. 30: Effect on BP of (a) *T. violacea* (60 mg/kg) alone, (b) phenylephrine (0.16 mg/kg), and (c) phenylephrine (0.16 mg/kg) co-infused with *T. violacea* (60 mg/kg) in animals pre-treated with prazosin (1 mg/kg).

This protocol was performed to observe if the pre-treatment of the animals with prazosin would alter the response of the rats to the infusion of phenylephrine alone, as well as the co-infusion of phenylephrine with *T. violacea* (see section 4.6.2. above). All the increases in BP

observed in this protocol were significantly ($p < 0.05$) less than those observed in the absence of prazosin (figure 4.27). The infusion of phenylephrine (0.02 – 0.16 mg/kg) alone, as well as the co-infusion of phenylephrine (0.01 – 0.16 mg/kg) with *T. violacea* (60 mg/kg) produced significant ($p < 0.05$) dose dependent ($p < 0.05$) increases in the SBP, DBP and MAP when compared to the values at the new 'baseline' produced by prazosin (1 mg/kg); with each dose of the phenylephrine co-infused with *T. violacea*, having similar final BP values as its corresponding dose (of phenylephrine) infused alone. The infusion of the least dose of phenylephrine alone, did not produce any increase in BP, therefore the values obtained with its co-infusion with *T. violacea* were significantly ($p < 0.05$) higher at this dose (figure 4.31). The infusion of phenylephrine alone as well as the co-infusion of phenylephrine with *T. violacea* did produce any change in HR, when compared to the 'baseline' values, except at the dose of 0.08 mg/kg where a significant ($p < 0.05$) increase in HR was seen with the infusion of the standard drug alone, and this was the only dose where the co-infusion of the standard drug with the MLE showed a reduction in the final HR value when compared to the value obtained with the standard drug alone); and 0.16 mg/kg (where a significant ($p < 0.05$) decrease in HR was seen with the co-infusion of the standard drug with the MLE) (figure 4.31 d).

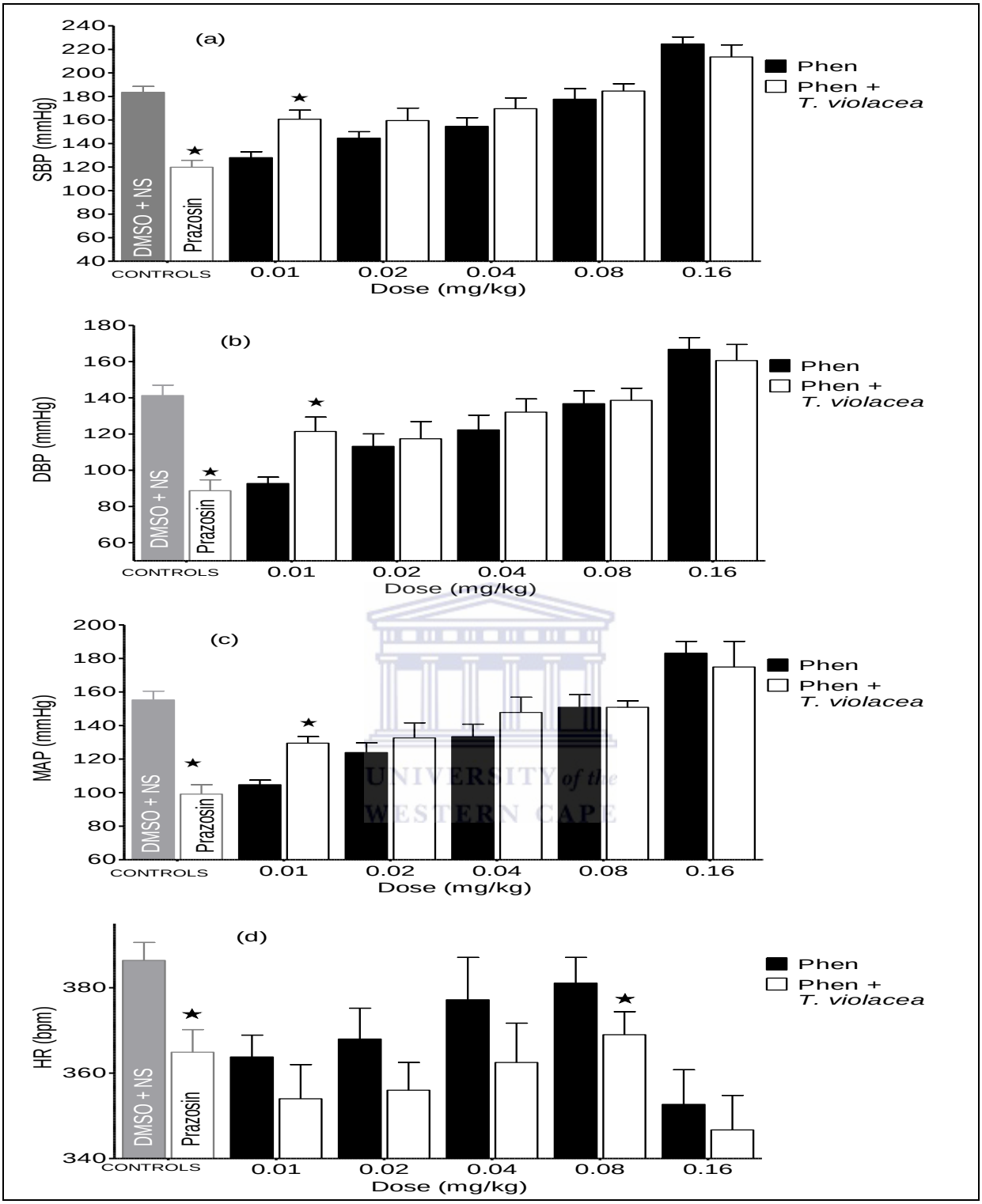

Figure 4. 31: Effect of phenylephrine (0.01 – 0.16 mg/kg) co-infused with *T. violacea* on the SBP (a), DBP (b), MAP (c), and HR (d) after pre-treatment of animals with prazosin. Values are presented as mean ± SEM. * indicates statistical significance.

7. DOES *T. VIOLACEA* ACT BY BLOCKING THE BETA I ADRENOCEPTORS?

7.1 Dobutamine dose response curve

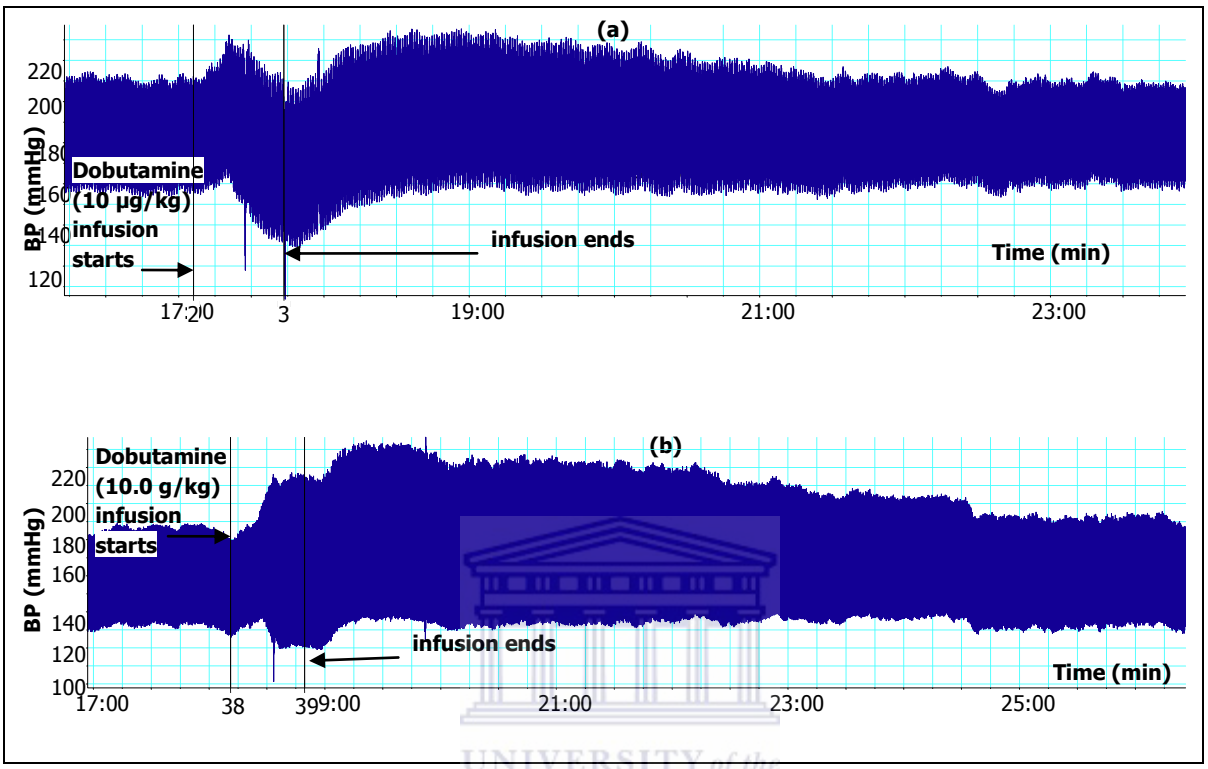

Figure 4. 32: Effect of dobutamine (10.0 µg/kg) on BP in two SHRs. Chart scaling 100:1

This experiment was carried out to investigate the contribution of the β_1 adrenoceptors to the reductions in BP and HR observed with the infusion of *T. violacea* in the rats. As shown in the tracings above, the effect of dobutamine (0.2 – 10.0 µg/kg) on BP was not predictable (figure 4.32), but partly dose dependent (figure 4.33 a). Dobutamine produced significant ($p < 0.05$) increases in BP at a few doses. Significant ($p < 0.05$) increases in (a) SBP from the baseline value of 190.2 ± 6.2 mmHg, were observed at the doses of 1.3 µg/kg (a 6.5 ± 1.1% increase to 202.6 ± 5.8 mmHg), 5.0 µg/kg (a 11.2 ± 1.2% increase to 211.5 ± 5.7 mmHg) and 10.0 µg/kg (a 17.3 ± 1.4% increase to 223.2 ± 6.4 mmHg); (b) DBP from the baseline value

of 135.6 ± 7.0 mmHg, was only observed at the dose of 10.0 µg/kg (a 10.9 ± 2.9% increase to 150.4 ± 6.7 mmHg); and (c) MAP from the baseline value of 153.8 ± 6.4 mmHg, were observed at the doses of 5.0 µg/kg (an 8.6 ± 1.7% increase to 167.0 ± 5.4 mmHg) and 10.0 µg/kg (a 13.5 ± 2.6% increase to 174.6 ± 6.8 mmHg) (figure 4.33 a). Dobutamine increased the HR in a dose-dependent manner from 394.0 ± 7.9 bpm at baseline, to 399.5 ± 7.2; i.e., by 1.4 ± 0.5% (maximum effect of lowest dose), and to 518.4 ± 8.3 bpm, i.e., by 31.6 ± 2.1% (maximum effect of highest dose) (figure 4.33 b).

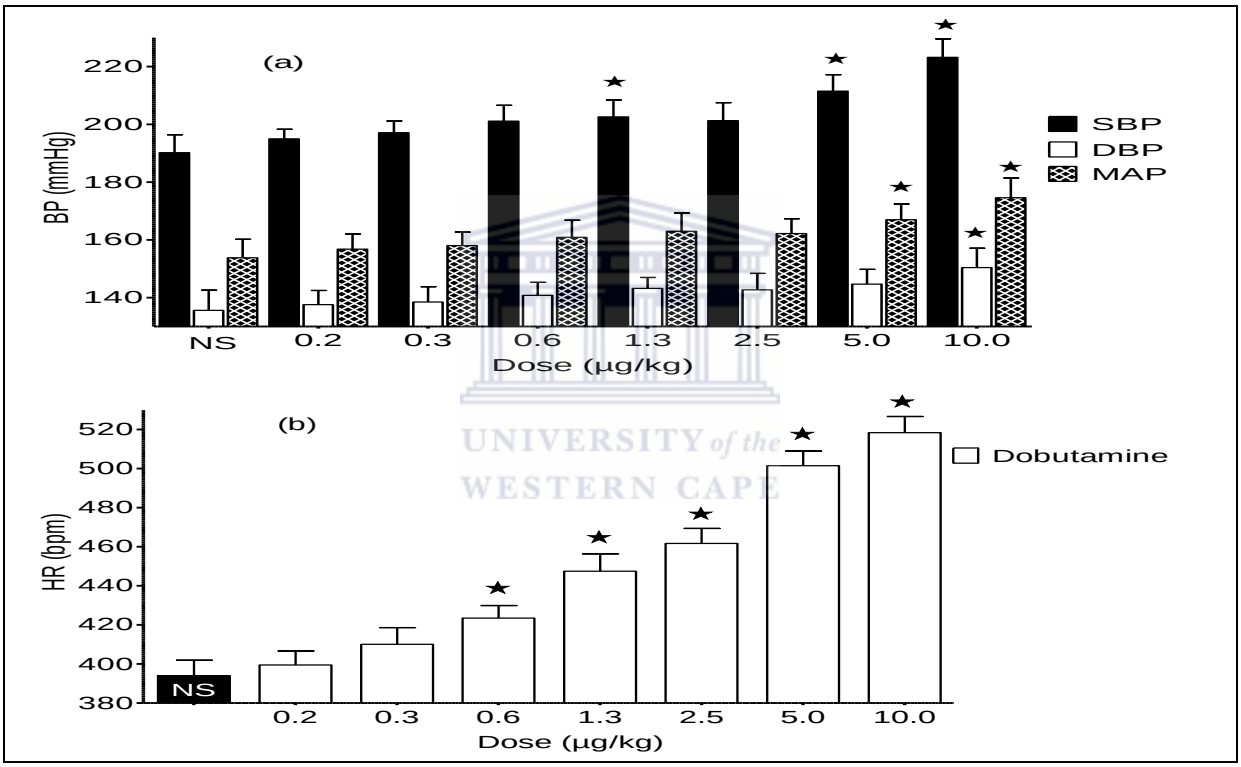

Figure 4. 33: Effect of dobutamine (0.2 – 10.0 µg/kg) on BP (a) and HR (b). Values are presented as mean ± SEM. * indicates statistical significance.

7.2. Effect of dobutamine (0.2 – 10.0 µg/kg) co-infused with T. violacea (60 mg/kg) on the BP and HR in SHR

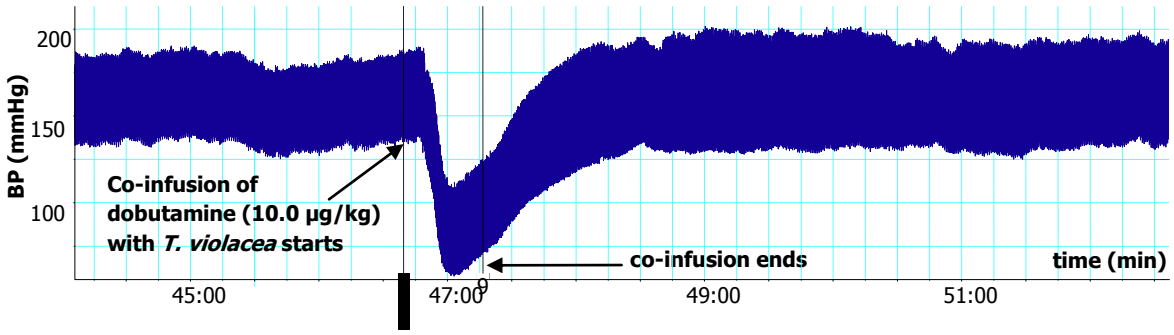

Figure 4. 34: Effect of dobutamine (10.0 µg/kg) co-infused with *T. violacea* (60 mg/kg) on BP in a SHR. Chart scaling 100:1

Compared to the values obtained with the infusion of the vehicle alone, (a) the infusion of *T. violacea* (60 mg/kg) alone significantly ($p < 0.05$) reduced the SBP, DBP, MAP and HR, without any significant change in pulse pressure (PP) observed (figures 4.34, 4.35); (b) the infusion of dobutamine (0.2 – 10.0 µg/kg) alone produced increases in the SBP, DBP, PP, MAP and HR, which were only significant ($p < 0.05$) at (i) the three highest doses (of 2.5 µg/kg, 5.0 µg/kg, and 10.0 µg/kg) with respect to the SBP, the PP and the MAP; and (ii) all doses above 0.3 µg/kg with respect to the HR (figure 4.34); (c). the co-infusion of dobutamine with *T. violacea* produced increases in SBP, DBP, PP, MAP and HR which were significant ($p < 0.05$) at (i) all doses with respect to the SBP, (ii) the doses of 0.2 µg/kg, 0.6 µg/kg, 1.3 µg/kg and 2.5 µg/kg with respect to the DBP, (iii) the doses of 0.2 µg/kg, 0.6 µg/kg, 1.3 µg/kg 2.5 µg/kg and 5.0 µg/kg with respect to the MAP, (iv) all doses above 1.3 µg/kg with respect to the HR (figure 4.35), (v) none of the doses given with respect to the PP (result not shown here).

Therefore, the final mean values obtained with the co-infusion of dobutamine with *T. violacea* when compared with the values obtained with the corresponding dose of dobutamine infused alone were (a) similar at all doses with respect to the SBP except at the doses of 1.3 µg/kg (at which there was a statistically significant ($p < 0.05$) increase) and 10.0 µg/kg (at which there was a statistically significant ($p < 0.05$) decrease) (figure 4.35 a); (b) similar at the doses of 0.3 µg/kg, 5.0 µg/kg and 10.0 µg/kg with respect to the DBP, but significantly ($p < 0.05$) higher at the doses of 0.2 µg/kg, 0.6 µg/kg, 1.3 µg/kg and 2.5 µg/kg (figure 4.35 b); (c) similar at the doses of 0.3 µg/kg, 0.6 µg/kg, 2.5 µg/kg and 5.0 µg/kg with respect to the MAP, while (i) significant ($p < 0.05$) increases were observed at the doses of 0.2 µg/kg and 1.3 µg/kg, and (ii) a significant ($p < 0.05$) decrease was observed at the dose of 10.0 µg/kg (figure 4.35 c); (d) similar at all doses with respect to the PP, except at the dose of 0.2 µg/kg (at which a significant ($p < 0.05$) reduction was observed) (result not shown here); (e) significantly ($p < 0.05$) lower at all doses with respect to the HR (figure 4.35 d).

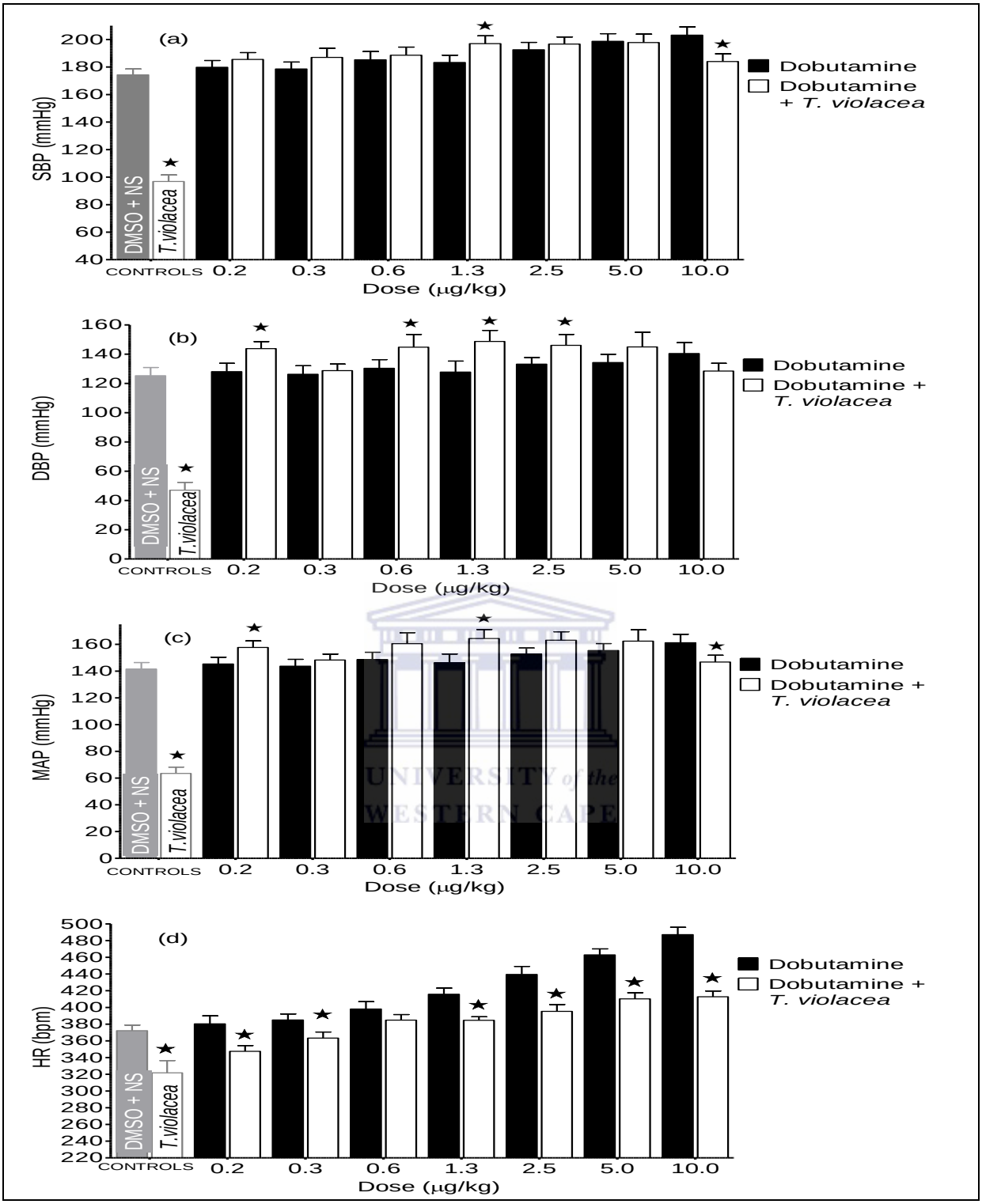

Figure 4. 35: Effect of dobutamine (0.2 – 10.0 µg/kg) co-infused with *T. violacea* (60 mg/kg) on the SBP (a), DBP (b), MAP (c), and HR (d) in SHR. Values are presented as mean ± SEM. * indicates statistical significance.

7.3. Propranolol dose response curve

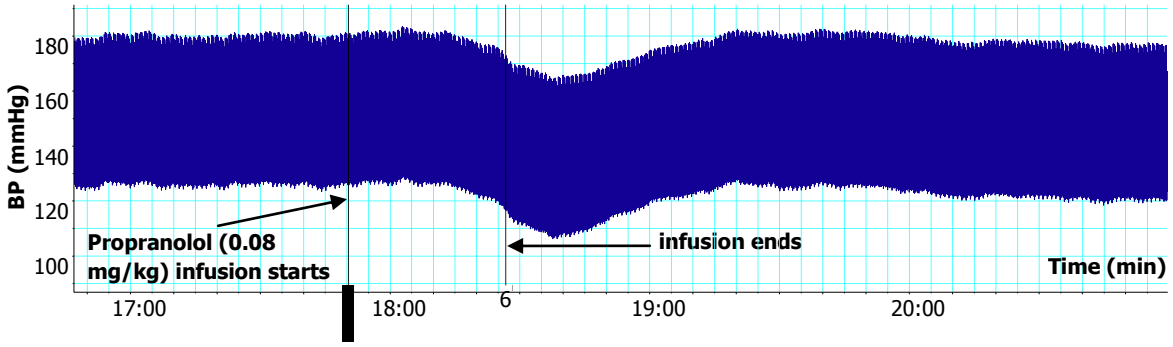

Figure 4. 36: Effect of propranolol (0.08 mg/kg) on BP in a SHR. Chart scaling 100:1

In this study, propranolol was used as a positive control for the possible effect of the MLE on the β_1 adrenoceptors, although propranolol is a non selective β adrenoceptor blocker which acts on both beta 1(β_1) and beta 2 (β_2) receptors (Dimo *et al.*, 2003; Lin *et al.*, 2007; Weir, 2009). In a dose-dependent manner, propranolol (0.1 – 3.2 mg/kg) reduced (a) the SBP from 189.7 ± 3.3 mmHg observed at baseline, to 179.6 ± 4.8 mmHg, i.e., by 1.2 ± 0.6% (maximum effect of lowest dose), and to 134.3 ± 7.6 mmHg, i.e., by 29.2 ± 3.5% (maximum effect of highest dose); (b) the DBP from 143.6 ± 4.5 mmHg observed at baseline, to 143.0 ± 5.3 mmHg, i.e., by 0.4 ± 0.8% (maximum effect of lowest dose), and to 90.0 ± 9.5 mmHg, i.e., by 37.3 ± 5.9% (maximum effect of highest dose); (c) the MAP from 159.1 ± 3.9 mmHg at baseline, to 158.2 ± 4.7 mmHg, i.e., by 0.6 ± 0.7 % (maximum effect of lowest dose), and to 104.9 ± 8.3 mmHg, i.e., by 34.1 ± 4.7% (maximum effect of highest dose) (figure 4.37 a); and (d) the HR from 371.3 ± 8.69 bpm observed at baseline, to 370.6 ± 5.9 bpm; i.e., by 0.2 ± 0.2% (maximum effect of lowest dose), and to 324.2 ± 11.2 bpm; i.e., by 12.7 ±1.4% (maximum effect of highest dose) (figure 4.37 b). Propranolol did not have any significant effect on PP (figure 4.36).

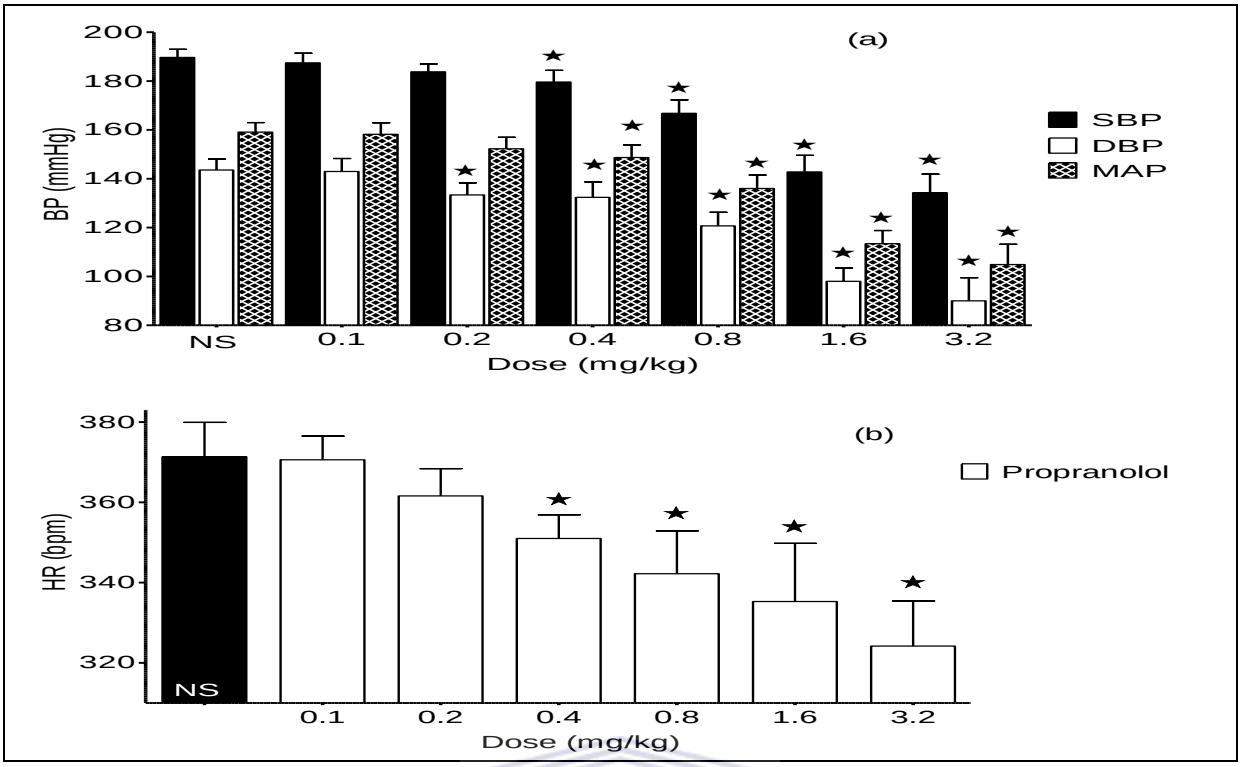

Figure 4. 37: Dose-response graph of the effect of propranolol (0.1 – 3.2 mg/kg) on BP and HR. Values are presented as mean ± SEM. * indicates statistical significance.

7.4. Experiment comparing the effect of T. violacea (60 mg/kg) with that of propranolol (1.6 mg/kg), during continuous infusion of dobutamine (2.3 mg/kg/hr)

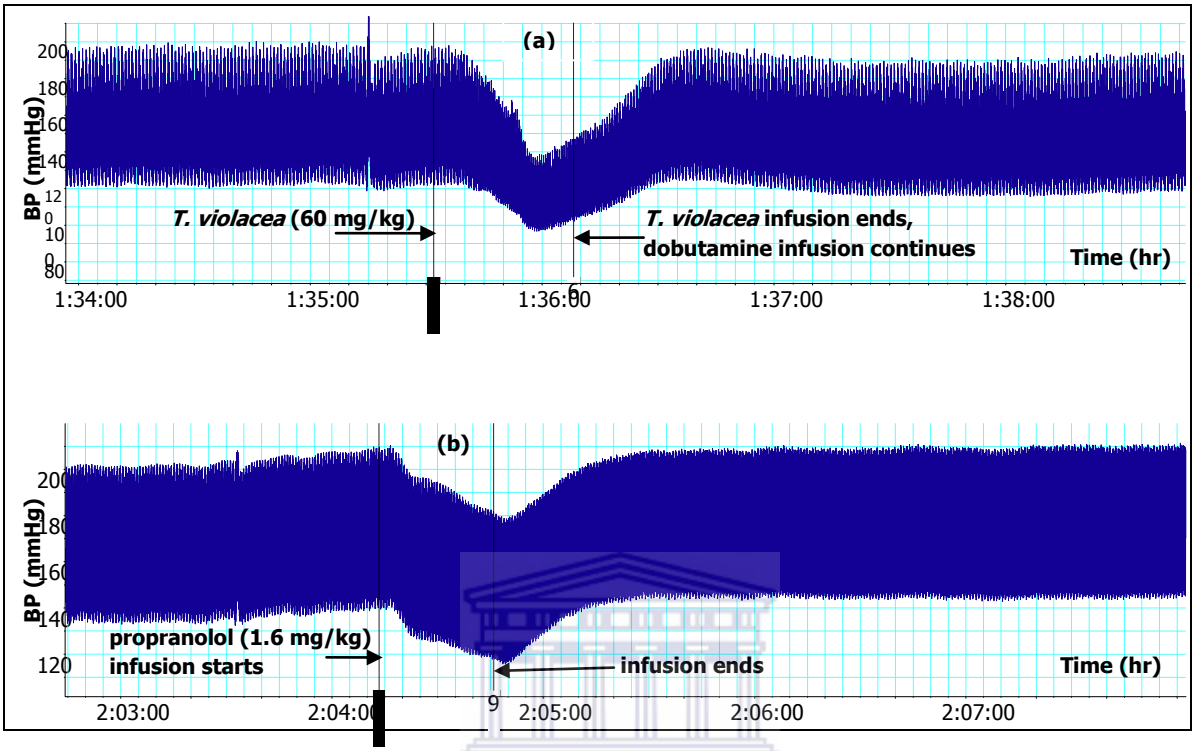

Figure 4. 38: Effect of *T. violacea* (60 mg/kg) (a) or propranolol (1.6 mg/kg) (b) on BP, during continuous infusion of dobutamine (2.3 mg/kg/hr) in a SHR. Chart scaling 100:1

The continuous infusion of dobutamine (2.3 mg/kg/hr) in the SHR did not produce a significant increase in BP from that at baseline, although the initial baseline readings are not shown here. Effort was made to keep the BP and HR due to dobutamine infusion steady for proper comparison of the effect of the standard drug with that of the test drug (*T. violacea*), and also to avoid over-stimulating the β_1 receptors and consequently, jeopardizing the survival of the animal for the duration of the experiment. During continuous infusion of dobutamine, *T. violacea* (60 mg/kg) significantly ($p < 0.05$) reduced (a) the SBP by 15.9 ± 3.1% (from 195.1 ± 6.3 mmHg to 165.0 ± 10.8 mmHg); (b) the DBP by 18.4 ± 4.2% (from

134.7 ± 8.5 mmHg to 111.4 ± 11.2 mmHg); and (c) the MAP by 15.6 ± 4.1 (from 154.7 ± 7.5 mmHg to 132.0 ± 11.4 mmHg). *T. violacea* (60 mg/kg) did not produce any significant reduction in HR. Propranolol (1.6 mg/kg) significantly ($p < 0.05$) reduced (a) the SBP by 17.3 ± 6.6% (from 199.4 ± 4.1 mmHg to 164.6 ± 12.2 mmHg); (b) the DBP by 18.3 ± 10.7% (from 139.0 ± 7.8 mmHg to 109.3 ± 7.8 mmHg); (c) the MAP by 19.3 ± 8.9% (from 159.0 ± 6.3 mmHg to 125.7 ± 9.5 mmHg); and (d) the HR by 14.8 ± 1.5% (from 423.9 ± 7.3 bpm to 361.1 ± 8.8 bpm). The observed reductions in SBP, DBP and MAP were similar with both the infusion of *T. violacea* and propranolol, however the reduction in HR produced by propranolol was significantly ($p < 0.05$) greater than that observed with the MLE (figure 4.39). Neither propranolol nor *T. violacea* had a significant effect on PP (result not shown here).

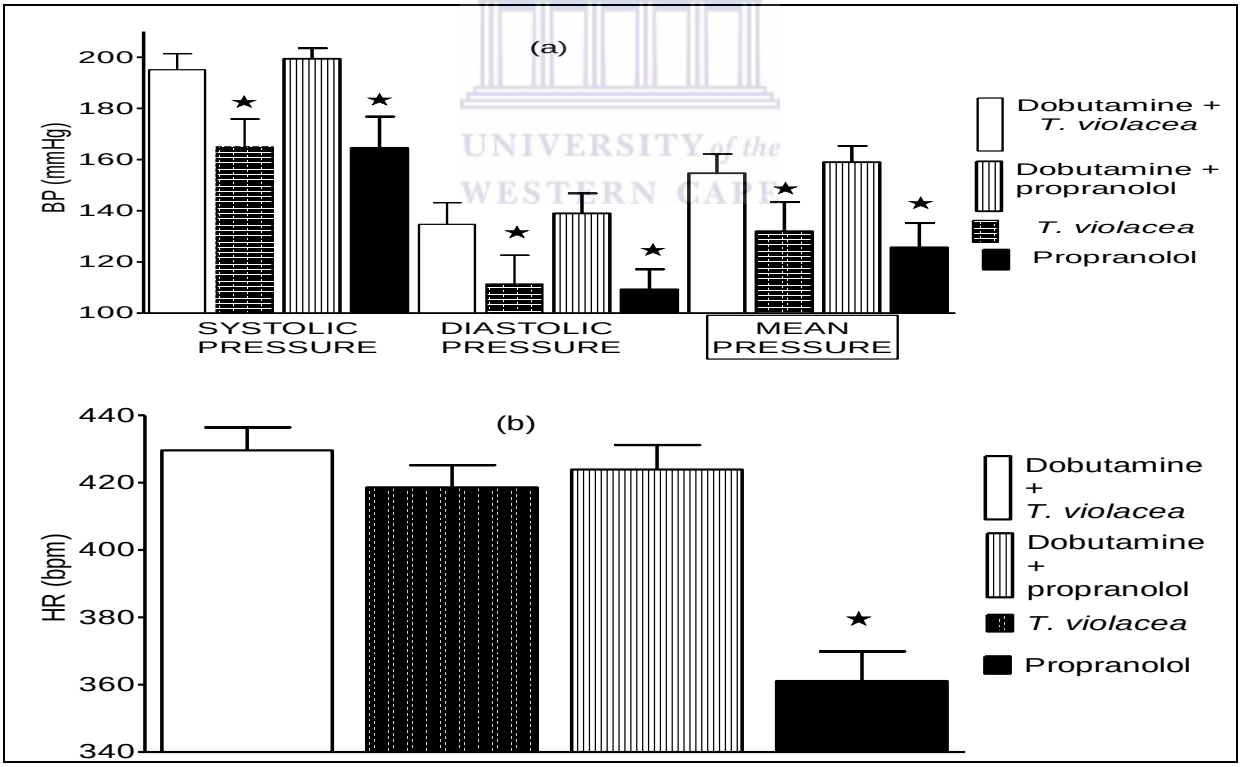

Figure 4. 39: Effects on BP (a) and HR (b) of propranolol (1.6 mg/kg) or *T. violacea* (60 mg/kg) during continuous infusion of dobutamine (2.3 mg/kg/hr) in the SHR. Values are presented as mean ± SEM. * indicates statistical significance.

8. DOES *T. VIOLACEA* ACT BY STIMULATING THE MUSCARINIC RECEPTORS?

8.1. Muscarine dose response curve

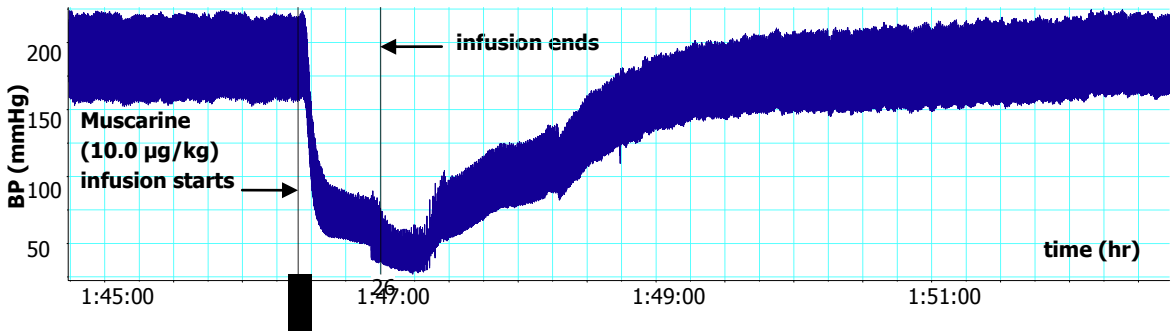

Figure 4. 40: Effect of muscarine (10.0 µg/kg) on BP in a SHR. Chart scaling 100:1

This experiment was performed to assess the effect of muscarine on BP and HR. In a dose-dependent fashion, muscarine (0.2 – 10.0 µg/kg) reduced (a) the SBP from 197.9 ± 5.6 mmHg at baseline, to 180.2 ± 3.8 mmHg, i.e., by 8.9 ± 1.9% (maximum effect of the lowest dose), and to 67.1 ± 3.6 mmHg, i.e., 66.1 ± 2.2% (maximum effect of the highest dose); (b) the DBP from 136.7 ± 8.2 mmHg at baseline, to 123.3 ± 7.1 mmHg, i.e., by 9.8 ± 2.3% (maximum effect of the lowest dose), and to 31.9 ± 6.8 mmHg, i.e., by 76.7 ± 5.4% (maximum effect of the highest dose); (c) the MAP from 157.3 ± 7.2 mmHg at baseline, to 123.3 ± 7.1 mmHg, i.e., by 9.4 ± 2.1% (maximum effect of the lowest dose), and to 44.1 ± 5.2 mmHg, i.e., by 71.9 ± 3.4% (maximum effect of the highest dose) (figure 4.40 a). Muscarine only produced significant ($p < 0.05$) reductions in HR from 382.6 ± 7.0 bpm at baseline, at the last 2 doses of 2.5 µg/kg (a 6.6 ± 0.9% decrease to 357.3 ± 8.3 bpm) and 10 µg/kg (a 55.6 ± 1.6% decrease to 169.7 ± 6.3 bpm) (figure 4.41 b).

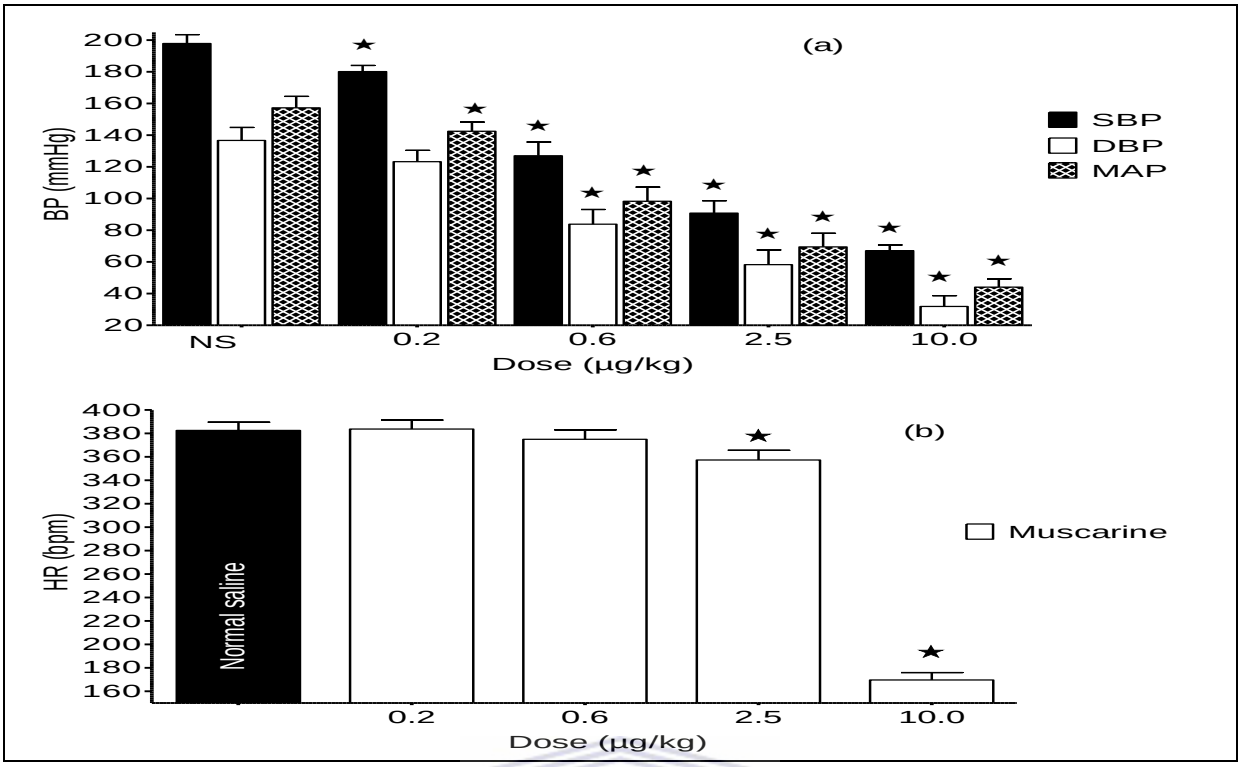

Figure 4. 41: Dose-response graph of the effect of muscarine (0.2 – 10.0 µg/kg) on systolic pressure (SBP), diastolic pressure (DBP), mean arterial pressure (MAP) and heart rate (HR). Values are presented as mean ± SEM. * indicates statistical significance.

8.2. Atropine dose response curve

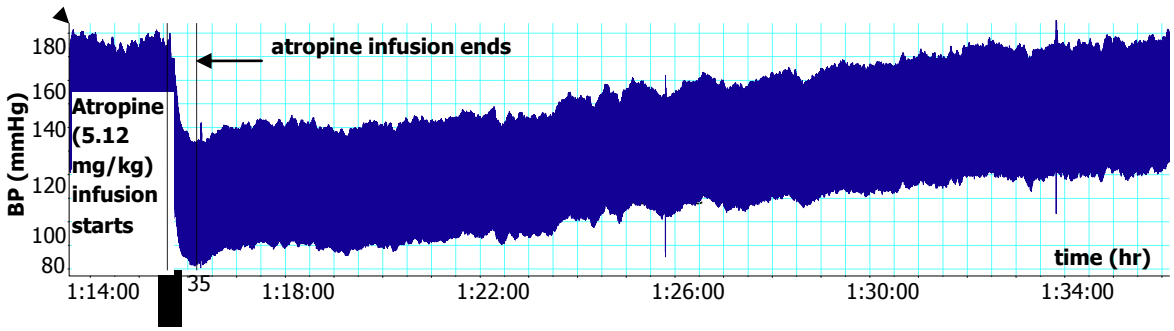

Figure 4. 42: Effect of atropine (5.12 mg/kg) on BP in a SHR. Chart scaling 100:1

In this study the doses lower than those presented here failed to produce any appreciable effect on either BP or HR. In literature (Brown & Taylor, 2011; Pappano, 2011), increases in BP were reported at doses lower than those presented here. This was not observed in these experiments. The doses presented here are the doses at which consistent effects on the BP were observed. In a dose-dependent fashion, atropine (0.02 - 20.48 mg/kg) reduced (a) the SBP from 188.5 ± 2.7 mmHg at baseline, to 168.8 ± 4.9 mmHg, i.e., by 2.8 ± 0.4% (maximum effect of the lowest dose), and to 125.5 ± 5.2 mmHg, i.e., by 33.4 ± 2.3% (maximum effect of the highest dose); (b) the DBP from 131.5 ± 4.4 mmHg at baseline, to 111.8 ± 7.7 mmHg, i.e., by 3.1 ± 1.6% (maximum effect of the lowest dose), and to 60.8 ± 9.4 mmHg, i.e., by 53.7 ± 6.1% (maximum effect of the highest dose); (c) the MAP from 150.2 ± 4.0 mmHg at baseline, to 131.0 ± 6.7 mmHg, i.e., by 2.6 ± 1.0% (maximum effect of the lowest dose), and to 81.2 ± 8.0 mmHg, i.e., by 46.0 ± 4.1% (maximum effect of the highest dose) (figure 4.43 a). The reductions in HR observed with atropine (0.02 – 20.48 mg/kg) when compared to the value of 373.2 ± 4.5 bpm at baseline, were not dose-dependent and significant ($p < 0.05$) reductions were only observed at the doses of 0.32 mg/kg (a 4.6 ± 2.7% reduction to 356.2 ± 12.0 bpm), 5.12 mg/kg (a 14.5 ± 2.5% reduction to 319.1 ± 14.0 bpm) and 20.48 mg/kg (a 33.8 ± 2.2% reduction to 246.9 ± 10.3 bpm) (figure 4.43 b).

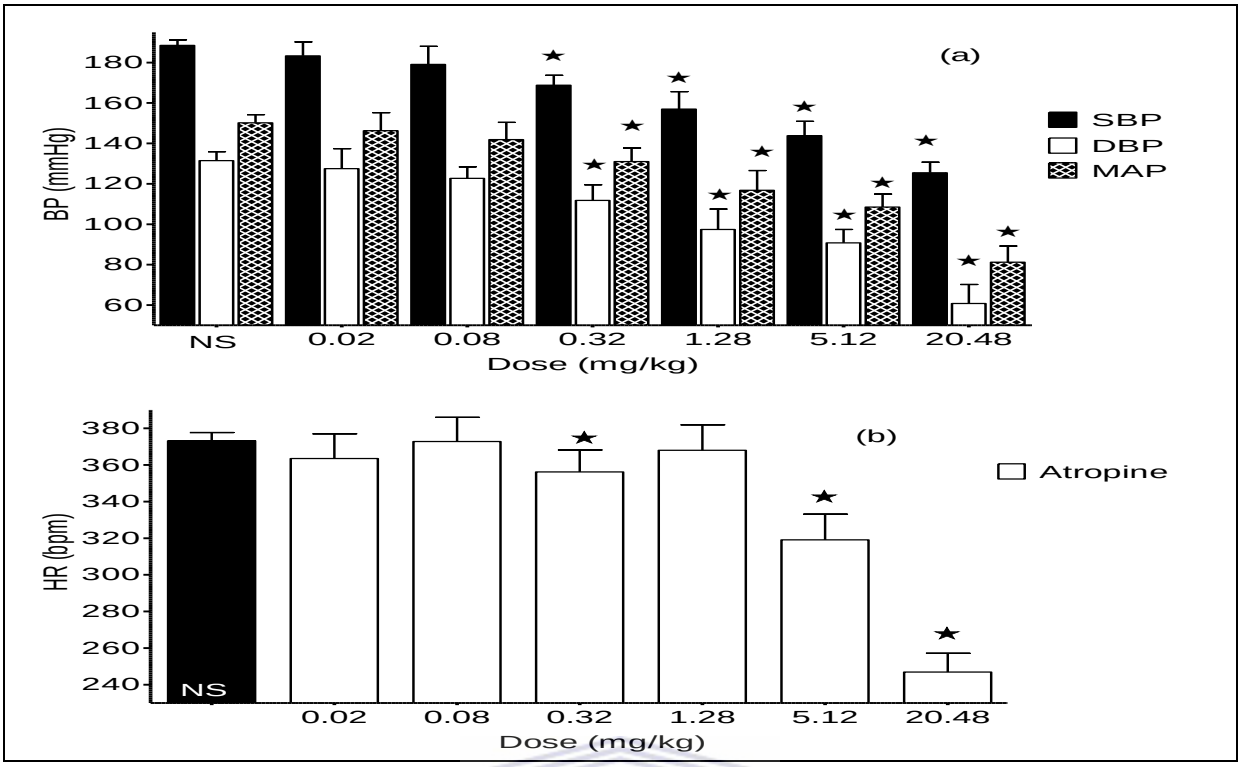

Figure 4. 43: Dose-response graph of the effect of atropine (0.02 – 20.48 mg/kg) on BP and HR. Values are presented as mean ± SEM. * indicates statistical significance.

8.3. Effect of T. violacea (30, 60 and 120 mg/kg) on BP and HR, after pre-treatment of animals with atropine (5.12 mg/kg)

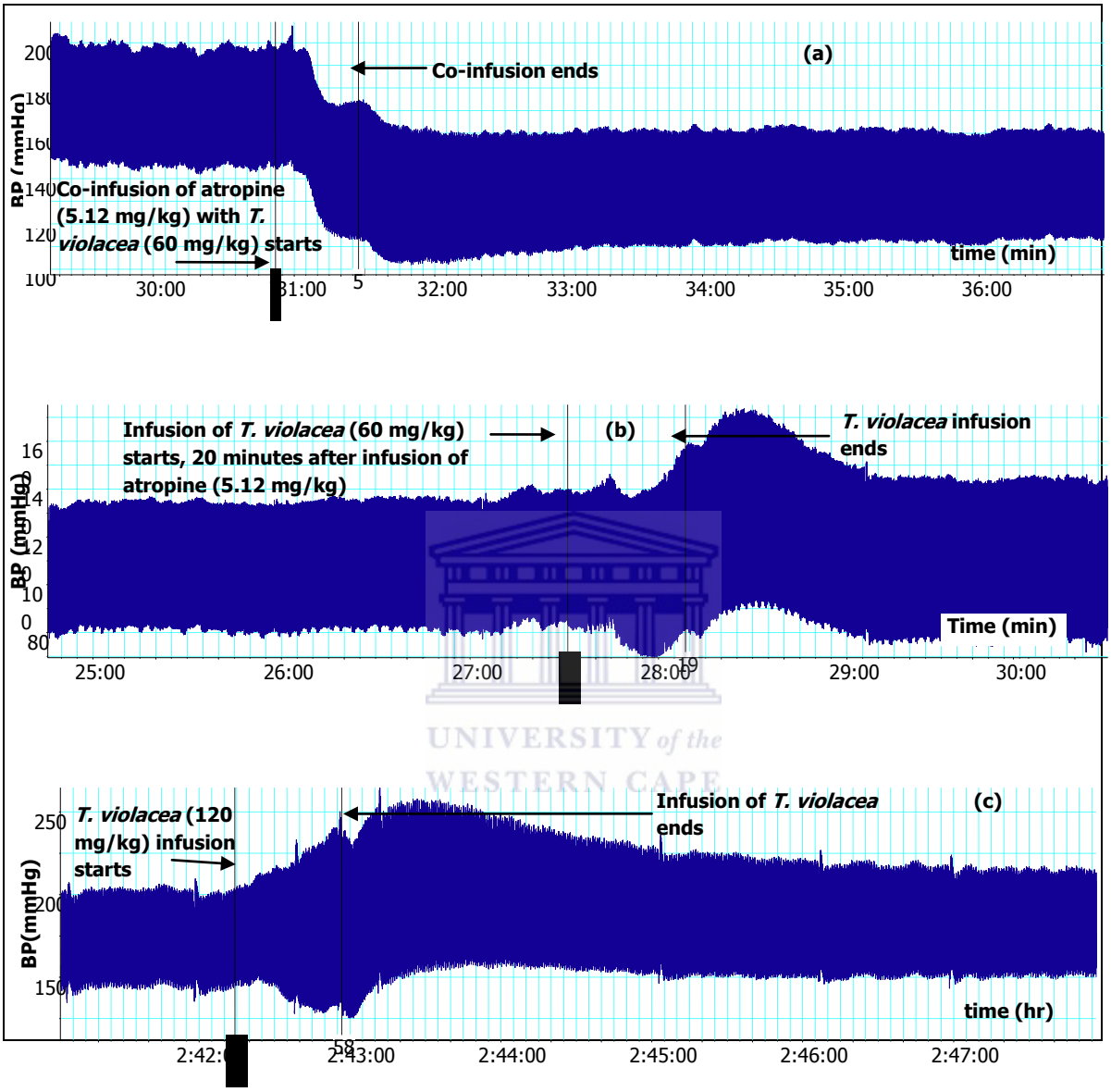

Figure 4. 44: (a) effect of co-infusing *T. violacea* (60 mg/kg) with atropine (5.12 mg/kg) on BP; effect of infusing *T. violacea* (b = 60 mg/kg, c = 120 mg/kg) on BP, 20 minutes after the pre-treatment of a SHR with atropine (5.12 mg/kg). Chart scaling 100:1

The effect of simultaneous co-infusion of atropine (5.12 mg/kg) with *T. violacea* (60 mg/kg) was similar to those normally observed with the infusion of *T. violacea* alone (figure 4.44 a).

However, the infusion *T. violacea*, 20 minutes after pre-treatment of the animals with atropine (5.12 mg/kg), led to significant increases in the BP of the rats (figure 4.44 b and c), as opposed to the hypotensive effect previously reported with the infusion of *T. violacea* (5 mg/kg – 150 mg/kg) (figure 4.4). The dose of atropine (5.12 mg/kg) was the dose at which 80% of maximum BP effect was observed with increasing dose of atropine in the previous experiment. After pre-treatment of rats with atropine, the infusion of *T. violacea* in a dose-dependent manner significantly ($p < 0.05$) increased (a) the SBP from 176.5 ± 4.6 mmHg observed with the infusion of the vehicle alone by 17.2 ± 1.3% (to 206.9 ± 6.3 mmHg), 24.9 ± 3.0% (to 220.4 ± 6.2 mmHg) and 33.3 ± 2.2% (to 235.2 ± 5.5 mmHg) at the doses of 30 mg/kg, 60 mg/kg and 120 mg/kg respectively; (b) the DBP from 128.4 ± 4.6 mmHg observed with the vehicle by 11.7 ± 2.0% (to 143.5 ± 7.7 mmHg), 17.5 ± 3.3% (to 150.9 ± 6.7 mmHg) and 21.0 ± 2.4% (to 155.4 ± 6.0 mmHg) at the doses of 30 mg/kg, 60 mg/kg and 120 mg/kg respectively; (c) the PP from 48.1 ± 1.6 mmHg observed with the vehicle by 18.7 ± 1.6% (to 63.4 ± 3.0 mmHg), 43.4 ± 4.8% (to 69.5 ± 2.1 mmHg) and 65.6 ± 4.3% (to 79.8 ± 1.9 mmHg) at the doses of 30 mg/kg, 60 mg/kg and 120 mg/kg respectively; (d) the MAP from 144.4 ± 4.5 mmHg observed with the vehicle by 13.8 ± 1.7% (to 164.4 ± 7.1 mmHg), 20.4 ± 3.1% (to 173.9 ± 6.5 mmHg) and 19.3 ± 4.3% (to 172.3 ± 8.7 mmHg) at the doses of 30 mg/kg, 60 mg/kg and 120 mg/kg observed with the infusion of 120 mg/kg respectively. The only significant ($p < 0.05$) change in the HR was the 3.4 ± 1.7% increase (from 384.3 ± 2.75 bpm to 397.4 ± 5.6 bpm) at the dose of 120 mg/kg of the MLE (figure 4.45).

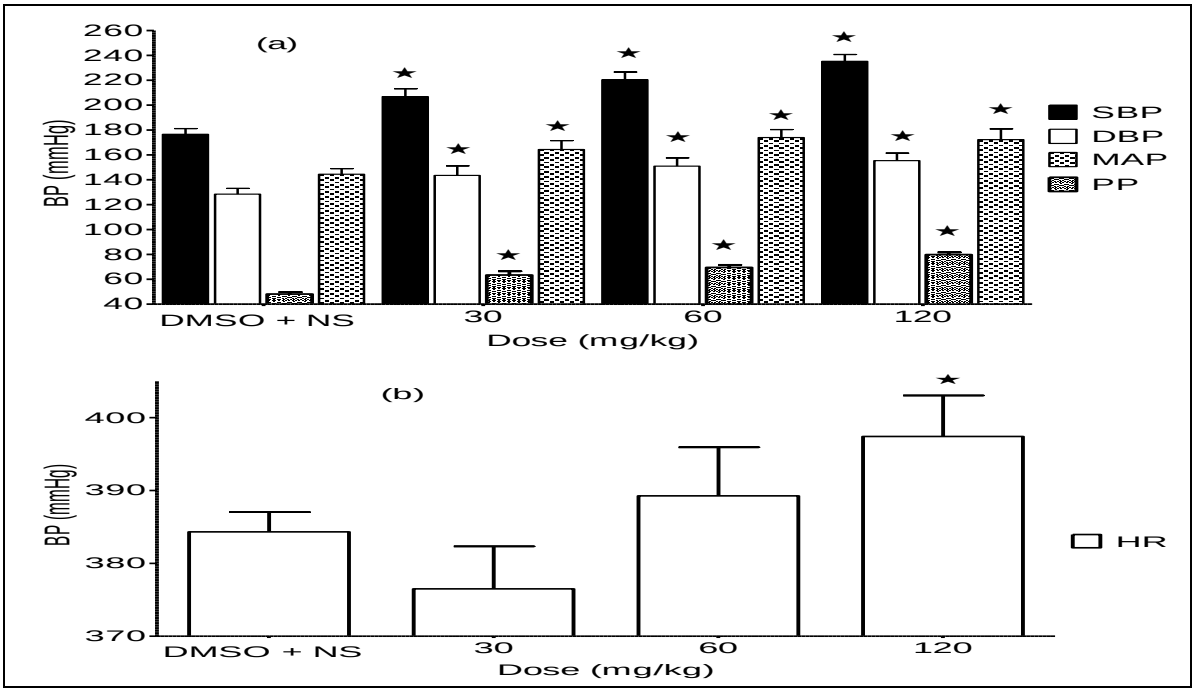

Figure 4. 45: Effect of *T. violacea* (30 mg/kg, 60 mg/kg and 120 mg/kg) on BP (a) and HR (b), after pre-treating SHRs with atropine (5.12 mg/kg). Values are presented as mean ± SEM. * indicates statistical significance.

8.4. Experiment comparing the effect of muscarine (2.5 µg/kg) with that of T. violacea (60 mg/kg) after pre-treatment of animals with atropine (5.12 mg/kg)

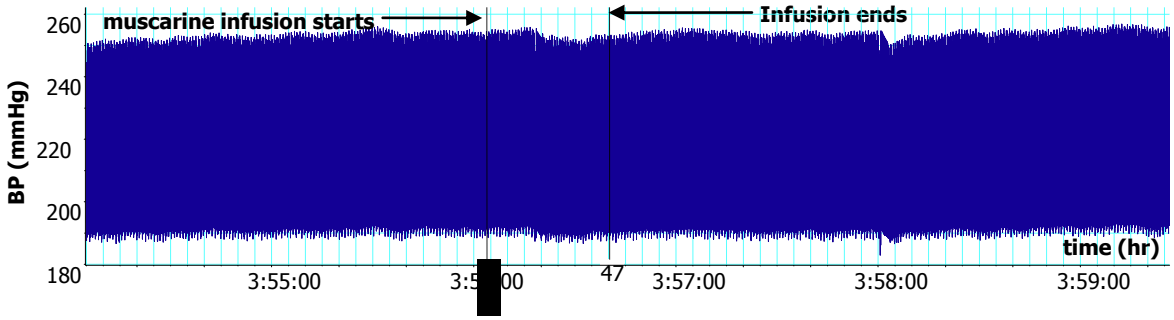

Figure 4. 46: Effect of infusing muscarine (2.5 µg/kg) on BP, 20 minutes after the pre-treatment of a SHR with atropine (5.12 mg/kg). Chart scaling 100:1

After pre-treatment of animals with atropine (5.12 mg/kg), the BP returned back to the initial baseline value, which was not the case with the HR (results not shown here). Subsequent infusion of muscarine (2.5 µg/kg) did not produce any change in BP and/or HR (as previously seen in figures 4.39 and 4.40), i.e., the BP and HR reducing effect of muscarine previously reported were nullified (figure 4.46). However, *T. violacea* (60 mg/kg) still produced significant ($p < 0.05$) 17.2 ± 3.6% increase in the SBP (from 192.1 ± 2.9 mmHg to 225.7 ± 9.8 mmHg) and 11.0 ± 3.5% increase in the MAP (from 166.0 ± 4.9 mmHg to 184.7 ± 9.9 mmHg). Consequently, the increases in SBP and MAP observed with the infusion of *T. violacea* were significantly ($p < 0.05$) higher than those observed with muscarine. Furthermore, the reductions in DBP and HR previous seen with muscarine (2.5 µg/kg) (figures 4.39 and 4.40) and *T. violacea* (60 mg/kg) (figures 4.4) were obliterated after pre-treatment of animals with atropine (5.12 mg/kg) (figure 4.47).

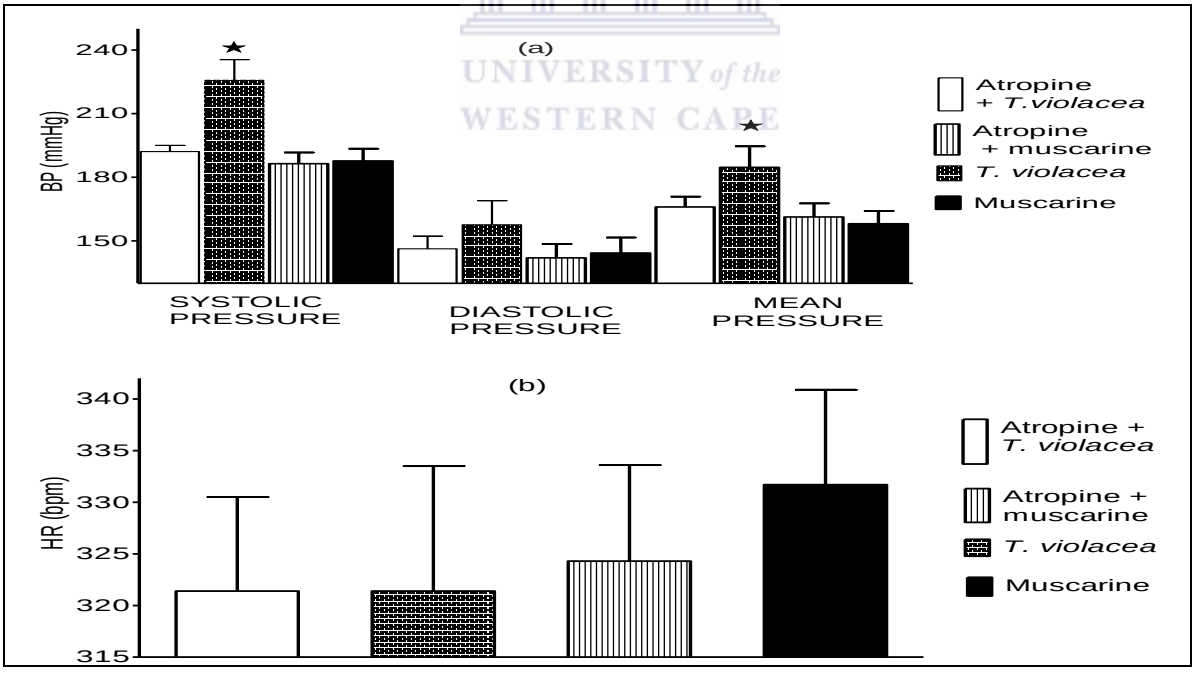

Figure 4. 47: Effects on BP (a) and HR (b) of muscarine (2.5 µg/kg) or *T. violacea* (60 mg/kg) infused 20 minutes after the pre-treatment of SHRs with atropine (5.12 mg/kg). Values are presented as mean ± SEM. * indicates statistical significance.

9. EFFECT OF CHRONIC ADMINISTRATION OF *T. VIOLACEA*, CAPTOPRIL OR THE VEHICLE (DMSO + NS) ON BODY WEIGHT, BLOOD PRESSURE, HEART RATE, PLASMA LEVELS OF ALDOSTERONE IN SHR

9.1. Body weight

Intraperitoneal administration of *T. violacea* (60 mg/kg/day), captopril (10 mg/kg/day) or the vehicle (DMSO + NS) into SHRs, for 21 days did not produce any statistically significant change in the mean body weight of any of the rat groups (figure 4.48).

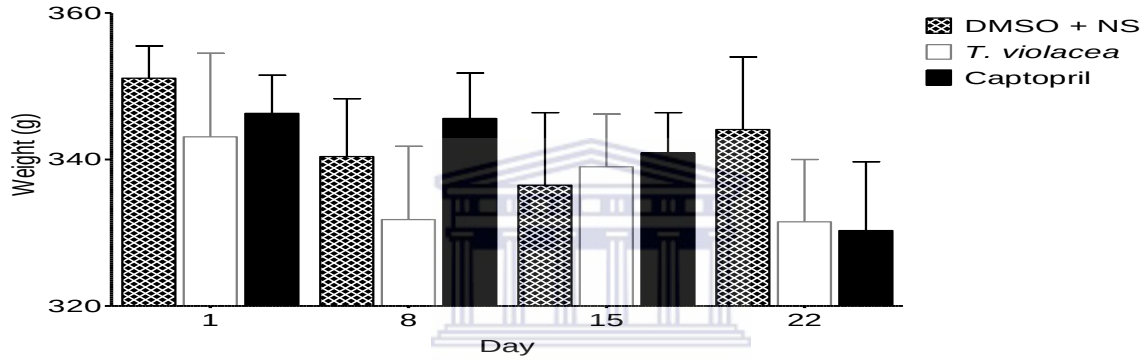

Figure 4. 48: Effect of chronic (21 days) intraperitoneal administration of *T. violacea* (60 mg/kg/day), captopril (10 mg/kg/day) or the vehicle (DMSO + NS) on the body weight (g) of SHR. Values are presented as mean ± SEM. * indicates statistical significance.

9.2. Blood pressure and heart rate

9.2.1. Non-invasive measurement

On the 8th day of the study, statistically significant ($p < 0.05$) reductions in the SBP of 4.1% (from 207.2 ± 2.5 mmHg to 198.6 ± 2.2 mmHg), 6.7% from 204.0 ± 3.9 mmHg to 190.3 ± 3.1 mmHg) and 11.7% (from 213.1 ± 6.2 mmHg to 188.2 ± 5.1 mmHg) were observed in the control, *T. violacea* and captopril groups respectively. On the 15th day, further statistically significant ($p < 0.05$) reductions in SBP of 2.0% (i.e., to 194.6 ± 3.7 mmHg), 3.1% (i.e., to

184.4 ± 3.6) and 13.1% (i.e., to 160.2 ± 4.5 mmHg) were observed in the control, *T. violacea* and captopril groups when compared to their respective values on the 8th day (figure 4.49 a). There was no significant change in the HR on the 8th day when compared to the respective values on the 1st day of study in any of the groups. However, significant ($p < 0.05$) reductions of 1.9% (from 396.5 ± 3.5 mmHg to 389.2 ± 2.3 mmHg) and 4.5% (from 392.1 ± 7.7 mmHg to 374.5 ± 4.8 mmHg) were observed in the control and *T. violacea* groups respectively on the 15th day when compared to the mean values observed on the 1st day of study (figure 4.49 b). The BP and HR were assessed in anaesthetized animals on the last day of study (day 22).

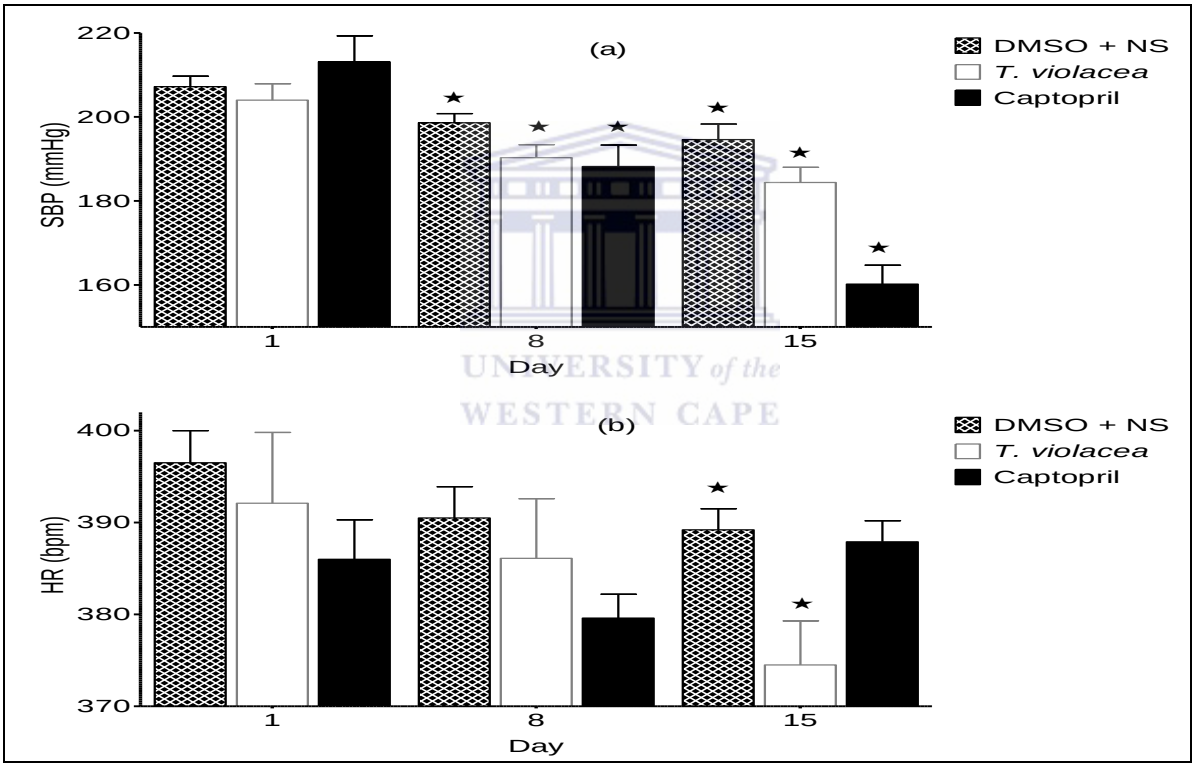

Figure 4. 49: Effect of chronic (21 days) intraperitoneal administration of the vehicle (DMSO + NS), *T. violacea* (60 mg/kg/day) and captopril (10 mg/kg/day) on SBP (mmHg) and HR (bpm). Values are presented as mean ± SEM. * indicates statistical significance.

9.2.2. Invasive measurement

On the 22nd day, as compared to the values obtained in the control group, significant ($p < 0.05$) reductions in the SBP (from 173.8 ± 5.5 mmHg to 151.3 ± 4.2 mmHg, i.e., by 11.1%), DBP (from 130.8 ± 2.3 mmHg to 111.0 ± 7.4 mmHg, i.e., by 10.4%) and MAP (from 145.0 ± 3.3 mmHg to 124.4 ± 6.3 mmHg, i.e., by 11.2%) were only observed in the captopril (10 mg/kg) treated group. The 5.5% reduction in HR (from 365.5 ± 6.9 bpm to 350.3 ± 13.6 bpm) observed in the captropril treated group when compared to the value obtained in the control group of rats was not statistically significant. The reductions in the SBP of 2.1% (from 173.8 ± 5.5 mmHg to 170.1 ± 7.5 mmHg), the DBP of 5.3 (from 130.8 ± 2.3 mmHg to 123.9 ± 7.3 mmHg) and the MAP of 3.4% (from 145.0 ± 3.3 mmHg to 140.1 ± 6.8 mmHg); which were associated with a 1.5% increase in HR (from 365.5 ± 6.9 bpm to 370.9 ± 14.1 bpm) in the *T. violacea* treated animals were not statistically significant when compared to the respective values observed in the control group (figure 4.50).

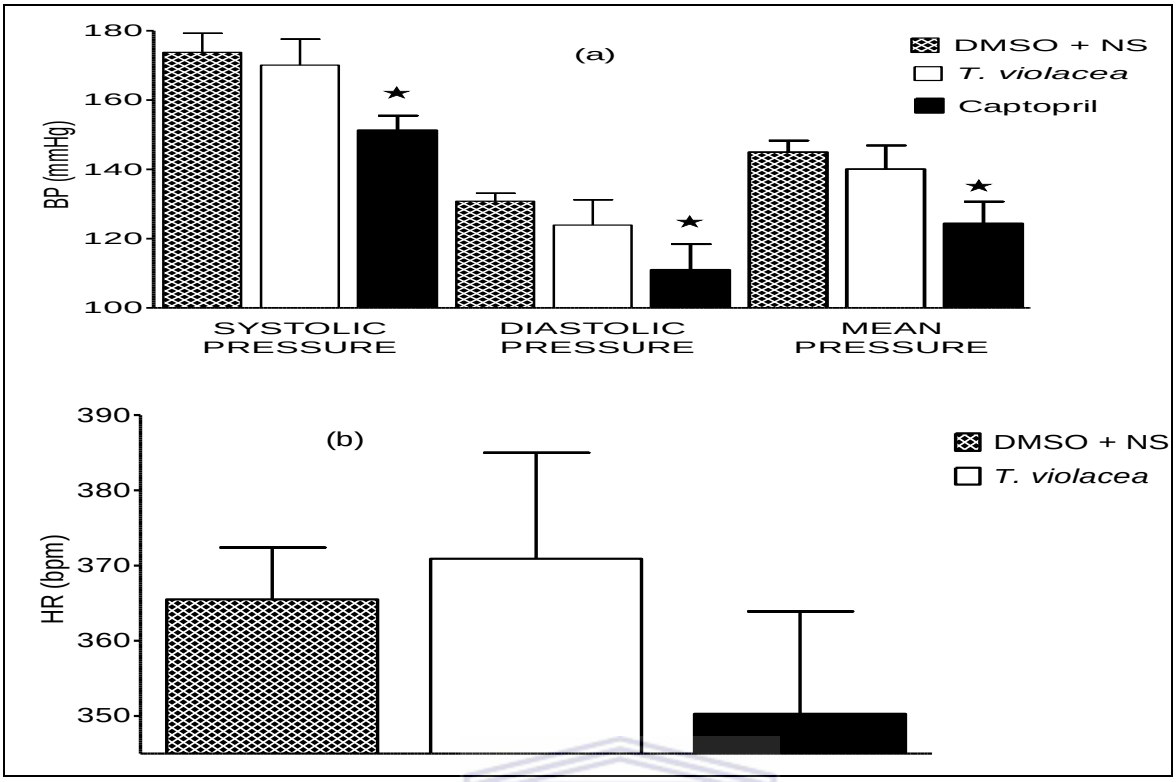

Figure 4. 50: Effect of chronic (21 days) intraperitoneal administration of the vehicle (DMSO + NS), *T. violacea* (60 mg/kg/day) or captopril (10 mg/kg/day) on BP (a) and HR (b) of male SHR. Values are presented as mean ± SEM. * indicates statistical significance.

9.3. Plasma aldosterone levels

Compared to the plasma aldosterone levels in the control group (1319.5 ± 76.7 pg/ml), significantly ($p < 0.05$) lower values where observed in the *T. violacea* treated animals (740.2 ± 65.0 pg/ml, i.e., by 43.9%), and the captopril treated animal (838.4 ± 77.9 pg/ml), i.e., by 36.5% (figure 4.51).

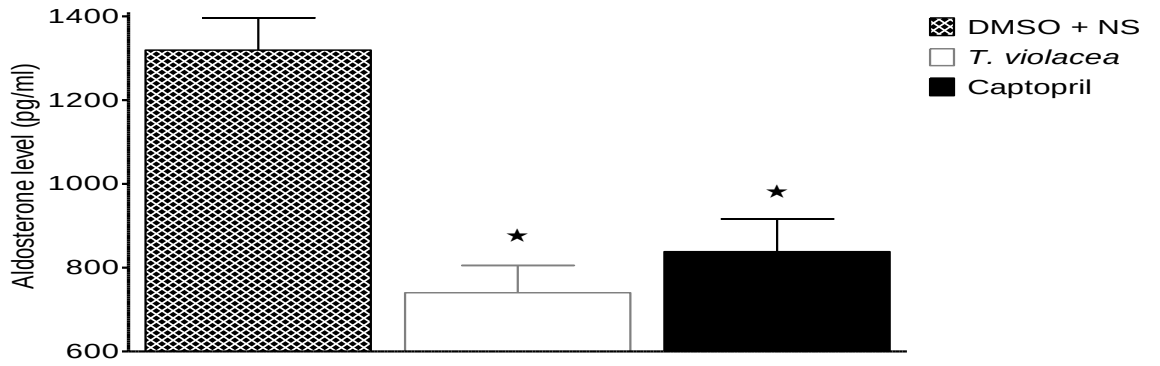

Figure 4. 51: Effect of chronic (21 days) intraperitoneal administration of the vehicle (DMSO + NS), *T. violacea* (60 mg/kg/day) or captopril (10 mg/kg/day) on serum aldosterone levels (pg/ml) of male SHR. Values are presented as mean ± SEM. * indicates statistical significance.

10. ANIMALS EXCLUDED FROM THE STUDY

On completion of the surgical procedures, anaesthetized SHR with SBP less than 150 mmHg and HR lower than 300 bpm, after the 30 minutes stabilization period given to each animals after the surgical procedure was completed were excluded from the study, and the total number of such animals in this study was 17.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

1. THE STABILITY OF THE BLOOD PRESSURE AND HEART RATE.

The animals used in the present study had sustained high blood pressure (BP) levels, i.e., were hypertensive, with systolic blood pressures (SBP) greater than 150 mmHg in the absence of any intervention/treatment (figures 4.1 – 4.3; table 4.1), thus, satisfying the criteria in literature used to classify hypertension (HTN) in the spontaneously hypertensive rat (SHR) model of human HTN. The HR of the SHR used in this study was only slightly higher than those of the normotensive animals used, and this is also in line with the observations in literature (Dickhout & Lee, 1998; Charles River Laboratories International, 2009; Valenti *et al.*, 2009; Williams, 2010). The BP and HR of the rats were largely constant during the period it took to complete each protocol, a benefit of conducting studies in the anaesthetized rat model (Hearse & Sutherland, 2000; Kurtz *et al.*, 2005). Furthermore, the vehicles, normal saline and/or dimethyl sulfoxide used in this study did not have any significant effect on either the BP or HR, and have been previously used separately, and together in similar ratios by previous researchers (figures 4.1 – 4.3) (Abdelrahman *et al.*, 2004; Lewis *et al.*, 2005; Park *et al.*, 2006; Ahn *et al.*, 2007; Ajay *et al.*, 2007; Chan *et al.*, 2007; Tosaka *et al.*, 2007; Mackraj *et al.*, 2008; Ramesar *et al.*, 2008; S. Y. Ryu *et al.*, 2008; H. H. Ryu *et al.*, 2011). The results obtained from assessing the blood oxygen, carbon (IV) oxide, bicarbonate as well as the base excess before and after the experiments were not shown in the results section as no significant changes were observed in any of the values.

2. EFFECT OF *TULBAGHIA VIOLACEA* ON BLOOD PRESSURE AND HEART RATE IN THE SPONTANEOUSLY HYPERTENSIVE RATS

T. violacea produced significant ($p < 0.05$) reduction in the SBP, DBP, MAP and HR in both the SHR (figures 4.4 and 4.5; table 4.2) and normotensive Wistar Kyoto (WKY) rats (the results obtained in the WKY rats are not shown here, since they were similar to those observed in the SHR). At doses above 30 mg/kg, *T. violacea* generally produced reductions in BP that were significantly ($p < 0.05$) more pronounced in the SHR, while producing reduction in HR that were significantly ($p < 0.05$) more pronounced in the WKY (results not shown here). The greater reduction in BP values seen in the SHR may largely be due to the much higher baseline BP values in the SHR (sometimes over 240 mmHg), when compared to the WKY (normally less than 140 mmHg). Consequently, an agent that reduces BP can only reduce BP by up to 140 mmHg in the WKY, while being able to reduce BP by up to 240 mmHg in the SHR.

The reduction in SBP and MAP observed in both SHR and WKY with the administration of *T. violacea* in the present study corroborates the findings of Mackraj *et al.* (2008) and Ramesar *et al.* (2008). Mackraj *et al.* (2008) reported that intraperitoneal administration of *T. violacea* (50 mg/kg body weight) reduced the SBP by 9.12 ± 0.31% (mean% decrease from day 0 to day 14) in the Dahl salt-sensitive (DSS) rat model, while the control group experienced an increase of 4.66 ± 0.56% in SBP at the end of the same period. Meanwhile, Ramesar *et al.* (2008) reported that intravenous (60 minutes) continuous co-infusion of *T. violacea* (50 mg/kg) with exogenous ang I (0.1 mg/kg) at a rate of 50 µL/min produced only a 2.2% increase in maximum mean arterial pressure when compared to the 14.5% increase observed with intravenous co-infusion of DMSO with exogenous ang I in WKY rats. The

associated reductions in HR (figure 4.5; table 4.2) in the present study, suggests an association between the BP and HR in the manner in which the MLE brings about its cardiovascular effect in both the SHR and WKY. There is no previous report on the effect of *T. violacea* on HR in literature. The momentary nature of the BP reduction observed in this study (figure 4.4) may suggest that the anti-hypertensive constituent(s) of the crude extract has a short half-life, and/or the crude extract also contain vasoconstrictory constituent(s) which antagonizes the initial hypotensive effect; thereby, returning the BP towards baseline, even though the MLE is still being infused into the animal. Another possible explanation could be that the baroreflex response to the decrease in BP produced by the MLE is strong enough to return the BP towards baseline level while the MLE is still being infused.

3. DOES *T. VIOLACEA* ACT BY INHIBITING THE ANGIOTENSIN I CONVERTING ENZYME?

The infusion of ang I alone produced significant ($p < 0.05$) increases in the SBP, DBP and MAP; which were associated with variable effects on the HR (figures 4.6 and 4.7). The absence of decreases in HR (figure 4.7) must be due to the ability of ang II (produced from ang I) to not only constrict blood vessels, but also its ability to blunt the baroreflex (Kobori *et al.*, 2007; Carlson & Wyss, 2008).

The MLE of *T. violacea* was able to reduce the increases in the BP produced by ang I (figures 4.8 and 4.9), suggesting that *T. violacea* antagonized the ACE, thereby, preventing the conversion of ang I to the potent vasoconstrictor, ang II. *T. violacea* has been previously

reported to have an ACE inhibitory activity *in- vitro* (Duncan *et al.*, 1999; Mackraj & Ramesar, 2007) and *in- vivo* in WKY rats (Ramesar *et al*., 2008).

The co-infusion of ang I with *T. violacea* led to reductions in HR, which were significant at two doses of the standard drug co-infused with the MLE. This may suggest that the MLE was able to restore the baroreflex (Kobori *et al.*, 2007; Carlson & Wyss, 2008) by reducing the amount of ang II produced. This argument is buttressed by the further increase in HR observed with the co-infusion of the highest dose of ang I with *T. violacea* (figure 4.9 d).

The infusion of captopril into the SHR produced significant ($p < 0.05$) reductions in BP which were not dose-dependent, or associated with significant changes in HR, except at the dose of 2.5 mg/kg (figures 4.10 and 4.11). It was also impossible to repeat doses of captopril in the same animal, as the greatest discernable decrease in BP, was observed with the first dose of captopril given to each animal. This may be due to the long half-life of captopril, which varies from 2 to 4 hours (Lechleitner *et al.*, 1990; Benowitz, 2011; Saseen & Maclaughlin, 2011).

Although, the dose of 2.5 mg/kg of captopril was observed to have produced 80% of the maximum reduction in BP observed in this study (figure 4.11), the dose of 10 mg/kg was subsequently used in this study to (a) ensure that the activity of enzymes are better inhibited, and (b) utilize a dose that has been previously used in a similar study in literature (Mackraj *et al.*, 2008; Ramesar *et al.*, 2008). After pre-treating animals with captopril, the hypertensive effect observed with randomly infused doses of ang I (3.1 – 100.0 µg/kg) were significantly

($p < 0.05$) attenuated (figures 4.12 and 4.13), when compared to the values observed in the absence of captopril (10 mg/kg) (figures 4.6 and 4.7). Reductions in HR, which were significant at the highest doses of ang I given, accompanied the attenuated BP effect (figure 4.13 b), and these are different from the lack of reductions in HR in animals that were not pre-treated (figure 4.7 b). This suggests that captopril adequately inhibited the ACE, and the subsequent actions of ang II which includes inhibiting the baroreceptor reflex (Smith & Vane, 2003; Chen *et al.*, 2007; Hoe *et al.*, 2007; Shin *et al.*, 2009; Thaveau *et al.*, 2010).

4. DOES *T. VIOLACEA* ACT BY BLOCKING THE ANGIOTENSIN II RECEPTORS?

In the present study, the infusion of ang II alone produced significant dose-dependent increases in BP, which were not associated with significant reductions in HR, except at the two highest doses given (figures 4.14 and 4.15), indicating baroreflex impairment. The normal baroreceptor reflex to increases in BP involves decreases in HR and sympathetic nervous system activity. Angiotensin II diminishes the sensitivity of this reflex by synaptically inhibiting it in the nucleus tractus solitarii, by increasing sympathetic nerve activity while decreasing the vagal tone to the heart. It also directly stimulates the myocardium, thus, shifting the operating 'set-point' for regulation of sympathetic outflow to higher BP (Isaacson & Reid, 1990; Kumagai & Reid, 1994; Averill, 2000; Averill & Diz, 2000; Bader *et al.*, 2001; Balt *et al.*, 2003; Diz *et al.*, 2008; Smith *et al.*, 2008; Hilzendeger *et al.*, 2010; Arnold *et al.*, 2011). This may explain the reductions in HR observed with the two highest doses of ang II given alone (figure 4.15). Impairment of the baroreflex has been previously reported in many experimental models of HTN, and is believed to be preceding the onset of HTN (Sanderford & Bishop, 2002; Veerasingham & Raizada, 2003).

The co-infusion of ang II (3.1 – 50.0 µg/kg) with *T. violacea* (60 mg/kg) generally did not lead to significant changes in SBP, DBP or MAP, when compared to the values observed in the absence of *T. violacea* (figures 4.16 and 4.17); except for the significant ($p < 0.05$) increases in SBP at the two lowest doses as well as the significant ($p < 0.05$) reductions in the DBP at the two highest doses; suggesting that the MLE may not reduce BP by blocking the angiotensin II type 1 (AT_1) receptors. The co-infusion of the standard drug with the MLE produced further increases in HR, which were significant ($p < 0.05$) at the two highest doses given (figure 4.17 d) when compared to the values obtained with the standard drug in the absence of the MLE (figure 4.15 b), suggesting that the crude extract may contain substances which may have cardio-excitatory potential, in the presence of ang II. Mackraj *et al.* (2008) previously observed that chronic *T. violacea* administration reduced systemic arterial BP in the DSS rat by decreasing renal AT_1 receptor gene expression and hence, modulating sodium and water homeostasis. A possible explanation for the apparent difference in the result obtained in this study and that of Mackraj *et al.* (2008) may be that *T. violacea* could inhibit the expression of new AT_1 receptor genes without blocking the function of receptors already present as suggested by the results of this study.

As a positive control for the action of the MLE on the (AT_1) receptor, a DRC of ang II (3.1 – 50.0 µg/kg) was conducted in rats pre-treated with losartan, a known ang II receptor blocker. The dose of 30 mg/kg (Wong *et al.*, 1990, 1995; Choi *et al.*, 2009a; Susic *et al.*, 2009b) was used as the lengthy half-life of losartan, which is about 2 hrs, and that of its terminal metabolites which is between 6 and 9 hrs (Lankford *et al.*, 1997; Gainer *et al.*, 1998; Warner *et al.*, 1999; Ripley & Hirsch, 2010) means that the conduction of a separate DRC for losartan in this study, would have required over 50 rats. The use of such a large number of animals for a protocol that is not crucial towards achieving the aims of this study is

discouraged by the Ethical clearance obtained for the study. Losartan produced significant ($p < 0.05$) and sustained decreases in the BP when compared to the baseline values with little or no effect on the heart rate (figures 4.18 and 4.19).

The observed increases in BP, after the pre-treatment of animals with losartan (figures 4.20 and 4.21), were significantly ($p < 0.05$) less than those previously observed in the absence of losartan (figures 4.14 and 4.15). The lack of any significant change in HR after pre-treatment with losartan was similar to the results previously obtained in the absence of losartan in the same study; suggesting that baroreflex was not restored even though the BP effect of ang II was attenuated (Averill & Diz, 2000; Veerasingham & Raizada, 2003).

In the experiment to compare the effect of the MLE (60 mg/kg) with that of losartan during continuous infusion of ang II, the reductions in the SBP, MAP and HR observed with the infusion of the MLE were more significant ($p < 0.05$) (figure 4.22 a, and 4.23) when compared to the corresponding values obtained with losartan (30 mg/kg) (figure 4.22 b., and 4.23). The equally significant ($p < 0.05$) reductions in BP values obtained with the standard drug lasted much longer, when compared to that obtained with *T. violacea* (which was momentary). *T. violacea* was therefore, infused before losartan in each animal to allow for comparison of BP and HR effect in same animals. This result furthermore suggests that the MLE may not act via the AT_1 receptors, since the BP profile and magnitude seen in the presence of ang II (figures 4.22 and 4.23) were similar to those seen in absence of the agonist (ang II) (figure 4.4 and 4.5).

5. DOES *T. VIOLACEA* ACT BY BLOCKING THE ALPHA I ADRENOCEPTORS?

Phenelyephrine is a potent selective α_1 adrenergic receptor agonist in arterioles and venules. The α_1 adrenergic receptors mediate the vasoconstrictory actions of catecholamines, especially noradrenaline, thereby, increasing BP when stimulated which is accompanied by a baroreflex mediated bradycardia (Katzung, 2004). The infusion of phenylephrine (0.01 – 0.16 mg/kg) produced dose-dependent increases in the mean values of the SBP, DBP and MAP, which were associated with dose-dependent reductions in HR (figures 4.24 and 4.25); i.e., the normal baroreflex response to increasing BP (Fazio *et al.*, 2001; Fletcher *et al.*, 2002; Veerasingham & Raizada, 2003; Ward & Abdel-Rahman, 2006).

The mean values of the SBP, DBP, MAP and HR attained when phenylephrine (0.01 – 0.16 mg/kg) was co-infused with *T. violacea* (60 mg/kg) (figures 4.26 and 4.27), were similar to the values obtained with the infusion of the standard drug alone (figures 4.24 and 4.25). The results obtained from this protocol suggest that *T. violacea* may not have any appreciable effect on the α_1 adrenergic receptors. Although the infusion of phenylephrine alone produced significant ($p < 0.05$) reduction in HR when compared to the values obtained with the vehicle alone, the co-infusion of phenylephrine with *T. violacea* did not produce significant reduction in HR, when compared to the values obtained with the vehicle alone (figure 4.25 d). The effect on HR obtained with the co-infusion of the MLE with phenylephrine is similar to that observed with the co-infusion of ang II with the MLE (figure 4.17 d), and suggests that the crude MLE contains some substances that may blunt the normal baroreflex response to phenyelphrine.

The experiment involving the co-infusion of phenylephrine was repeated, in rats pre-treated with prazosin, to investigate if prazosin (a α_1 adrenergic receptor antagonist) will give answers to questions that were not answered above. The dose of prazosin (1 mg/kg) used in this study, has been previously used by other researchers (Nagai *et al.*, 2003; Dabire, 2004; Antunes *et al.*, 2006; Wang *et al.*, 2006; Braga *et al.*, 2008; PintÉRovÁ *et al.*, 2009; Wong *et al.*, 2011) to block the α_1 adrenergic receptors. Its half-life of approximately 3 hours (Biaggioni & Robertson, 2011) made it impossible to carry out a DRE. Prazosin (1 mg/kg) lead to prolonged statisitical significant ($p < 0.05$) reduction in SBP, DBP, MAP and HR in the SHR (figures 4.28 and 4.29).

Apart from the significantly ($p < 0.05$) reduced maximum BP responses observed with both the infusion of phenylephrine alone as well as the co-infusion of phenylephrine with the MLE (figure 4.30) when compared to those seen in the absence of prazosin (figure 4.28 and 4.29), the pre-treatment of animals with prazosin in this study did not lead to any different pattern in the BP and/or HR effect observed with the standard drug alone, or when co-infused with the MLE (figures 30 and 31). This may further suggest that the α_1 adrenergic receptors are not involved in the anti-hypertensive effect of the MLE. On HR, the co-infusion of the MLE with the standard drug produced reductions in the HR, which was significant ($p < 0.05$) at the dose of 0.08 mg/kg when compared to the infusion of the standard drug alone, in the protocol that involved the pre-treatment of animals with prazosin (figure 4.31d), as opposed to the lack of any change in HR in the protocol that did not involve the pre-treatment of the animals with prazosin (figure 4.22d). This may suggest that the presence of prazosin might have blocked the constituents of the MLE which might have blunted the baroreflex in figure 4.22d.

6. DOES *T. VIOLACEA* ACT BY BLOCKING THE BETA I ADRENOCEPTORS?

Although, dobutamine (0.2 – 10.0 µg/kg) led to dose-dependent increases in HR, which were significant ($p < 0.05$) at all doses greater than the two lowest doses, its effect on SBP, DBP, PP and MAP were not necessarily dose-dependent even though significant ($p < 0.05$) increases were observed at the highest doses with these parameters (figures 4.32 and 4.33). The result obtained in this DRE, agrees to a large extent with what is in literature, wherein dobutamine has been reported to have positive inotropic and chronotropic effects on the heart, with its effect on BP being variable (Romson *et al.*, 1999; Rang *et al.*, 2000; Brunton *et al.*, 2007; Fang & Rocco, 2011; Maclaren *et al.*, 2011). Dobutamine is a racemic mixture, having equal amounts of left- and right- enantiomers of a chiral molecule that stimulates β_1 and β_2 adrenergic receptors. The (–) enantiomer is an agonist for α adrenergic receptors, while the (+) enantiomer is a very weak partial agonist. In humans, the cardiac effects of dobutamine predominates, as the vasoconstrictory effects that might have been mediated by its action on α adrenergic receptors are neutralized by its vasodilatory actions via the β_2 adrenergic receptors. At low doses, the inotropic effect is greater than the chronotropic effect, and is associated with modest decrease in peripheral vascular resistance. In animals, administration of dobutamine at rates lower than 20 µg/kg/minute, has been previously reported to produce increased inotropism, modest chronotropism, and virtually no change in BP (Fang & Rocco, 2011; Maclaren *et al.*, 2011). The rate of infusion of 2.3 mg/kg/hr, used in this study was greater than 20 µg/kg/minute, and may explain the pronounced chronotropism associated with the modest inotropism (figure 4. 33). Although both subtypes of the β receptor present in the heart are excitatory and their stimulation leads to an increase in cardiac output, the β_1 receptors are the primary adrenergic receptors in the heart (Freeman *et al.*, 2006; Cruickshank, 2007; McCorry, 2007).

The typical BP profile previously seen in this study for the MLE (figure 4.4) was also seen with the co-infusion of the MLE with the standard drug (figure 4.34). Since, the MLE was used as an antagonist in the present study, it was the difference between the resultant BP and HR effect observed when co-infusing dobutamine (the standard drug) with the MLE, and the resultant BP and HR effect of the standard drug alone that was used as a yardstick of how potent the effect of the MLE was on the particular mechanism under review. Co-infusing dobutamine (0.2 – 10.0 µg/kg) with the MLE produced significant ($p < 0.05$) reductions in the HR values when compared to the values obtained with the infusion of the standard drug alone (figure 4.35 d). The co-infusion of dobutamine (0.2 – 10.0 µg/kg) with *T. violacea* (60 mg/kg) led to further increases in BP when compared to the values obtained with the infusion of the dobutamine alone. These increases were observed to be statistically significant ($p < 0.05$) at most doses with respect to the DBP, but only at a dose with respect to the SBP, and at two doses with respect to the MAP (figure 4.35). Consequently, there were reductions in the PP at virtually all but one dose, which were however, not statistically significant (results not shown here). The results obtained suggest that the MLE has significant effect on both the HR and cardiac contractility (based on the reduction in the PP seen when dobutamine was co-infused with *T. violacea*), when compared to that observed when dobutamine was infused alone. This result strongly suggests that the MLE has an inhibitory action on the β_1 adrenoceptor, which may partly explain the previously observed bradycardia produced by the infusion of the MLE alone in the SHR (figure 4.5 d), and may explain the resultant hypotension (figures 4.4 and 4.5). The product of the TPR and the CO gives one the value of the arterial BP (Levine, 1997; Levick, 2003). The DBP represents the lowest value to which BP falls in the arteries before the next systolic ejection, and is influenced by the HR and the total peripheral resistance (TPR, which is the combined resistance to blood flow in the arterioles). The faster heart beats, the less likely the heart muscle are able to sufficiently relax

prior to the next systole, therefore, the DBP will rise (Bell, 2008). There is no previous report on the effect of *T. violacea* on DBP (figures 4.4 and 4.5 b). The further increase in DBP observed when dobutamine was co-infused with the MLE as compared to when dobutamine was infused alone (figure 4.34 b) throws up an interesting question. The answer may be that the reduction in DBP which the infusion of the MLE alone produces (figure 4.5 b), might not only have been obliterated by the presence of dobutamine, but that some other constituents in the crude extract itself is responsible for the further rise in the DBP observed.

In a related experiment, propranolol was used as a positive control used to compare the effect of the MLE on the effect of β_1 adrenoceptors. Propranolol is a non selective β adrenoceptor blocker, with actions on both the β_1 and β_2 receptors (Dimo *et al.*, 2003; Lin *et al.*, 2007; Weir, 2009). In a dose-dependent manner, propranolol (0.1 – 3.2 mg/kg) reduced the SBP, DBP, MAP and HR; but had no effect on the PP (figures 4.36 and 4.37).

During continuous infusion of dobutamine, *T. violacea* (60 mg/kg) significantly ($p < 0.05$) reduced the SBP, DBP and MAP, but did not produce a significant reduction in HR. Propranolol which was infused at the dose which gave 80% of the maximum reduction in BP in the DRE (1.6 mg/kg) (figure 4.37) on the other hand; significantly ($p < 0.05$) reduced the SBP, DBP, MAP and HR. Neither propranolol nor *T. violacea* had a significant effect on the PP (figures 4.38 and 4.39). This result furthermore buttresses the argument that the MLE may act through the β_1 adrenoceptors in the SHR. Agents that inhibit these receptor are very useful for lowering BP in mild to moderate HTN, since their stimulation leads to increased in HR, CO, and ultimately BP (Katzung, 2004; Cheng, 2009; Weir, 2009).

7. DOES *T. VIOLACEA* ACT BY STIMULATING THE MUSCARINIC RECEPTORS?

Muscarine chloride was used as a positive control for the possible effect of the MLE on the muscarinic receptors. Muscarine (0.2 – 10.0 µg/kg) significantly ($p < 0.05$) reduced the SBP, DBP, MAP in a dose dependent manner when compared to values at baseline. These reductions in BP were only associated with reduction in HR at the two highest doses (figures 4.40 and 4.41).

In literature, muscarine (a parasympathomimetic agent) has been reported be have activity similar to that of acetyl choline, although being more potent. Previous reports in literature alludes to the fact that muscarine reduces BP, while atropine (a parasympatholytic agent) has been reported in literature to have various effects on BP; from increases (Bartholow, 1908; Fraser, 1957; Wess *et al.*, 1987), to no effect in WKY rats and SHR (Lazartigues *et al.*, 1999; Mestivier *et al.*, 2001), and to immediate and dose-dependent reductions, at the doses of 5-50 mg/kg given intravenously in conscious unanaesthetized WKY rats and hypertensive rats (SHR, and Sprague-Dawley rats made hypertensive by subcutaneous implantation of deoxycorticosterone acetate) (Abraham *et al.*, 1981a, 1981b; Cantor *et al.*, 1983). Furthermore, Abraham and co-workers (1981a) observed that i) the hypertensive rats showed greater responsiveness to the hypotensive action of atropine, even though the plasma concentration of atropine was similar in all groups of rats, ii) the pre-treatment of animals with phentolamine, a non-selective α – adrenergic antagonist, abolished the hypotensive action of atropine, iii) atropine blocked the pressor effect of phenylephrine in all the animals, and the dose-response curve was shifted to the right to a similar degree in all animals (both

normotensive and hypertensive), iv) the reduction in BP were associated with increases in HR.

In the present study, the infusion of atropine doses lower than 0.02 mg/kg did not produce any significant change in BP or HR, as opposed to the increases in BP and HR mentioned in some literature (Bartholow, 1908; Fraser, 1957; Wess *et al.*, 1987). In the study conducted by Lazartigues *et al.* (1999), increases in HR were observed in the WKY, but not in the SHR. Meanwhile, Muntzel *et al.* (1997) observed that atropine (200 µg/kg) increased HR, but did not affect BP in SHR. In literature, the net cardiovascular effects of atropine in patients with normal hemodynamics have been reported to be mild: increases in HR may occur, but there is little effect on BP; but the cardiovascular effects of exogenous direct-acting muscarinic agonists are easily prevented (Brown & Taylor, 2011; Pappano, 2011). Therefore, the results obtained in the present study; i.e., dose-dependent significant reductions in BP observed with the infusion of atropine (0.02 - 20.48 mg/kg) which were associated with reductions in HR at highest doses when compared with the respective values at baseline (figures 4.42 and 4.43); corroborates the results obtained by Abraham *et al*., (1981a; 1981b) and Cantor *et al*., (1983) who reported reduction in BP with atropine infusion, but disagrees with Abraham *et al*., (1981a) with respect to the effect on HR. The lack of tachycardia, in this study, with the infusion of atropine in the SHRs used in this study has been previously suggested to be possibly due to a depressed parasympathetic nervous activity in hypertensives (van Zwieten *et al.*, 1995), although some other investigators (Abraham *et al.*, 1981a; Lazartigues *et al.*, 1999) opined that it may be due to a lack of vagal tone in free moving conscious animals as opposed to anaesthetized animals.

In the experiments to observe the effect of simultaneously co-infusing atropine (5.12 mg/kg) with *T. violacea* (30, mg/kg, 60 and 120 mg/kg) (figure 4.5), the results obtained did not show the usual BP and/or HR profile seen with the infusion of *T. violacea* at these doses. The prominent BP profile and magnitude was that of atropine (figure 4.44 a). Infusion of agonists was done 20 minutes after infusing atropine in most studies in which atropine was used to block the muscarinic receptors (Muntzel *et al.*, 1997; Dimo *et al.*, 2003; Delphine Holopherne *et al.*, 2008; Khwanchuea *et al.*, 2008; Lessa *et al.*, 2008; Liu *et al.*, 2008; Rattmann *et al.*, 2008; Shirasaka *et al.*, 2009; De Menezes *et al.*, 2010).

Consequently, this protocol was repeated, but this time with the agonist infused at least 20 minutes after pre-treating the animals with atropine. The BP of the animals returned towards baseline, but the HR stayed down after treatment with atropine (5.12 mg/kg) (results not shown here). The infusion of the MLE (30 mg, 60 mg and 120 mg/kg), led to significant ($p < 0.05$) dose-dependent increases in BP (i.e., the SBP, DBP, PP and MAP) of the rats (figures 4.44 b, 4.44 c and 4.45), as opposed to the dose dependent reduction previously reported with the infusion of the MLE alone (5 – 150 mg/kg) (figures 4.4 and 4.5) suggesting that it takes atropine some time to adequately block the muscarinic receptors, which must have been the reason why previous researchers allowed some time before the infusion of agonists after infusing atropine. The only significant ($p < 0.05$) change in HR was the 3.4 ± 1.7% increase observed with the infusion of the dose of 120 mg/kg (figure 4.45). This result strongly suggests that the MLE indeed acts through the muscarinic receptors.

After the pre-treatment of the animals with atropine, the effect of muscarine (2.5 µg/kg) on BP and HR were nullified (figures 4.46 and 4.47). However, the infusion of *T. violacea* (60

mg/kg) produced significant ($p < 0.05$) increases in SBP and MAP respectively (figure 4.44 b and 4.47). Consequently, the increases in SBP and MAP observed with the infusion of *T. violacea* were significantly ($p < 0.05$) higher than those observed with muscarine after pre-treatment of animals with atropine. The HR reducing effect previously observed with the infusion of the MLE prior to the treatment of the same animals with atropine was abolished after atropine pre-treatment (figure 4.47). It can be inferred from this experiment, that both muscarine and *T. violacea* produce bradycardia, and the consequent reduction in BP by actions on the muscarinic receptors in the heart. The increase in BP observed with the infusion of the MLE after atropine pre-treatment suggests that the crude extract may also contain pressor agents, whose actions may become prominent when one of the mechanisms via which the MLE brings about its hypotensive effect is blocked.

8. EFFECT OF CHRONIC (21 DAYS) INTRAPERITONEAL ADMINISTRATION OF THE VEHICLE (DMSO + NS), *T. VIOLACEA* OR CAPTOPRIL ON THE BODY WEIGHT, BLOOD PRESSURE AND PLASMA ALDOSTERONE LEVEL

8.1. Body weight

The administration of *T. violacea* (60 mg/kg/day), captopril (10 mg/kg/day) or the vehicle (DMSO + NS) intraperitoneally for 21 days, did not produce any statistically significant change in the mean body weight of any of the groups of SHR (figure 4.48).

8.2. Blood pressure and heart rate

8.2.1. Non-invasive blood pressure and heart rate measurement

The mean values of the SBP obtained in all the groups of animals used, were significantly ($p < 0.05$) lower after seven days of treatment, when compared to the values obtained on the first day of the study; despite a prior 14 day period of acclimatizing the animals to the setup used for the tail-cuff measurement. Furthermore, statistically significant ($p < 0.05$) reductions in SBP were observed in the control, *T. violacea* and captopril groups respectively on day 15 when compared to the values obtained on day 8 of the study. The observed reductions were greatest in the captopril group, and least in the control group of animals. On the final day of the experiment (day 22), the BP and HR were assessed in anaesthetized animals (figure 4.49). The observed reductions in BP may be partly due to the animals having more time to become better acclimatized to the glass restrainer used to limit their movement during tail-cuff measurements, as the days passed by. The intra-peritoneal treatment of animals did not produce statistically significant changes in HR after the first week of treatment in any of the groups. However, significant ($p < 0.05$) reductions were observed in the control and *T. violacea* groups respectively on the 15th day when compared to the mean values observed on the 1st day of study (figure 4.93). The reductions in HR in the *T. violacea* - treated and - captopril treated rats may also be partly due the animals becoming better accustomed to the small space, being more relaxed, and consequently, reducing sympathetic nervous system activation, which normally would increase HR and BP, as part of the 'fight', 'fright' or 'flight' response (Kurtz *et al.*, 2005; Freeman *et al.*, 2006; Fox *et al.*, 2007; Hainsworth *et al.*, 2007; McCorry, 2007; Reil & Bohm, 2007; Craven, 2008).

In a review by Kurtz *et al.* (2005), the authors were of the opinion that the indirect methods used in experimental animals share some of the advantages of their use in humans. These includes the fact that they do not require surgery, can be used to obtain repeated measurements of SBP in conscious animals during studies of short or long duration, require less expensive equipment than some direct methods (e.g., telemetry) and can also be less expensive to operate, can be used to screen for systolic HTN or substantial differences in SBP among large numbers of animals. They also share the same disadvantages of indirect office methods of BP measurement widely used in clinical and epidemiological studies that form the scientific basis for current clinical practices in HTN. The disadvantages includes the fact that they only measure BP in a very small sample of cardiac cycles, they impose significant thermal and restraint stress that disturbs multiple aspects of the cardiovascular system such as BP, HR and stress hormones, despite the fact that they are non-invasive and investigators try their best to train and acclimatize animals to undergo the procedures. Furthermore, the accuracy of these methods in animals, particularly the tail-cuff methods, is open to question despite the presence of several published studies purporting to validate cuff methods since most of the studies were based on faulty analytic techniques involving correlations between cuff measurements of BP and direct measurements of BP simultaneously or subsequently obtained with arterial catheters in the same animal; instead of using more appropriate techniques such as agreement analysis. Finally, these methods are not well suited to measuring diastolic pressure.

8.2.2. Invasive measurement blood pressure and heart rate measurement

Significant ($p < 0.05$) reductions in the mean values of the SBP, DBP and MAP were observed in the captopril (10 mg/kg) treated group, but was not observed in the *T. violacea*

(60 mg/kg) treated group, when compared to the values obtained in the control group. The starting SBP (obtained via the tail-cuff transducer) was not statistically different between the groups. On HR, there was no statistically significant difference in HR between the groups at the end of the experimental period (figure 4.50). This suggests that chronic, 21 - day administration of *T. violacea* (60 mg/kg/day) may not reduce BP or HR in the SHR. This is contrary to the previous work of Mackraj *et al.* (2008) in DSS rats. The results may also buttress the observation that the BP and HR effect of the MLE of *T. violacea* in the acute experiments performed in this study were momentary. On the other hand, chronic treatment of animals with captopril produced significant reduction in BP, but not in HR. This observation is in line with reports in literature (Lechleitner *et al.*, 1990; Smith & Vane, 2003; Hu *et al.*, 2007; Havranek, 2008; Mackraj *et al.*, 2008; Zicha *et al.*, 2008; Jovanovic *et al.*, 2009; Montenegro *et al.*, 2009; Shin *et al.*, 2009).

8.3. Plasma aldosterone levels

The mean concentration of aldosterone found in the serum of the animals treated with *T. violacea* (60 mg/kg) and captopril (10 mg/kg) after 21 days were significantly ($p < 0.05$) lower, when compared to the mean concentration of aldosterone observed in the control group (which were treated with a mixture of DMSO and NS). There was no statistically significant difference between the mean concentration of aldosterone observed in the *T. violacea* treated group and that observed in the captopril treated group (figure 4.51). The results obtained in the protocol are in agreement with the previous work of Mackraj *et al.* (2008) who observed that *T. violacea* (50 mg/kg/day) decreased aldosterone secretion in DSS rats. Aldosterone regulates electrolyte, fluid balance and BP homoeostasis (Connell & Davies, 2005). It also mediates maladaptive tissue remodelling throughout the CVS and

central nervous system (CNS), thereby, worsening the HTN (Nistala *et al.*, 2009; Zannad *et al.*, 2009; Aoki *et al.*, 2010; Briet & Schiffrin, 2010; De-An *et al.*, 2010; Whaley-Connell *et al.*, 2010; Lymperopoulos *et al.*, 2011). Therefore, a reduction in its plasma levels is advantageous to the animal.

9. LIMITATION OF THE STUDY

The scope of this study was limited due to lack of funds and access to some equipment which would have enabled the researcher to (a) investigate more mechanisms via which the MLE may mediate its anti-hypertensive effect, such as the calcium channels, the alpha 2 adrenergic receptors and the possible effect of the MLE on diuresis; (b) isolate and identify the different constituents of the crude extract and possibly link each constituent to its own individual BP and/or HR effect; and (c) quantify the amount of the different constituent in the extract, and the amount in plasma after administration into animals.

10. CONCLUSION

At the end of the study, all the objectives of the study were achieved. The crude methanol leaf extract of *Tulbaghia violacea* was observed to reduce BP and HR in the male SHR. The BP and HR reducing effect of the MLE may involve a) the inhibition of the ACE, b) the inhibition of the β_1 adrenoceptors, c) the stimulation of the muscarinic receptors and d) a reduction in the level of aldosterone in plasma. The results also suggest that the MLE may not act through the angiotensin II receptors or the α_1 adrenergic receptors.

11. RECOMMENDATIONS

Tulbaghia violacea may be useful as an antihypertensive agent in humans, although further studies are required to ascertain its safety and optimal dose for such an application. Such studies would include, in addition to those mentioned under the limitations, isolating and removing constituents that may negate the potency of the anti-hypertensive constituent(s). It will also be crucial to investigate the effect of temperature on the active constituent(s), as well as the first pass metabolism, the bioavailability when taken orally, the rate of metabolism in the body, the distribution, the half life, toxicity and also the route(s) of elimination from the body of its constituents. Finally, an assessment into its interaction with other herbs, drugs or food in both experimental animals and in clinical trials would be required.

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