

***In vivo* effects of *Crinum macowanii* on the Rat Cardiovascular System**

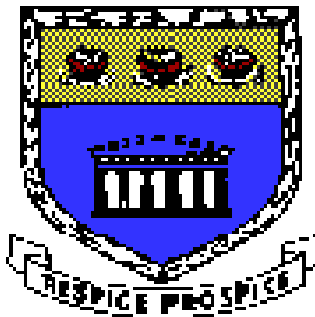
Kirstin Gracia Persson

A thesis submitted in partial fulfillment of the requirements for the degree of Magister Scientiae in the Department of Medical Bioscience, University of the Western Cape.

May 2007

UNIVERSITY of the
WESTERN CAPE

Supervisor: A.P. Burger, Co-Supervisors: Prof P. Mugabo & Prof I. Green



***In vivo* effects of *Crinum macowanii* on the Rat Cardiovascular System**

Keywords

In vivo

Crinum macowanii

Aqueous extract

Pre-treatment

Rats

Blood pressure

Heart rate

Adrenaline

Atropine

Atenolol

Prazosin

Verapamil

Reserpine



Abstract:

Crinum macowanii (*C. macowanii*) (Amaryllidaceae) as authenticated by Mr. F. Weitz at the Herbarium, University of the Western Cape, is widely used as a traditional remedy and is thought to have therapeutic value (Fennell and van Staden 2001). The objective of this study was to determine the cardiovascular effects of the crude aqueous extract of *Crinum macowanii* on the rat and to determine the effect of pre-treatment drugs on *Crinum macowanii* effects in *in vivo*, anaesthetized normotensive, male Wistar rats (200-250 g). Rats were pre-treated (i.v.) with 80% of the concentration giving the maximal effect of pre-treatment drugs *in vivo* prior to *Crinum macowanii* infusions. *Crinum macowanii* produced a significant dose dependent increase in heart rate ($P = 0,0011$) and highly significant ($P < 0,0001$) increases in systolic and diastolic pressures at all doses. The effect of the aqueous extract of *C. macowanii* on all parameters assessed was similar ($P > 0,01$) with pre-treatment of atropine (1,2 mg/kg), atenolol (6,0 mg/kg), prazosin (400 mg/kg), and reserpine (0,6 mg/kg). In rats pre-treated with verapamil (4,8 mg/kg) the effect of *C. macowanii* on the heart rate was similar ($P > 0,01$), but its effects on the systolic and diastolic blood pressure were significantly ($P < 0,01$) decreased. The results showed that the mechanism of action of *Crinum macowanii* may involve calcium channels (verapamil is a Ca^{+} channel blocker). Further studies are needed to fully elucidate the mechanism of action of *Crinum macowanii*.

Key words:

Crinum macowanii; *in vivo*; Pre-treatment; atropine; atenolol; prazosin; reserpine; verapamil; anaesthetized normotensive rats; blood pressure; heart rate

References

Fennell, C.W. and van Staden, J. 2001. *Crinum* Species in traditional and modern medicine. *Journal of Ethnopharmacology*, 78:15-26.

DECLARATION

I declare that “*In vivo* effects of *Crinum macowanii* on the Rat Cardiovascular System” 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: Persson, Kirstin G.

Signed: _____

Date: _____



DEDICATION

Thanks be to the Lord who allowed me the room to grow and learn as He remained faithful to me throughout this whole process. Without Him I can do nothing. Thanks also to the people closest to me including my family and friends who believed in me and spoke truth into my life when I needed to hear it the most.



ACKNOWLEDGEMENTS

I am grateful to Mr. A. Burger, Professor P. Mugabo, and Professor I. Green for their supervision, instruction and useful insights. Thanks to Professor Madsen for his meticulous and flawless work on statistical results. Thanks also to Mr. Y. Alexander for his technical support.



CONTENTS

Title Page	i
Keywords	ii
Abstract	iii
Declaration	iv
Dedication	v
Acknowledgements	vi
Contents	vii
List of tables and figures	xii

Chapter 1

Introduction and Literature Review

1.1 Introduction	1
1.2 Literature Review	4
1.2.1 Traditional medicinal uses of plant life	4
1.2.2 Prevalence of use of plant species in Africa	6
1.3 Crinum species	7
1.3.1 Phytochemical analysis of Crinum species	7
1.3.2 Medical uses of Crinum species	8
	vii

1.4 <i>Crinum macowanii</i>	9
1.4.1 General description of <i>Crinum macowanii</i>	9
1.4.2 Alkaloids and medicinal uses of <i>Crinum macowanii</i>	10
1.5 Aims and objectives	10
1.6 Hypothesis	11

Chapter 2

The Cardiovascular System

2.1 General anatomy and physiology	12
2.2 Electrical conducting system of heart	14
2.3 Blood flow through heart	16
2.3.1 Systemic blood flow through heart	16
2.3.2 Pulmonary blood flow through heart	16
2.3.3 Pulmonary and systemic circulation of blood	17
2.4 Blood supply to heart	17
2.4.1 Arterial blood supply	17
2.4.2 Cardiac output	19
2.4.3 Stroke volume	20
2.4.4 Heart rate	21
2.4.5 Peripheral resistance	22
2.4.6 Venous blood supply	23
2.4.7 Valves of the heart	24
2.4.8 Reactions of valves due to pressure	24
2.4.9 Mean arterial pressure	26
2.4.10 Nervous regulation of cardiac function	26

Chapter 3

Materials and Methods

3.1 Raw plant material and extraction process	38
3.1.1 Plant collection	38

3.1.2 Aqueous extraction	38
3.2 Animals	38
3.2.1 Collection	38
3.2.2 Preparation of <i>in vivo</i> system	39
3.3 <i>In vivo</i> parameters assessed	41
3.4 <i>In vivo</i> equipment	41
3.5 Chemicals used for extraction	42
3.6 <i>In vivo</i> testing drugs	42
3.6.1 Control	42
3.6.2 Testing drugs	42
3.7 Data analysis	43
3.8 Protocol	45
3.8.1 Model for drug dosing (Protocol 1 and Protocol 2)	45
3.9 Ethical considerations	47
3.9.1 Animal rights	47
3.9.2 Humaneness	48
3.9.3 Significance	48
3.9.4 Personal obligation	48



Chapter 4

Results and Discussion

4.1 Individual dose response curves	49
4.1.1 Effect of adrenaline	52
4.1.1.1 Effect on heart rate	52
4.1.1.2 Effect on systolic blood pressure	53
4.1.1.3 Effect on diastolic blood pressure	54
4.1.2 Effect of <i>Crinum macowanii</i>	55
4.1.2.1 Effect on heart rate	55
4.1.2.2 Effect on systolic blood pressure	57
4.1.2.3 Effect on diastolic blood pressure	58
4.1.3 Effect of atropine	59

4.1.3.1 Effect on heart rate	59
4.1.3.2 Effect on systolic blood pressure	60
4.1.3.3 Effect on diastolic blood pressure	61
4.1.4 Effect of atenolol	62
4.1.4.1 Effect on heart rate	62
4.1.4.2 Effect on systolic blood pressure	63
4.1.4.3 Effect on diastolic blood pressure	64
4.1.5 Effect of prazosin	65
4.1.5.1 Effect on heart rate	65
4.1.5.2 Effect on systolic blood pressure	66
4.1.5.3 Effect on diastolic blood pressure	67
4.1.6 Effect of verapamil	68
4.1.6.1 Effect on heart rate	68
4.1.6.2 Effect on systolic blood pressure	69
4.1.6.3 Effect on diastolic blood pressure	70
4.1.7 Effect of reserpine	72
4.1.7.1 Effect on heart rate	72
4.1.7.2 Effect on systolic blood pressure	73
4.1.7.3 Effect on diastolic blood pressure	74
4.2 Effect of <i>Crinum macowanii</i> combined with control drugs	75
4.2.1 Effect of <i>Crinum macowanii</i> when pre-treated with atropine	75
4.2.1.1 Effect on heart rate	75
4.2.1.2 Effect on systolic blood pressure	76
4.2.1.3 Effect on diastolic blood pressure	77
4.2.2 Effect of <i>Crinum macowanii</i> when pre-treated with atenolol	79
4.2.2.1 Effect on heart rate	79
4.2.2.2 Effect on systolic blood pressure	80
4.2.2.3 Effect on diastolic blood pressure	81
4.2.3 Effect of <i>Crinum macowanii</i> when pre-treated with prazosin	83
4.2.3.1 Effect on heart rate	83
4.2.3.2 Effect on systolic blood pressure	84

4.2.3.3 Effect on diastolic blood pressure	85
4.2.4 Effect of <i>Crinum macowanii</i> when pre-treated with verapamil	87
4.2.4.1 Effect on heart rate	87
4.2.4.2 Effect on systolic blood pressure	88
4.2.4.3 Effect on diastolic blood pressure	89
4.2.5 Effect of <i>Crinum macowanii</i> when pre-treated with reserpine	91
4.2.5.1 Effect on heart rate	91
4.2.5.2 Effect on systolic blood pressure	92
4.2.5.3 Effect on diastolic blood pressure	93
4.2.6 Effect of <i>Crinum macowanii</i> when pre-treated with adrenaline	95
4.3 Discussion	95
4.3.1. Adrenaline	95
4.3.2. <i>Crinum macowanii</i>	96
4.3.3. Atropine	96
4.3.4. Atenolol	97
4.3.5 Prazosin	98
4.3.6 Verapamil	98
4.3.7 Reserpine	100
Chapter 5	
5.1 Summary and Conclusions	102
5.2 Recommendations	104
References	105



LIST OF TABLES AND FIGURES

Figure 1.1: <i>Crinum macowanii</i>	9
Figure 2.1: Cross section of the mammalian heart	13
Figure 3.1: Male Wistar Rats	39
Figure 3.2: <i>In vivo</i> anaesthetized normotensive rat model	41
Table 3.1: Protocol for individual dose response curves	46
Table 3.2: Protocol for <i>Crinum macowanii</i> dose response curves with pre-treatment	46
Table 4.1: Dose response curve findings for pre-treatment drugs	51
Figure 4.1: Effect of adrenaline on HR (dose response curve)	52
Figure 4.2: Effect of adrenaline on HR (percentage change graph)	53
Figure 4.3: Effect of adrenaline on SBP (dose response curve)	53
Figure 4.4: Effect of adrenaline on SBP (percentage change graph)	54
Figure 4.5: Effect of adrenaline on DBP (dose response curve)	55
Figure 4.6: Effect of adrenaline on DBP (percentage change graph)	55
Figure 4.7: Effect of <i>Crinum macowanii</i> on HR (dose response curve)	56
Figure 4.8: Effect of <i>Crinum macowanii</i> on HR (percentage change graph)	56
Figure 4.9: Effect of <i>Crinum macowanii</i> on SBP (dose response curve)	57
Figure 4.10: Effect of <i>Crinum macowanii</i> on SBP (percentage change graph)	57
Figure 4.11: Effect of <i>Crinum macowanii</i> on DBP (dose response curve)	58
Figure 4.12: Effect of <i>Crinum macowanii</i> on DBP (percentage change graph)	58
Figure 4.13: Effect of atropine on HR (dose response curve)	59
Figure 4.14: Effect of atropine on HR (percentage change graph)	59
Figure 4.15: Effect of atropine on SBP (dose response curve)	60
Figure 4.16: Effect of atropine on SBP (percentage change graph)	60
Figure 4.17: Effect of atropine on DBP (dose response curve)	61
Figure 4.18: Effect of atropine on DBP (percentage change graph)	61
Figure 4.19: Effect of atenolol on HR (dose response curve)	62
Figure 4.20: Effect of atenolol on HR (percentage change graph)	63
Figure 4.21: Effect of atenolol on SBP (dose response curve)	63
Figure 4.22: Effect of atenolol on SBP (percentage change graph)	64

Figure 4.23: Effect of atenolol on DBP (dose response curve)	64
Figure 4.24: Effect of atenolol on DBP (percentage change graph)	65
Figure 4.25: Effect of prazosin on HR (dose response curve)	65
Figure 4.26: Effect of prazosin on HR (percentage change graph)	66
Figure 4.27: Effect of prazosin on SBP (dose response curve)	66
Figure 4.28: Effect of prazosin on SBP (percentage change graph)	67
Figure 4.29: Effect of prazosin on DBP (dose response curve)	67
Figure 4.30: Effect of prazosin on DBP (percentage change graph)	68
Figure 4.31: Effect of verapamil on HR (dose response curve)	69
Figure 4.32: Effect of verapamil on HR (percentage change graph)	69
Figure 4.33: Effect of verapamil on SBP (dose response curve)	70
Figure 4.34: Effect of verapamil on SBP (percentage change graph)	70
Figure 4.35: Effect of verapamil on DBP (dose response curve)	71
Figure 4.36: Effect of verapamil on DBP (percentage change graph)	71
Figure 4.37: Effect of reserpine on HR (dose response curve)	72
Figure 4.38: Effect of reserpine on HR (percentage change graph)	72
Figure 4.39: Effect of reserpine on SBP (dose response curve)	73
Figure 4.40: Effect of reserpine on SBP (percentage change graph)	73
Figure 4.41: Effect of reserpine on DBP (dose response curve)	74
Figure 4.42: Effect of reserpine on DBP (percentage change graph)	74
Figure 4.43: Effect of <i>Crinum macowanii</i> on HR when pre-treated with atropine (dose response curve)	75
Figure 4.44: Effect of <i>Crinum macowanii</i> on HR when pre-treated with atropine (percentage change graph)	76
Figure 4.45: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with atropine (dose response curve)	77
Figure 4.46: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with atropine (percentage change graph)	77
Figure 4.47: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with atropine (dose response curve)	78

Figure 4.48: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with atropine (percentage change graph)	78
Figure 4.49: Effect of <i>Crinum macowanii</i> on HR when pre-treated with atenolol (dose response curve)	79
Figure 4.50: Effect of <i>Crinum macowanii</i> on HR when pre-treated with atenolol (percentage change graph)	80
Figure 4.51: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with atenolol (dose response curve)	81
Figure 4.52: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with atenolol (percentage change graph)	81
Figure 4.53: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with atenolol (dose response curve)	82
Figure 4.54: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with atenolol (percentage change graph)	82
Figure 4.55: Effect of <i>Crinum macowanii</i> on HR when pre-treated with prazosin (dose response curve)	83
Figure 4.56: Effect of <i>Crinum macowanii</i> on HR when pre-treated with prazosin (percentage change graph)	84
Figure 4.57: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with prazosin (dose response curve)	85
Figure 4.58: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with prazosin (percentage change graph)	85
Figure 4.59: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with prazosin (dose response curve)	86
Figure 4.60: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with prazosin (percentage change graph)	86
Figure 4.61: Effect of <i>Crinum macowanii</i> on HR when pre-treated with verapamil (dose response curve)	87
Figure 4.62: Effect of <i>Crinum macowanii</i> on HR when pre-treated with verapamil (percentage change graph)	88

Figure 4.63: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with verapamil (dose response curve)	89
Figure 4.64: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with verapamil (percentage change graph)	89
Figure 4.65: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with verapamil (dose response curve)	90
Figure 4.66: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with verapamil (percentage change graph)	90
Figure 4.67: Effect of <i>Crinum macowanii</i> on HR when pre-treated with reserpine (dose response curve)	91
Figure 4.68: Effect of <i>Crinum macowanii</i> on HR when pre-treated with reserpine (percentage change graph)	92
Figure 4.69: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with reserpine (dose response curve)	93
Figure 4.70: Effect of <i>Crinum macowanii</i> on SBP when pre-treated with reserpine (percentage change graph)	93
Figure 4.71: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with reserpine (dose response curve)	94
Figure 4.72: Effect of <i>Crinum macowanii</i> on DBP when pre-treated with reserpine (percentage change graph)	94

CHAPTER 1

1.1 Introduction

At the International Conference on Primary Health at Alma Ata in 1978 the World Health Assembly called on nationals to give a high priority to traditional health systems and traditional medical drug policies (Normann, Snyman, and Cohen 1996). In recent times, there has been a major shift back to the use of traditional medicine, particularly for those nations who have so far removed themselves from the uses of herbal remedies and plant medicinals. For many nations though, traditional uses of plants and herbs have been and will always be a mainstay in their lifestyle and culture. South Africa is one of these nations. It is a nation that prides itself in bio-cultural diversity with a rich extant of herbal medical traditions that stretches back to the Paleolithic time period (Normann, Snyman and Cohen 1996).

Traditional medicine is defined as the total knowledge and practices based on indigenous belief systems (Tabuti, Dhillion and Lye 2003). This form of medicine is used in the health maintenance and prevention or complete elimination of physical and/or mental disorders (Tabuti, Dhillion and Lye 2003). The World Health Organization encourages the promotion and development of traditional medicine for vast usage because it is not only safe and economical for developing countries, but it is widely accepted by most populations because people are very accustomed to it (WHO 2002).

It is not effective enough to only utilize past knowledge of traditional medicine for the treatment and cure of certain diseases seen in modern times. It has been found that combining the knowledge of traditional medicine with conventional medicine is advantageous to the advancement of medicine (Tabuti, Dhillion and Lye 2003). In fact, traditional medical practitioners (also known as "traditional healers") if able to gain new knowledge of the discoveries made in medicine will also advance their traditional medical practices. The testing of traditional plants and their physiological effects is an efficient way to advance and bridge both

traditional and conventional medicine. As the field of traditional medicine is cultivated, safer ways of practicing traditional medicine and using plants to their full benefit will be implemented into the health system.

Most of the early written medical records were scripted at least 3500 years ago, although there are recordings of traditional plant usages in Egypt around 1600 B.C. and in the Chinese Yin Dynasty even earlier than that time period (Normann, Snyman, and Cohen 1996). Traditional Chinese medicines that have a history of antibacterial and anti-rheumatism agents have been used as therapeutic drugs some 2000 years ago (Dawei *et al* 2004).

The first people who laid the foundation for traditional medical techniques were, however, Egyptian Africans. Later on, this knowledge spread, extending to Bantu people groups within Africa and then to other nations abroad. For instance, the early Zulus were known to have used different forms of roasted plant bulbs to cure backaches (Fennell and van Staden 2001). They would bind the bulbs to varicosities by using strap-like leaves to hold the bulb in place. These were said to help soothe swollen joints and reduce the swelling of the strained area (Fennell and van Staden 2001).

The Zulu, however, were not the only Bantu people to practice these forms of medication. The Tswana would drink crushed leaves and stalks that were brewed together (regularly a cup of boiling water would be mixed with a half cup of chopped leaves and then left to stand for 5 minutes) in order to aid in continuous urine flow in the bladder. This brew was also used as a cure for kidney infections, a practice that is still in use today (Fennell and van Staden 2001).

Although medicinal practices have changed, much of the use of traditional plant life and the practices therein have remained intact. In fact, most of the African world still consults traditional healers and use holistic ways of prevention and cure for diseases (Hamilton 1993). The nature of disease has changed since the early history of medicine. Hypertension (also known as high blood pressure), a disease that had not been widely reported in the earlier days of medicine, has now

become a major disease in both the Western world and developing countries (Whelton, He and Louis 2003). Regular blood pressure is defined as a systolic blood pressure equal to or less than 120 mmHg, and a diastolic blood pressure equal to or less than 80 mmHg. People who have blood pressure readings of 130-139 mmHg for systolic and 85-89 mmHg for diastolic are considered hypertensive (Whelton, He, and Louis 2003).

In the early 1990's there were six million cases of hypertension documented in South Africa. Most of these were reported to have been in urban black communities (Seedat 1996). A second confirmation of this report quickly arose in 1996 when a Demographic and Health Survey for the prevention of hypertension in South Africa was launched. It was conducted on 13,802 randomly chosen South Africans aged 15 years and older. Using a cutoff point of 140/90 mmHg, blood pressures of people who were visited in their homes were recorded. The average prevalence of hypertension was 22.9% in men and 24.6% in women (Steyn *et al* 2001).

Although important public health initiatives have transpired in order to reduce the incidences of hypertension in many communities, the reports of hypertension have escalated in the developing world. Many believe that this is due to rapid Westernization and the adoption of many lifestyle trends therein (Seedat 1996). Hypertension is a common health problem in the world that is continuously undiagnosed. When hypertension is actually identified few are treated with the correct medication (Steyn *et al* 2001). It is of vital importance that more research is performed in this area of medicine. One of the advantages of experimentation in the area of hypertension is that there are ample plants with anti-hypertensive effects that are available for testing. The more information that is discovered in the area of traditional plant medicine, the better opportunity for prevention and cure for diseases, such as hypertension.

Chapter one of this thesis contains an introduction and literature review, which expounds upon the early and more modern uses of traditional medicines. It gives a full description of the Amaryllidaceae family and Crinum species. It also describes the Crinum *macowanii* plant and its medicinal uses. Chapter two presents the general anatomy and physiology of the heart muscle. It

then explains the electrical conducting system of the heart. The blood flow through the heart is then described including both the pulmonary and systemic circulation. The arterial and venous blood flow is the next to be explained followed by the valves of the heart. The intrinsic and extrinsic controls of the heart including the nervous control regulation are then clarified. The final part of chapter two describes the anaesthetized normotensive rat model used as an animal model for evaluating cardiovascular changes. It expresses the advantages and limitations of the *in vivo* model.

The third chapter portrays the methods and the materials used in the *in vivo* experiment. The materials used and expounded on include the drugs and the physical equipment. The methods of the *in vivo* experiments cover the preparation and cannulation of the animal. The protocol, and the parameters assessed throughout the experiments. There is then a short data analysis explanation followed by ethical considerations.

Chapter four expounds on the results and discussion of the *in vivo* experiments. These results consider changes in systolic and diastolic pressures and heart rate in the *in vivo* study. Chapter five contains general conclusions and recommendations.

1.2 Literature Review

1.2.1 Traditional medicinal uses of plant life

Traditional medicine is a dynasty of unvarying knowledge that has been passed on through generations of people. It has evolved within communities as people have worked to discover new ways of bettering the process of applying these medicines. Traditional medicine includes using natural resources such as herbs, leaves, bark, roots, bulbs and flowers for therapeutic remedies. In these cases, the resources are usually collected after they have been dried and crushed and are ready for use as a powder in a decoction or poultice (Normann, Snyman, and Cohen 1996). There are many traditional plants that are now identified and used on a regular basis in the African society. For example, eucalyptus trees that are grown in many places in Africa are

commonly used in the treatment of typhoid and enteric fever. Wild dagga, which are plants with tiers of bright colored orange flowers and pungent leaves (scientifically called “*Leonotus leonurus*”) have been used to treat hypertension, diabetes, colds, and headaches, Bitterbossie also known as “Christmas berry” is an evergreen shrub which grows on sand dunes and produces pink-colored flowers followed by “Christmas” berries. This plant is used in the treatment of arthritis, diabetes, and toothaches and as a blood purifier. Ysterhout scientifically called “*Dodonaea viscosa*” is a common widespread shrub with greenish-purple leaves and winged fruit. Ysterhout is used in the treatment of colds and inflammation (Normann, Snyman, and Cohen 1996). These plants and many others are commonly used among indigenous people on a daily basis.

Over the past twenty years interest in medicinal plants in many societies has grown due to the use of herbal products as an effective source of self-medication by the general public in all nations. In fact, Chinese traditional medicine is used in 40% of the cases at the primary health care level (Normann, Snyman, and Cohen 1996). As medicine advances all over the world there have been new and useful discoveries made from the isolation of plant life. For example, in India the plant “*Coleus forskolii*” has been discovered to be an effective treatment for glaucoma (Normann, Snyman, and Cohen 1996).

China has recently discovered a treatment for malaria in the form of derivatives of the Chinese medicinal plant “*chloroquine-resistant*” (Normann, Snyman, and Cohen 1996). These are just some of the many nations that have cultivated the use of higher plant life for effective medication. Many of these plants are not used in their original form but have been combined with other traditional drugs and changed to accommodate their specific uses.

In the U.S. it is estimated that 25% of prescription drugs come directly or indirectly from higher plant sources. 74% of these drugs were discovered by scientific investigation of traditional remedies (Normann, Snyman, and Cohen 1996). There are many pharmaceutical drugs that are in use today that have been derived from plants. Some examples of these are:

Aspirin- Derived from the plant “*Filipendula ulmaria*” and is commonly used as an anti-inflammatory and a pain reducer (Normann, Snyman, and Cohen 1996).

Codeine- Derived from the traditional plant “*papaversomniferum*” and is known to ease pains or suppress a cough (Normann, Snyman, and Cohen 1996).

Reserpine – Derived from the plant “*rauvolfia serpentina*” and is used in treatment of hypertension (Normann, Snyman, and Cohen 1996).

These are just a few examples of the thousands of drugs that have their roots in traditional medicine and are derivatives of plant life.

1.2.2 Prevalence of use of plant species in Africa

Traditional medicine is an integral part of South African cultural life and it is estimated that indigenous medicines are used by an estimated 27 million South Africans (Mander 1997). The use of indigenous and indiginised medicines is vastly practiced not only to enhance personal well being, but also to treat physical conditions. It has been reported that 700 indigenous plant species are used for medicinal purposes in South Africa alone and that 64% of the entire world population uses traditional medicine for the treatment of ailments and for general healthcare (Fennell and van Staden 2001). In Africa, these figures skyrocket to 80% because of lack of medical personnel available and the higher costs of imported pharmaceuticals. Furthermore, 75% of South Africans consult a traditional healer on a regular basis (Hamilton 1993). This is largely due to the belief system that medicines that are plant based actually hold unique supernatural powers that propagate the use of these natural medicines among the African population. In the rural areas, Africans have the ability to identify and harvest their own herbal remedies. In more urbanized settings, it is more common to purchase traditional medicines from a herbalist or "muti" shops (Veale *et al* 1992). Traditional healers continue to hold a prominent role in African society, as they are the most knowledgeable about medicinal plants and their prescription.

There are many benefits to testing plants. As medicine advances, the evaluation of herbal remedies proves that there are many unknown and possible severe side effects that could be cause for concern. It is essential to scientifically test plants if the promotion and development of

these traditional medicines are to be cultivated. It is also important to research the comprehensive phytochemical effects that the extract of a certain plant can have on health and well being. It is of vital importance to use the crude aqueous extract of traditional plants to their full medicinal potential as it encourages the path forward in cure and therapy for diseases.

1.3 Crinum species

1.3.1 Phytochemical analysis Crinum species

The Amaryllidaceae active compounds are known for their pharmacological and microbiological activities including antiviral, anti-tumoral, and anticholinergic effects (Pham *et al* 1998). The reason why Crinums are used for medicinal purposes may possibly be due to their alkaloid constituents because there are certain alkaloids that are common to a variety of Crinum species (Fennell and van Staden 2001). An analysis of the phytochemicals of this species reported more than 150 different alkaloids contained in this plant. Over 100 alkaloids of the Amaryllidaceae type have been identified in the last twenty-years (Fennell and van Staden 2001). The highest concentrations of active compounds can be located in the bulbs or in the epidermis of the outer scale of the leaves. There are also cells that are referred to as “mucilage filled raphide cells” occurring in all plants, which contain many alkaloids (Fennell and van Staden 2001).

From the time of Hippocrates, there were many crude extracts of the Amaryllidaceae family that had shown anti-tumoral and antiviral effects (Wildman 1960). Lycorine, which is the principle alkaloid found in many different species of the Amaryllidaceae family has been proven to inhibit the growth of *in vivo* tumors and actually reduce harmful cellular activity in bone marrow (Ghosal, Saini and Razdan 1985). This discovery has afforded more research of the cytotoxic effects of calprotectin, which has advanced cancer research (Ghosal, Saini, and Razdan 1985).

Lycorine has also exhibited effects on anti-viral activity because of its distinct structure containing two hydroxy groups. In this case, the antiviral activity is due to the ability to inhibit multiplication of RNA and DNA viruses (Lewis 1990). The ability of Lycorine to block protein

synthesis achieves a decrease in the amount of the virus that is transmitted to other cells. In this way it exhibits major medicinal effects on diseases such as poliomyelitis and herpes type 1 virus (Harborne and Baxter 1993).

1.3.2 Medicinal uses of *Crinum* species

In the Amaryllidaceae family, *Crinum* plants were highly regarded by botanists who used the phytochemicals for all different types of ailments. Although the knowledge of these plants was first discovered by the traditional healers the commencement of documentation began with Victorian plant hunters, early missionaries and colonial herbalists (Bryant 1966). Some of the most commonly known effects of this plant include use of it as an analgesic, a violent emetic, and a lactate exhibitor (Bryant 1966).

There are, however, many different types of *Crinum* yielding different medicinal effects. For example, *Crinum bulbispermum* is one of the favored medicinal herbs by many tribes. The southern Sotho have reported using the leaves or crushed bulbs for treating ailments such as colds and coughs by the drinking of a strong liquid that contains soluble parts of the plant roots (Roberts 1990). The bulbs also have positive external effects on wounds by drawing out infections and treating hemorrhoids. The bulbs have also been used as a treatment of rheumatism and chronic backaches by the Zulu and Tswana tribes by applying the roasted bulb to aching joints. Fennel and van Staden report the use of *Crinum moorei* in weight loss inducing therapy and to increase lactation in animals and humans (Fennell and van Staden 2001).

There is much room for growth and expansion in the discovery of new phytochemical activity in *Crinum* species because only 27 species out of the 170 different compounds (most of which are alkaloids) have been investigated (Fennell and van Staden 2001). The use of plants as sources of pharmaceutical drugs is growing. The ethnobotanical uses of the plant *Crinum* is regularly explained on the basis of chemical and physiological studies performed on it. As a result of *Crinum* being widely used as a traditional remedy, it has been thought that this plant contains

therapeutic value (Fennell and van Staden 2001).

1.4 *Crinum macowanii*

1.4.1 General description of *Crinum macowanii*

Crinum macowanii is reported to have its origin on Mt. Meru, in Tanzania. The plant is referred to as “dururu” in Shona vernacular or “umduze” among the Zulus (Nair *et al* 2000). It mainly grows in seasonally flooded grassland plains and the savannah region within deciduous woodland areas. It most frequently grows in areas with large seasonal variations in water supply and will take root in black cotton soils (Fangan and Nordal 1993). These plants that are sometimes referred to as “bush” or “march lily” produce turnicate bulbs that lie dormant at certain times of the year. The bulbs are large, typically 200 mm or more in diameter (Njagi 2004). Their flowers are large and their structure resembles the umbels of a lily (Fennell and van Staden 2001). Under field conditions, the reproduction of this plant is slow. This truth, coupled with the overuse of *Crinum macowanii* in tribal practices threatens the life of this plant.



Figure 1.1 *Crinum macowanii* (Archer and Condry 1999)

1.4.2 Alkaloids and medicinal uses of *Crinum macowanii*

There are a vast number of alkaloids that have been found in the plant *Crinum macowanii*. Alkaloids are known as naturally occurring substances that are not necessarily vital to the organism that produce them but they are an after product of plant processes. Sometimes alkaloids are also called “metabolites” (Meyer *et al* 1996). The three principle alkaloids of *Crinum macowanii* are lycorine, crinine, and powerline (Njagi 2004). There are eight other alkaloids that were previously discovered and isolated. These include: macowine, krepowine, buphanidine, crinamidine, undulatine, cheyille, 4 α - dehydroxycrinamabine, and 1-epideacetyl bowdendine. The final alkaloid lycorinine was one that was discovered very recently (Njagi 2004). *Crinum macowanii* active compounds are most frequently used medicinally for their antiviral and antifungal activities. A phytochemical analysis of these plants, like many other Amaryllidaceae that are endemic to southern Africa, proves that there is vast research still to be done in order to discover more potential medicinal uses.

1.5 Aims and objectives

The primary aim of this project is to perform a pharmacological screening of *Crinum macowanii* for the purpose of determining the effects of this plant on the cardiovascular system. The steps therein are as follows:

- 1) Determine the effects of the crude aqueous extract of *Crinum macowanii* on blood pressure and heart rate in the *in vivo* rat via independent dose response curves.
- 2) Determine by which mechanism *Crinum macowanii* causes the noted effect by pre-treating the rats with possible agonist or antagonist drugs with cardiovascular effects and creating dose response curves to view the effect of *Crinum macowanii* when pre-treated with “known” drugs.

1.6 Hypothesis

The hypothesis of this project is that *Crinum macowanii* will have cardioactive effects on the heart. The primary postulation is that this plant will have actions and activity on the cardiovascular system. It is hypothesized that the crude aqueous extract of *Crinum macowanii* may lower both the heart rate and blood pressure.



CHAPTER 2

The cardiovascular system

2.1 General anatomy and physiology

Although the heart performs a large amount of work, the human heart is roughly the size of a clenched fist (Martini, Timmons, and Tallitsch 2003). The human heart is a cone shaped organ that weighs between 250-350 g (Martini, Timmons, and Tallitsch 2003). The rat heart, compared to the human heart, is similar in structure and physiology as both rats and humans are mammals. Therefore, the rat model was chosen for this project.

The heart is located in the thorax against the anterior chest wall, in what is referred to as the “pericardial cavity”. The pericardial cavity is between the pleural cavities in the mediastinum, which contains the thymus, esophagus and the trachea (Martini, Timmons, and Tallitsch 2003). The heart is positioned in a cavity of the body that is posterior to the sternum and costal cartilages and rests on the superior surface of the diaphragm (Marieb and Mallatt 2001). The heart is positioned obliquely in the pericardial cavity, with the lower point (or apex) lying slightly to the left of the midline, and it sits at an angle in relation to the longitudinal axis of the body. There are four corners of the heart when looking at it anatomically, with the “base” as the broad posterior surface (Marieb and Mallat 2001).

The heart is enclosed by three layers of tissue. The pericardium almost completely surrounds the heart and the fibrous portion is the tougher outer layer of the heart. The inner layer is the serous portion and has contact with the surrounding lungs (Opie 1998). The serous portion includes the parietal layer, which is the inner surface of the fibrous pericardium, and the visceral layer (or epicardium), which is part of the heart wall (Marieb and Mallat 2001). The layers of the pericardium are separated by fluid, which allows the heart movement while contracting and relaxing (Opie 1998).

The heart wall, as mentioned before, also has three layers including the superficial epicardium, the middle myocardium, and the deep endocardium. The bulk of the heart wall is made of thick, contractile cardiac muscles cells. These are referred to as the myocardium or simply myocytes and are amply supplied with blood cells (Opie 1998).

Internally, there are four chambers of the heart. The two superior or atrial chambers are called “receiving chambers” because they receive blood from pulmonary and systemic circulation by way of the veins (Thibodeau and Patton 1993). The two lower chambers are called “pumping chambers” and receive blood from the atria and pump blood out of the heart to the arteries.

The heart is divided in half running longitudinally by what is referred to as the interventricular or interatrial septum. This line cuts through the top atrial chambers and the bottom ventricular chambers. The other division exists by the marking of two different grooves including the coronary sulcus, and the anterior interventricular sulcus. These both run latitudinally dividing the right and left ventricle. The posterior side of the heart is largely made of the left atrium and left ventricle, which forms the left border as well (Hall-Craggs 1995).

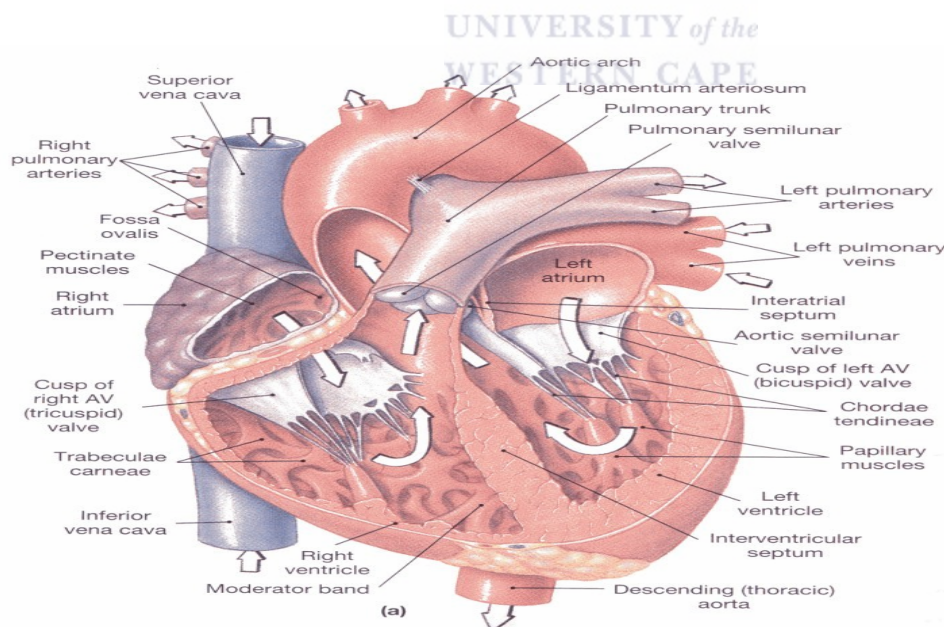


Figure 2.1 Cross section of the mammalian heart (Silverthorn 2003)

The cardiac cycle generates continuous blood flow to all the tissues in the body. The stages of the cardiac cycle must correspond perfectly to ensure adequate cardiac system operation. There are five basic steps in the full cardiac cycle:

- 1) The cycle starts with an electrical stimulus that leads to contraction of the cardiac muscle.
- 2) Then a contraction of the cardiac muscle generates changes in pressure and blood volume (systole).
- 3) There is a mechanical opening and closing of the heart valves that controls the direction of the blood flow.
- 4) This is followed by a relaxation of the cardiac muscle (diastole).
- 5) Diastole is followed by the generation of the first and second heart sounds, which result from the closure of heart valves (Meyer *et al* 2002).

2.2 Electrical conducting system of heart

The cardiac muscle tissue (myocardium) of the heart wall has many branching cells that are joined into a bigger mass by intercalated discs. A single intercalated disc has gap junctions and this allows the larger areas of the cardiac muscle to act as a single unit. The single unit or joined cells are called a functional “syncytium” (Thibodeau and Patton 1993). Due to the syncytium structure, the muscle cells can pass an action potential along the heart wall, and therefore, stimulate contractions. The myocardium is created by two separate fiber bundles. One bundle is for the atrium and the other is for the ventricles. The myocardium in the ventricles is thicker than either atrium because more force is needed to pump ventricular blood. The myocardium of the left ventricle, however, is thicker than that of the right ventricle because the left ventricle pushes blood through most vessels of the body whereas the right ventricle only pushes blood through the lungs. Neither of the myocardium bundles are physically connected therefore there is need for an atrioventricular conducting system for synchronized electrical activity (Hall-Craggs 1995). The conduction system of the heart is essential for the correct sequencing of the atrial and

ventricular contractions. The components of the conducting system include the following elements, the sinoatrial node, the internodal fibers, the atrioventricular node, the atrioventricular bundle, the right and left bundle branches, and the Purkinje fibers (Hall-Craggs 1995). The sinoatrial node (SA node) also referred to as the “pacemaker,” is a strip of myocytes that are located at the posterior wall of the right atrium, close to the superior vena cava (Meyer *et al* 2002). Anatomically, the sinoatrial node is spindle shaped and its dimensions are 20 x 3 mm in the human (Opie 1998). When an electrical current flows to the muscle fibers that form most of the heart, each fiber that is excited fired an action potential. The action potential, in turn, initiates the rise in intracellular calcium ion concentration that activates the myofibrils to contract. The myofibril is considered the contractile machinery of the cell. The firing of the action potential causes the phenomenon of depolarizing across both atria. Some of the signals that are sent from the pacemaker are also sent to the atrioventricular node located in the inferior part of the interatrial septum. The impulses then pass through the atrioventricular bundle, also known as the “bundle of His” (Hall-Craggs 1995). This bundle runs through the interventricular septum and divides the bundles into right and left branches or the “crus” (Hall-Craggs 1995). The right bundle branch runs towards the apex of the heart. As the impulse runs down the bundle branches, these branches turn into what is referred to as conduction myocardium or “Purkinje fibers.” The left crus runs down the left surface of the septum and breaks into two strands consisting of the left papillary muscle and the left ventricular muscle (Hall-Craggs 1995). The blood from the left ventricle is ejected into the aorta to feed the systemic circuit. The right ventricle ejects blood into the pulmonary arteries to feed the pulmonary circuit. In short, the electrical impulses flow like such: SA node->AV node->Bundle of His-> left and right bundle branches->Purkinje fibers (Opie 1998).

2.3 Blood flow through the heart

2.3.1 Systemic blood flow through the heart

The systemic circulation is responsible for bringing oxygen rich blood from the heart to the rest of the body's cells, and then returning the carbon dioxide rich blood back to the heart. The right atrium chamber receives blood from the systemic circuit and the right ventricle discharges the blood into the pulmonary circuit (Opie 1998). The heart beats, causing the atria to contract followed by the ventricles. Both of the ventricles contract at the same time and eject equal volumes of blood into the pulmonary and systemic circuits. Each circuit, both pulmonary and systemic begins and ends at the heart. Blood flows from the heart *via* the left ventricles to the blood vessels, then to all parts of the body (except the lungs). Then the blood flows back to the right atrium of the heart. The blood moves from the arteries to the arterioles then to the capillaries. Capillaries are microscopic vessels carrying blood from small arteries to small veins, or from arterioles to venules that are the basic connecting vessels between arteries and veins. The blood then flows out of each organ by way of its venule and then to its veins, which drain the blood to the superior and inferior vena cava (Opie 1998). The superior and inferior vena cava drains blood from the upper and lower limbs respectively (Opie 1998). There is a vital 2-way exchange of substances that occur between the blood and the cells. Two great veins return venous blood to the heart via the right atrium to complete the systemic circulation. The blood comes full circle back to the starting point of the left ventricle after passing through the pulmonary circulation.

2.3.2 Pulmonary blood flow through the heart

The pulmonary circuit transports carbon dioxide rich blood from the heart to the gas exchange surfaces of the lungs, and then returns the oxygen rich blood back to the heart (Martini *et al* 2003). The left atrium collects blood from the pulmonary circuit, and the left ventricle ejects blood into the systemic circuit. The venous blood moves from the right atrium to the right ventricle and then to the pulmonary artery. From there it moves to the lung arterioles and

capillaries. The exchange of gas occurs between the blood and the air on the capillary beds of the lungs. It is here that the deoxygenated blood is turned into oxygenated blood (Martini *et al* 2003). The oxygenated blood moves to the lung venules and returns to the four pulmonary veins by the left atrium of the heart. The left atrium then pushes the blood to the left ventricle and it is again pumped through the systemic circulation.

2.3.3 Pulmonary and systemic circulation of blood:

The following is a general representation of the entire blood circulation in the body:

Superior/Inferior vena cava-> right atrium-> tricuspid valve-> right ventricle-> pulmonary semi-lunar valve->pulmonary trunk-> pulmonary arteries-> lungs->pulmonary veins-> left atrium-> bicuspid-> left ventricle-> aortic semi-lunar valve-> aorta-> systemic circulation (Martini *et al* 2003).



2.4 Blood supply to the heart

2.4.1 Arterial blood supply

The arteries traditionally take the blood away from the heart into the systemic circulation. The small arteries are known as arterioles. There are basic principles of the heart's blood supply that are important to know in order to understand blood flow. These are as follows:

- 1) Both ventricles receive their blood supply from the branches of the right and left coronary arteries
- 2) Each atrium receives blood from the small branches of the corresponding coronary arteries
- 3) The most abundant supply of blood goes to the left ventricular myocardium because the left ventricle performs the most work (Thibodeau and Patton 1993).

The two major arteries supplying the myocardial cells with blood are two vessels known as the right and left coronary arteries. The branch of the left coronary artery is the anterior intraventricular artery and supplies blood to the anterior parts of the heart. The left marginal

artery branches from the left coronary artery and supplies blood to the lateral wall of the left ventricle. The right coronary artery extends to the posterior part of the heart. The right marginal artery is a larger branch of the right coronary artery and supplies blood to the lateral wall of the right ventricle. The posterior interventricular artery supplies the posterior and inferior part of the heart with blood. The atrioventricular nodal artery supplies the atrioventricular bundle and branches. The right coronary artery is usually smaller than the left and supplies a smaller area of the myocardium (Meyer *et al* 2002).

The main factor determining arterial blood flow is the volume of blood that is in the arteries, which is proportional to arterial blood pressure (Thibodeau and Patton 1993). The blood pressure is the force that causes blood to flow through the arteries, capillaries and veins. It originates when the heart's pumping forces the blood against the walls of the blood vessels. The stretching of the elastic arteries helps to maintain constant blood flow. Arterial blood pressure (BP) can be calculated by multiplying cardiac output (CO) and peripheral vascular resistance (PVR) ($BP = SV * HR * PVR$). More simply stated: $BP = CO * PVR$ (Opie 1998).

If the arterial blood pressure is high for some reason, then the volume of blood that is in the arteries also tends to be high. If the arterial blood pressure tends to be low, then the volume of blood is lower as well. An increase or decrease in either of these physiological factors affects the other.

Since the heart can pump blood into the large arteries more quickly than the arterioles and capillaries can accommodate it, there is always considerable pressure in the arteries (Naqvi and Blaufox 1998). Blood pressure is highest in the aorta because that is where the blood leaves the heart. It progressively diminishes in the smaller blood vessels and eventually is at its lowest pressure in the veins (Naqvi and Blaufox 1998). There are many external factors determining the arterial blood flow and pressure but two of the most pertinent are blood flow produced by the heart (cardiac output) and the resistance of the blood vessels to the blood flow (systemic vascular resistance otherwise called peripheral resistance).

There are different physiological mechanisms that maintain normal blood pressure. Structures, such as the kidneys, brain, heart, endocrine glands, and blood vessels are involved in the regulation of blood pressure. The two most influential mechanisms are the autonomic nervous system and hormones.

The autonomic nervous system receives information from pressure sensitive nerve endings (baroreceptors) and this is communicated to the brainstem. When pressure decreases, it causes an activation of the sympathetic nervous system, which increases the contractility of the heart through the β -receptors (Tortora and Grabowski 2000). Sympathetic stimulation simultaneously causes vasoconstriction of both the arterial and venous circulation through the α -receptors.

Hormonal mechanisms also affect the blood flow by causing vasoconstriction, vasodilation, and blood volume changes (mentioned previously). Adrenaline and noradrenaline, which are secreted from the adrenal medulla, respond to sympathetic nervous system stimulation (Tortora and Grabowski 2000). Both of these catecholamines act rapidly by increasing cardiac output and causing vasoconstriction (explained later).

2.4.2 Cardiac output

Cardiac output (CO) is the volume of blood ejected by a ventricle (left or right) per minute. It is a product of two factors being stroke volume (SV) and heart rate (HR): $CO (Q) = SV * HR$ (units = litres per minute (Opie 1998)). Of the three major determinants of cardiac output, the most important factor is heart rate. The other two factors include pre-load and after-load (Opie 1998).

Stroke volume is the volume of blood that each ventricle pumps per cardiac cycle. Heart rate is defined as the number of times the heart beats in a minute. The contraction of the heart is called systole. Therefore, when the volume of blood is pumped through by a single contraction the result is called systolic discharge (Thibodeau and Patton 1993). When the heart contracts, pressure is exerted on the blood within the ventricles. As discussed before, the blood in the right

ventricle is pumped into the pulmonary circulation and the blood in the left ventricle is pumped into the systemic circulation.

In a 70 kg male, normal stroke volume readings are around 70ml or 50-70% of ventricular end diastolic volume (Thibodeau and Patton 1993). In the rat, cardiac work is defined by the double product (systolic heart rate * blood pressure) (American Heritage Stedman's Medical Dictionary 2004).

Two of the important factors affecting cardiac output are exercise and stress (Meyer *et al* 2002). The difference between cardiac output during rest and cardiac output during exercise is called the cardiac reserve (Tortora and Grabowski 2000). A normal cardiac output is 5 L/min at rest and 25 L/min during exercise, giving a cardiac reserve of 20 L/min (Tortora and Grabowski 2000).

2.4.3 Stroke volume

There are mechanical, neural, and chemical factors that regulate the beating of the heart (Thibodeau and Patton 1993). Stroke volume (SV) of the heart is dependent on ventricular filling, the length of the muscle fiber and tension, contractility of the heart, and blood flow resistance (Meyer *et al* 2002). Ventricular end-diastolic volume also known as “pre-load” is the stretching of the cardiac muscle fibers dependent on the volume of blood in the ventricle at the end of diastole. This is a mechanical factor of the heart and the utmost volume that the ventricular fiber can handle before the release. The right and left atrium pressure determines the filling of the ventricles. The increase in pressure in the atrium results in a larger ventricular filling. The larger the filling in the ventricle, the more the fibers stretch within physiological limits. The further the fiber stretches, the larger the potential stroke volume of the heart. A larger stroke volume, in turn, means a higher cardiac output (Meyer *et al* 2002).

Aortic output (Qa) equals the amount of fluid pumped by the left ventricle per minute. The tension length of the muscle fiber or the forcefulness of contraction of individual ventricular

muscle fibers is the largest determinant of the aortic output. The more the cardiac muscle fibers stretch, the greater the tension and stronger the contraction of the muscle. The energy of contraction is proportional to the initial length of fibers. This is known as “Starlings Law” (Meyer *et al* 2002). Initial fiber length determines stroke volume, because it determines how far the fiber can actually stretch.

“Afterload” is defined as the resistance against the ejection of blood or the load that the heart must eject blood against (Opie 1998). Initial fibers length is dependent on end diastolic volume, which depends on filling pressure. The left ventricle afterload is equal to aortic pressure during diastole. The left ventricle must overcome vascular resistance before the semi-lunar valve opens. The aortic pressure that is put on the vessel wall increases vascular tension to eject blood. The ventricular pump overcomes the resistance, or afterload across the vessels. The energy required to overcome this vascular resistance affects the stroke volume, which in turn, affects cardiac output.

2.4.4 Heart rate

The heart rate (HR) determines the time available to fill the ventricles. An increase in heart rate will lead to an increase in cardiac output. The adjustment of heart rate is the regulatory mechanism of tissue perfusion. The heart rate is controlled both neurologically and chemically. Neurological chemical control is the finest control of the heart rate and the cardiac output.

The heart rate is largely determined by hormones, oxygen intake and carbon dioxide output (Thibodeau and Patton 1993). Nutrients and various drugs also have a major impact on the heart rate (Thibodeau and Patton 1993). Chemically, adrenaline and noradrenaline are the most commonly known determinates of heart rate. The heart rate and stroke volume change the cardiac output of the ventricles, which in turn, affect the blood pressure. Any external factor that causes the heart to beat more quickly or to beat more strongly affects all of these other factors, including cardiac output and blood volume and pressure. A normal resting heart rate in a human

is 60-100 bpm. A resting heart rate below 60 bpm is considered bradycardia and a heart rate above 100 bpm is considered tachycardia (Thibodeau and Patton 1993).

2.4.5 Peripheral resistance

Peripheral resistance (PR) is the resistance that blood encounters passing through small arteries or arterioles. In essence it is the resistance to the flow of blood because there is friction between the blood and the vessel wall. The two main factors controlling peripheral resistance are the viscosity of the blood and the diameter of the arterioles (Thibodeau and Patton 1993). The first factor of viscosity is due to the general makeup of the blood and the fact that the blood is not always liquified but thicker, therefore challenging its fluidity. Viscosity is determined by the amount of proteins that are present in the blood. If there is a higher blood protein count, then there will most likely be a higher viscosity of the blood, which makes blood flow more difficult. The diameter of the small arteries and arterioles, also known as the lumen, is adjusted actively (Thibodeau and Patton 1993). The arterioles have a muscular coat that allows for constriction or dilation in proportion to the blood flow. Peripheral resistance is vital to the establishment and maintenance of arterial blood pressure because it controls the amount of blood flowing out of the arteries into the arterioles. The greater the peripheral resistance to the outflow, the less outflow. The constriction of these vessels increases the peripheral resistance, which in turn increases the blood pressure. When the arterioles are relaxed and dilated the blood pressure and peripheral resistance decreases.

Total peripheral resistance refers to the cumulative resistance of thousands of arterioles in the body and is approximately equal to the resistance of the arterioles (as discussed above). Arterioles are referred to as resistance vessels because of their resistance to blood flow (Naqvi and Blafox 1998). Total peripheral resistance is equal to mean arterial pressure (MAP) divided by cardiac output (CO): (MAP / CO) .

2.4.6 Venous blood supply

The veins are large blood vessels that return the blood from the tissues back to the heart. The lungs oxygenate blood and send it back to the heart via the pulmonary veins. All veins except the pulmonary vein contain deoxygenated blood, and the smaller veins are commonly called venules. There are three major routes for the blood that is taken from the systemic circulation back to the heart. These are the coronary sinus, the superior and the inferior vena cava. They are located in the upper posterior right atrium (Hall-Craggs 1995).

The venous blood drainage is made up of a system of veins converging at the coronary sinus and opening into the right atrium of the heart. The great cardiac vein is the primary vein that originates in the myocardium and removes the blood from the left side of the heart. The great cardiac vein is located in the anterior interventricular sulcus and is joined by the left marginal vein along with the posterior vein of the left ventricle forming the coronary sinus. There are small and middle cardiac veins that are located near the place of coronary termination. The small cardiac vein drains the right side of the myocardium.

The superior vena cava, as the name describes, is located on the superior side of the right atrium and delivers venous blood to the heart from the head, neck, upper limbs and chest. The inferior vena cava, is the vein that delivers venous blood from the rest of the trunk, the viscera and the lower limbs of the body and is located inferior to the right atrium (Martini *et al* 2003). The veins of the body consistently reflect intra-atrial pressures. For the *in vivo* system of the normotensive rat model, the jugular vein is cannulated to record the pressures of the atria. This has been found to be an effective way to track and maintain atrial pressure throughout the experiment (discussed later).

2.4.7 Valves of the heart

The valves of the heart play a pertinent role in proper heart function because they enforce one-way blood flow through the heart. There are four main valves of the heart. There are paired atrioventricular (AV) valves, dividing the atria from the ventricles and aortic and pulmonary semilunar valves, which traffic blood flow through the aorta and pulmonary systems. The right atrioventricular valves are referred to as tricuspid valves because they have three cusps that open and close to permit or restrict blood flow. The left atrioventricular valves are commonly referred to as the bicuspid valve because they contain two cusps (Meyer *et al* 2002). At the junction of the ventricles and great arteries are aortic and pulmonary semilunar valves. These are called semilunar because when they are closed they are likened to a semi-lunar shape and also having three cusps. Both semilunar valves prevent the back flow of blood from the great arteries to the ventricles (Hall-Craggs 1995). The ventricular valves of the heart contain papillary muscles and a strong band projecting from the muscle known as chordae tendinae, which is attached to papillary muscles (Meyer *et al* 2002). The chordae tendinae and papillary muscles are the actual regulators of the opening and closing motions of the valves because they are able to be stretched and take form again, depending on the function performed. Once stretched the chordae tendinae serve as an anchor for the cusps to keep them in a closed position, in prevention of ventricular reflux. The heart valves push open for blood to flow freely and shut to prevent a back flow of blood. For example, both atrioventricular valves prevent back flow of blood from the ventricles to the atria. When the ventricles are relaxed, the cusps of the AV valves lay in the ventricular chambers. When the ventricles contract the pressure within rises and it eventually forces the blood upward against cusps so that they close (Meyer *et al* 2002).

2.4.8 Reactions of valves due to pressure

The blood flows because a specific pressure gradient is in effect between different parts of the heart (Thibodeau and Patton 1993). In short, the blood continues to circulate the way it does, from the left ventricle and eventually back to the right atrium of the heart because a certain blood

pressure gradient is in effect between the two structures. The blood pressure gradient includes the blood pressure in one structure as compared to the blood pressure in another structure. A typical blood pressure of the ventricular blood when contracting and moving blood to the aorta, is 120 mmHg. A normal blood pressure when the left ventricle relaxes is 80 mmHg (Thibodeau and Patton 1993). The average blood pressure gradient would then be around 100mmHg because of the average taken from both contraction and relaxation. When the right atrium receives the venous return of blood from the systemic circulation, the superior and inferior vena cava open into the posterior part of the heart and there is no valve involved. Once in the atrium, however, the valve between the right atrium and right ventricle close so that there is no blood flow to the ventricle. As the pressure builds in the right atrium with the continuous deposit of blood, the atrioventricular valve opens to allow the free flow of blood. The valve only opens when the ventricle is in the state of relaxation, also known as diastole. When the AV valves are open, 70% of the blood entering into the ventricle, from the atria flows in without resistance (Meyer *et al* 2002). During atrial systole or the contraction phase, the pressure in both the atria and ventricle increases. Although the contraction is weak in the atrium, it is strong enough to force the rest of the blood (30%) into the open ventricle. The intra-atrial pressure that is produced with the atrial contraction results in ventricular filling pressure. When the ventricle fills and the pressure increases, the atrioventricular valve closes with the contraction of the right ventricle. The ventricular filling pressure determines the ventricular end-diastolic volume (VEDV) and ventricular end-diastolic pressure (VEDP). The volume and pressure produced by the ventricles sets the “pre-load” for the next ventricular contraction (“pre-load” discussed earlier). These pressures force the semi-lunar valves to open. The blood from the ventricle is forced through the valve to the pulmonary trunk. The blood shoots through the pulmonary arteries, away from the heart, to be oxygenated by the capillary beds of the lungs (Marieb *et al* 2001). Once the oxygenated blood is returned to the heart, it empties into the left atria. The left atria fills, and the bicuspid (also known as the “mitral”) valve opens to allow consistent blood flow into the left ventricle. Once the bicuspid valve closes, the pressure inside the left ventricle increases as it did in the right ventricle. The chordae tendinae are stretched and the ventricle contracts more, which raises the interventricular pressure. As a result of this, the aortic semi-lunar valve is forced open.

The blood then shoots through the aortic valve allowing the ventricles to relax (Marieb *et al* 2001). In post ejection, the ventricular pressure decreases and the blood naturally starts to flow from the aorta back into the ventricle. The aortic semilunar valve closes to prevent back flow and the pressure in the aorta slightly rises. Only when the ventricular pressure exceeds the pulmonary trunk or aortic pressures, can the ventricle decrease in volume. Ventricular contraction starts before ventricular emptying and this period, when the ventricular volume does not decrease, is known as isometric contraction. Ventricular contraction is known as ventricular systole. In a 300 gram rat, ventricular pressure is regularly 176-197mmHg, and the systolic blood pressure is 116-145mmHg (Livius *et al* 2000).

2.4.9 Mean arterial pressure

Pulse pressure represents the difference between systolic and diastolic pressures, or the amount of systolic pressure that increases above diastolic during systole equals pulse pressure (systolic - diastolic). The mean arterial pressure (MAP) is the average pressure in the arterial system throughout the cardiac cycle. The accurate determination of mean blood pressure is complicated. It is approximated by the equation of (systolic and diastolic) divided by 2 equals mmHg ($120 + 80 / 2$ equals 100 mmHg). A more simple expression of mean arterial blood pressure is to multiply cardiac output by peripheral vascular resistance ($MAP = CO * PVR$) (Livius *et al* 2000).

2.4.10 Nervous regulation of cardiac function

The heart has both intrinsic and extrinsic regulations to control it. Intrinsic regulation results from the normal functional characteristics of the heart and is not dependent on neural or hormonal regulation. It functions in both *in vivo* and *in vitro* experiments. The extrinsic regulation of the heart is governed by neural and hormonal control function when the heart is *in vivo*. Nervous control regulation is an example of an extrinsic control factor of the heart. The sinoatrial and atrioventricular nodes and the ventricular myocardium are innervated by

sympathetic and parasympathetic receptors. The sympathetic nervous system activates a sympatho-adrenal or fight or flight response (Tortora and Grabowski 2000). In this response the sympathetic fibers secrete acetylcholine (chemical neurotransmitter) and this in turn activates the secretion of epinephrine or norepinephrine. Epinephrine and norepinephrine are catecholamines (explained later) produced from the adrenal medulla and do not only increase the heart rate and force of contraction but also enhance and prolong the effects of the sympathetic nervous system. Chronotropism is another word describing the rate at which the heart contracts or the heart rate (as discussed earlier). Sympathetic receptors speed the heart rate up, giving a positive chronotropic effect.

The parasympathic nervous system is a part of the autonomic nervous system. The parasympathetic receptors slow the heart rate down, giving a negative chronotropic effect but can only decrease the cardiac output by 10-20% upon the release of acetylcholine. Acetylcholine binds to channels that make the membrane more permeable to K^+ (Tortora and Grabowski 2000). Resting membrane potential becomes hyperpolarized and the heart rate decreases.

The sympathetic and parasympathetic receptors work in a similar way in inotropism as in chronotropism. Inotropism is the force produced by the contracting ventricular muscle fibers that must overcome aortic pressures and pump blood into the systemic circulation (Thibodeau and Patton 1993). The sympathetic fiber stimulation increase the force of the ventricular myocardium producing a positive inotropic effect and can increase output by 50-100% over resting values (Tortora and Grabowski 2000). The parasympathetic fibers however, reduce the force of the myocardial contraction, which puts less stretch on the fibers and produces a negative inotropic effect. Although the extrinsic regulations of the heart are governed by neural and hormonal functions, sympathetic and parasympathetic systems can be controlled to a certain extent. For example, the suppression of the cardiac sympathetic fibers has been known to decrease the average heart rate of 70 beats / minute to 60 beats/ minute (Meyer *et al* 2002). The stimulation of α -receptors by the sympathetic system produces a contraction of the vascular smooth muscles, increases the arterial resistance and increases the blood pressure (Meyer *et al* 2002). The β_2 -

receptor stimulation produces smooth muscle vasodilatation, but still promotes sympathetic stimulation (Meyer *et al* 2002). The β -adrenoreceptors are mainly found in the muscular wall of the heart and the α -adrenoreceptors are found in the coronary vessels. The parasympathetic receptors supply the cardiovascular system through the right and left vagal nerves. These nerves have a vital effect on the heart, because the vagal nerves innervate the sinoatrial and atrioventricular nodes as well as the atrial muscle fibers (Thibodeau and Patton 1993). The stimulation of the vagal fibers consistently has a negative chronotropic and inotropic effect on the heart as it decreases the transmission of impulses through the atrioventricular node and fibers. This in turn, decreases the heart rate (Thibodeau and Patton 1993). The common chemicals of the heart, adrenaline and noradrenaline react with β -adrenoreceptors of the sinoatrial node, the myocardium and the conduction system of the heart.

Intrinsic regulation of the heart is regulation within the heart without neural or hormonal regulation. Pre-load and after load effects are the main intrinsic regulation controls. Pre-load, as discussed above represent the stretching of the ventricles due to the amount of blood returning from the venous system. When the preload increases the cardiac output increases as well. Starling's law of the heart applies to intrinsic regulation because there is a greater contraction with the stretching of the cardiac muscle cells, which in turn, leads to a greater stroke volume (as described above). Another pre-load effect is the stretching of the right atrial wall, which stimulates the firing of the SA node and increases the heart rate. After load effects include creating pressure within the ventricle, which it must overcome in order to send blood up the aorta. This pressure does not necessarily influence stroke volume unless the pressure exceeds 170 mmHg (Tortora and Grabowski 2000).

Catecholamines are chemical compounds that circulate in the blood stream which are derived from an amino acid called tyrosine (Tortora and Grabowski 2000). Catecholamines are water-soluble and 50% of them are bound to plasma proteins that circulate in the blood stream (Tortora and Grabowski 2000). The three most abundant catecholamines in the body are epinephrine, norepinephrine and dopamine (Nackley *et al* 2007). Catecholamines, which act like hormones

throughout the body, are released by the adrenal glands. Epinephrine and dopamine act as neurotransmitters in the central nervous system but as hormones throughout the blood stream (Nackley *et al* 2007). Norepinephrine is a neurotransmitter of the peripheral sympathetic nervous system but is also present in the blood (as explained above). When released in the blood, catecholamines increase the heart rate and blood pressure but also reduce the amount of blood going to the skin and increase the blood flow to the major organs (such as the heart).

The heart houses different receptors that can be stimulated to react upon the introduction of various drugs. The five main receptors of the heart include α_1 , α_2 , β_1 , β_2 and C^{2+} channel receptors. Each of these receptors has the ability to produce positive or negative cardiogenic effects, dependent on what drug is introduced to the receptor. Muscarinic receptors of the heart, postganglionic sympathetic nerve terminal blockers and catecholamine depletion also have cardiovascular effects and are highlighted below.

Alpha-1 receptors are primarily found in the smooth muscle and have a principal effect of vasoconstriction in the blood vessels (as mentioned above). Blood vessels containing α_1 receptors are present in the skin and the gastrointestinal tract. During the fight-or flight response the vasoconstriction of the vessels results in decreased blood flow to these organs (Nackley *et al* 2007).

Adrenaline is a widely known catecholamine that works through α_1 receptors for the purpose of constricting the blood vessels, which increases peripheral resistance allowing the blood to be shunted to the body's core (Willems *et al* 2000). The sympathetic nervous system, acting through the splanchnic nerves (paired nerves innervating the viscera) to the adrenal medulla, stimulates the release of adrenaline (Willems *et al* 2000). These nerves release acetylcholine by preganglionic sympathetic fibers which act on acetylcholine receptors (Padley 2005). This depolarizes the cell and voltage-gated calcium channels allows an influx of calcium through (Dolphin 2006). This release of calcium causes exocytosis of chromaffin granules which secretes adrenaline into the bloodstream (Willems *et al* 2000).

The α_1 receptor effects include constriction of vessels in the skin, mucosae, subcutaneous tissues, splanchnic area and kidneys (Willems *et al* 2000). The constriction of the cerebral and pulmonary arteries can also be seen along with positive inotropic and chronotropic effects (Willems *et al* 2000).

At low dosages adrenaline has been reported to cause increased cardiac output, increased circulating volume and increased venous return. The net implications at lower doses is that systolic blood pressure will rise and diastolic may fall slightly (Wurtman *et al* 1964). Adrenaline also increases respiratory rate, tidal volume and minute ventilation (Wurtman *et al* 1964).

At higher dosages a rise in systemic venous return and systolic and diastolic blood pressure can be seen. A decrease in cardiac output also occurs with higher doses (Wurtman *et al* 1964).

Intravenous infusion of adrenaline is immediate and intense. With this type of infusion there is a rapid onset with a longer duration of the drug effects. Although the half-life of infused adrenaline is short, lasting between 3-5 minutes, the cumulative effect of adrenaline can be maintained for a long period of time (Cameron, Gunsher and Hariharan 1990). Constant adrenaline plasma levels can be maintained with continuous infusion but longer times in between infusions are necessary in order to re-attain baseline values (Cameron, Gunsher and Hariharan 1990). Intravenous adrenaline has a longer half-life in the brain lasting between two to two and one half hours (Steinman, Smerin and Barchas 1969).

Prazosin also works via α_1 vascular smooth muscle receptors to lower blood pressure by inhibiting the postsynaptic α_1 adrenoceptors (Silke, Hendry and Taylor 1981).

Studies have shown that prazosin competitively antagonizes the pressor effect of phenylephrine, which is a α_1 agonist. Prazosin also suppresses the pressor effect of norepinephrine (Silke, Hendry and Taylor 1981). By inhibiting the vasoconstrictive effect of released catecholamines in

circulation, prazosin dilates the peripheral blood vessels, therefore lowering blood pressure (Silke, Hendry and Taylor 1981). Although prazosin has little effect on cardiac output, the antihypertensive effect is traditionally not accompanied by reflex tachycardia making it a reliable testing drug (Silke, Hendry and Taylor 1981).

In certain studies prazosin had dose dependent reductions in mean arterial pressure and increases in serum renin activity (Graham and Pettinger 1979). Due to its selectivity for the post-synaptic α_1 receptor, however, the reduction in arterial pressure caused a lesser increase in serum renin than non-selective α_1 receptor blockers (Graham and Pettinger 1979).

Prazosin has a high affinity for plasma protein with protein binding at 97% and a half-life of two to three hours (Wang 2006).

Alpha-2 receptors are located in pre- and postsynaptic nerve terminals of the central and peripheral nervous system. Pre-synaptic α_2 receptors are an important part of the negative feedback control of noradrenaline release while postsynaptic α_2 receptors activate the smooth muscle of the blood vessels and cause constriction (Osawa *et al* 1990). Each subtype of the α_2 receptors are linked to proteins known as G_i proteins which are bound to the inside surface of the cell membrane (Osawa *et al* 1990). G proteins, short for guanine nucleotide bonding proteins, are receptor-coupled proteins that activate intracellular messenger systems to the cells of the cardiovascular system (Osawa *et al* 1990). G proteins are the most important signal transducing molecules in the cells. When a G protein- coupled receptor is activated, it causes the receptor to change its shape and bind to the G protein. G-proteins are linked to adenylyl cyclase, which dephosphorylates ATP to form cyclic AMP (cAMP) (Osawa *et al* 1990). G_i proteins specifically decrease cAMP and protein kinase activation, which decreases the heart rate and inotropy by inhibiting adenylyl cyclase (Osawa *et al* 1990).

Beta-1 blockers work through the sympathetic nervous system to impede the action of endogenous catecholamines (adrenaline and noradrenaline). Although there are technically three

types of β receptors (β_1 , β_2 and β_3), β_1 and β_2 are the only ones discussed throughout this study (β_3 receptors induce lipolysis but do not have direct implications on the heart) (Mongillo *et al* 2006).

Beta-1 receptors are found in the heart and the cerebral cortex (Nackley *et al* 2007). The β_1 receptors activate Gs proteins, which produce the opposite effect of the G_i proteins by increasing cAMP and activating a protein kinase that increases calcium via calcium channels and release of calcium by the sarcoplasmic reticulum in the heart (Nackley *et al* 2007). These actions increase the heart rate and inotropy of the heart.

Adrenaline not only works via α_1 mediation, but also has implications for β_1 receptors. Stimulation of β_1 cardiac receptors by adrenaline causes positive chronotropic and inotropic effects by increasing the rate and force of contractions (Cameron, Gunsher and Hariharan 1990). There is also increased AV conduction resulting in an increase in cardiac output and adrenaline has also been known to cause spontaneous firing of Purkinje fibers and initiate spontaneous myocardial contraction in asystole (Cameron, Gunsher and Hariharan 1990).

The affinity of adrenaline for β_1 receptors is greater than its affinity for α_1 receptors. At low doses and through a slower IV infusion the β_1 effects of adrenaline predominate (Cameron, Gunsher and Hariharan 1990). The capacity for vasodilation of the β_1 receptors is limited, however, when both are activated (Willems *et al* 2000).

Atenolol is a beta-adrenergic blocking agent that works through β_1 receptors to impede the action of the sympathetic nervous system through receptors in the heart and juxtaglomerular apparatus (Hayashi *et al* 2007). Since the sympathetic nervous system stimulates the pace the heartbeat, and atenolol blocks the actions of this system, the force of the heart muscle contraction is decreased (Hayashi *et al* 2007). This reduction, in turn, reduces the muscle oxygen demand of the heart.

Although atenolol does not produce vasodilatation, it does redistribute coronary circulation to ischemic places and decrease the release of rennin from the kidney, which lowers the blood pressure (Pederson and Cockcroft 2007). Unlike other β_1 blockers, atenolol does not metabolize in the liver, but is absorbed and eventually eliminated by renal excretion (Pederson and Cockcroft 2007).

Atenolol has been proven effective for the treatment of hypertension and cardiovascular disease but studies have shown that it may be less effective in the reduction of stroke and cardiovascular mortality (Pederson and Cockcroft 2007). This is possibly due to its inability to reduce central aortic pressure. Vasodilating β_1 blockers (e.g. carvedilol and nebivolol) have hemodynamic effects, which produce decreases in peripheral pressure. This may improve cardiovascular outcomes as compared to atenolol (Pederson and Cockcroft 2007).

Peak plasma levels are reached within five minutes of intravenous administration of atenolol and the elimination half-life is approximately six to seven hours (Wang *et al* 2006).

Beta- 2 receptors also activate the uptake of cAMP and are located in the lungs, smooth muscle, and cerebellum. In smooth muscle, β_2 receptors cause a relaxation in the walls thereby causing vasodilation (Nackley *et al* 2007). The stimulation of β_2 receptors relaxes the bronchial smooth muscles, thereby increasing vital lung capacity. Beta-2 receptors also relax the uterine muscles and promote the release of insulin (Nackley *et al* 2007). Due to the stimulation of adenylyl cyclase, however, there are similar actions seen in β_1 and β_2 receptors.

Adrenaline also has implications for β_2 receptors, although not as pronounced as with α_1 and β_1 receptors. As seen in a study done by Xiao and Cheng, β_2 receptors failed to hasten the relaxation of ventricular myocytes from adult rats and mice unless coupling to the G_i -protein was inhibited in order for the G_s - protein to be uncovered (1999). Also, only in neonatal, not adult rat cardiac myocytes, did the stimulation of β_2 -adrenergic receptors cause calcium transient quickening and

cell shortening by a cAMP-dependent mechanism (Steinberg 1999). Alpha-1 and Beta-1 receptor effects on the heart are more frequently studied and well known.

Calcium channel blockers are a class of drugs noted for their ability to block the entry of calcium into the muscle cells of the heart and arteries, thereby decreasing contraction of the myocardium and vasodilating the arteries (Grossman and Messerli 2004).

For muscles to contract, L-type voltage gated calcium channels must open to allow smaller amounts of calcium through. L-type channels produce large and sustained conduction and are inactivated slowly (Porter, Makuck and Rivkees 2002). The L-type channels are responsible for the plateau phase of action potentials and are regulated by cAMP-dependent protein kinases (Porter, Makuck and Rivkees 2002). After the channel opens, a larger influx of calcium is released from the sarcoplasmic reticulum stimulating the contractile apparatus and the binding of myosin cross bridges to multiple cytosolic calcium buffers (Porter, Makuck and Rivkees 2002). A thin filament known as protein troponin C then binds to calcium and activates the myofilaments which in turn, contract the muscle (Porter, Makuck and Rivkees 2002).

Calcium channel blockers work through L-type voltage gated calcium channels in the heart and blood vessels to prevent calcium levels from increasing during the plateau phase of the action potential (Grossman and Messerli 2004). This leads to a slower contraction of the heart and a decrease in total peripheral resistance in the blood vessels (Garvey 1969). The blood pressure decreases when the resistance in the blood vessels decreases.

Verapamil is a calcium channel blocker that blocks the movement of calcium into the muscle cells of the coronary arteries and relaxes the arterial muscles (Garvey 1969). This is done by decreasing the rate of recovery of the slow channel in the atrioventricular conduction system and the sinoatrial node and, therefore, depressing the sinoatrial node pacemaker activity and slowing conduction. Verapamil exhibits a negative inotropic effect, which reduces the myocardium consumption and has the ability to overcome reflex sympathetic responses due to the lowering of

blood pressure (Garvey 1969). Verapamil also has negative chronotropic and dromotropic effects, which reduce the myocardium oxygen consumption by slowing the conduction through the sinoatrial and atrioventricular nodes, respectively (Garvey 1969).

Verapamil has a half-life of two to seven hours and 90% of the drug binds to plasma proteins (McAllister and Kirsten 1982).

Muscarinic receptors are membrane-bound acetylcholine receptors found in the parasympathetic nervous system. These receptors are most sensitive to muscarine, which duplicates the actions of neurotransmitters (Bugajski 2007). Muscarine is an alkaloid that is derived from amino acids. Smooth muscle muscarinic receptors regulate cardiac contractions, gut motility and bronchial constriction (Bugajski 2007). The muscarinic receptors that promote vasodilation are located in the endothelial cells and are not innervated but secrete nitric oxide when encountering an agonist (Cheng *et al* 2007). Nitric oxide diffuses to the vascular smooth muscle cells and activates guanylate cyclase (a lyase enzyme that breaks down chemical bonds), which eventually causes relaxation of the vascular muscles (Bugajski 2007).

The muscarinic M₂ receptors are located in the sinoatrial node of the heart and are most noted in cardiovascular research for their involvement in vagal stimulation (Bugajski 2007). The vagal parasympathetic nerves innervate the release of acetylcholine in the heart for neurotransmission. Acetylcholine binds to muscarinic receptors on cells located in the sinoatrial and atrioventricular nodes (Bugajski 2007). Muscarinic receptors are then coupled to the Gi-protein and decrease cAMP (Bugajski 2007).

When vagal activity is increased in the sinoatrial node, the pacemaker cells decrease their action potential activity, which causes decreases in heart rate, increases in glandular secretory activity, and stimulation of smooth muscle contractions (Cheng *et al* 2007).

Atropine is a naturally occurring alkaloid of “*atropa belladonna*” and is a competitive antagonist of muscarinic cholinergic receptors (Lee 2007). Atropine works by binding to the muscarinic receptors thereby preventing acetylcholine from activating the receptor. In this way, atropine blocks the vagal nerve activity preventing the effects of catecholamines on the body (Lee 2007).

There is little effect seen on systemic vascular resistance or myocardial contraction with the administration of atropine. Atropine, however, is used in cases of bradycardia and electrical mechanical dissociation (Ohuchi *et al* 2005). In therapeutic doses, there is no significant effect on the peripheral blood vessels but there is marked vasodilatation at toxic doses (Ohuchi *et al* 2005). Heart rate, however, is minimally affected by low increasing doses of atropine (Ohuchi *et al* 2005).

Atropine has a plasma half-life of two to three hours with the most common compound used in medicine being atropine sulfate (Lee 2007).

Post-ganglionic nerve terminal blockers are drugs that irreversibly bind to storage vesicles of monoamine neurotransmitters (vesicular monoamine transporters) in the adrenergic neuron impairing the storage of biogenic amines by interfering with the uptake mechanism (Olivares 2006). This results in the depletion of norepinephrine, dopamine and serotonin in the central and peripheral nervous system and reduces the reuptake of catecholamines by adrenergic nerve terminals (Gilman *et al* 1990). In turn, this means that there are subsequent decreases in peripheral vascular resistance and blood pressure, which is accompanied by bradycardia (Olivares 2006). Cardiac output, renal blood flow and the filtration rate of the glomerular capillaries also decrease upon the introduction of reserpine (Olivares 2006).

Reserpine is an indole (derived from the amino acid tryptophan) alkaloid from the dried root of “*Rauwolfia serpentina*” with anti-hypertensive effects that reduce mortality (Olivares 2006). Reserpine inhibits normal sympathetic activity in the central and peripheral nervous system by binding to storage vesicles and preventing the normal storage of catecholamines at the pre-

synaptic, central nervous system and peripheral neuron (Gilman *et al* 1990). As reserpine binds to the storage vesicle, it causes catecholamines to leak into the synapse, making them unavailable for release when pre-synaptic neurons are stimulated (Gilman *et al* 1990).

Studies have shown that reserpine administration is associated with increased levels of tyrosine hydroxylase activity in the mesenteric artery, mesenteric vein, and adrenal medulla (Kohler, Berkowitz and Spector 1975). Tyrosine hydroxylase is the enzyme responsible for catalyzing L-tyrosine (an amino acid) to dihydroxyphenylalanine (DOPA), which is a precursor for dopamine and, in turn, adrenaline and noradrenaline (Kohler, Berkowitz and Spector 1975). Tyrosine hydroxylase is found in the cytosol of all cells containing catecholamines and is the rate-limiting step in the production of catecholamines (Kohler, Berkowitz and Spector 1975). The increase of tyrosine hydroxylase is related to catecholamine depletion because it must replace the catecholamines that are depleted by the administration of reserpine. Hartman *et al* also concurs that reserpine increases the levels of tyrosine hydroxylase in the heart muscle when administered and therefore depletes catecholamines via storage vesicles (1992).

The catecholamine depletion caused by reserpine is reported to be slower and less complete in the adrenal medulla than in other tissues (Kohler, Berkowitz and Spector 1975). Reserpine is characterized by a slow onset and with sustained effects in the blood stream. Mean plasma levels peak two and a one half hours after administration but has a half-life up to four and one half hours in phase one (Hartman *et al* 1992). Although intravenous infusion allows for 100% bioavailability of most drugs, the bioavailability of reserpine is only 50% after IV infusion (Olivares 2006).

The receptor that an unknown drug uses can be strongly suggested by *in vivo* trials. This suggestion can be made when testing the unknown drug alongside other drugs whose receptor is known. An ideal *in vivo* model for determining the effects and receptors of an unknown drug is the anaesthetized normotensive rat model, which is detailed in the next chapter (Lockett 1951).

CHAPTER 3

Materials and methods

3.1 Raw plant material and extraction process

All materials that were used were of standard analytical grade.

3.1.1 Plant collection

The *Crinum macowanii* bulbs were collected at New Plant Nursery, George. A total number of 35 bulbs were collected in order to perform all of these experiments.

3.1.2 Aqueous extraction

The first step in the procedure for making the aqueous extraction was to wash and dry the bulbs. The bulbs were dried in an oven at 30 degrees for 15 days until constant mass. The dry plant material was then milled to a fine powder. 252.3 g of the powder material was used for extraction. A soxhlet extraction with distilled water was carried out for 48 hours. The soxhlet extraction included boiling the solvent while percolating and evaporating, which yielded the extract and recovered solvent. The extract was then evaporated (40°C) until dry using a rotovapour (RE300B Barloworld Scientific). The extract was then put in a -85 degrees freezer (Snijders Scientific) for 24 hours after which it was put on freeze dryer (Freeze Zone6; Labconco) for 48 hours until it became a dried powder. The yield of the freeze-dried powder was 43.55 g. The dried powder was kept in a sealed amber coloured bottle at a constant temperature of 4 degrees C. The freeze-dried powder was reconstituted with normal saline to make an aqueous extract (Njagi 2004).

3.2 Animals

3.2.1 Collection

The animals used for this study were healthy male Wistar rats within the weight range of 200-250 g. They were less than 4 months old and the total number of rats used in these experiments was 100.



Figure 3.1 Male Wistar Rats (200-250 g)

3.2.2 Preparation of *in vivo* system

The first steps of the *in vivo* experiments included preparing the instruments and the operating table. The next step was to clean the tracheal cannula, and the arterial and venous catheters.

The arterial cannula, which cannulates the femoral artery, was then attached to tubing which leads to a heparin pump, which prevented any blood coagulation. The venous cannula was filled with a 10% solution of heparin, and the tubing was attached to a three-way valve of the saline/drug pump.

The rat was then collected and after weighing, the animal was anaesthetized with sodium pentobarbitone (i.p.). Sodium pentobarbitone was used as an anesthetic because there were no significant heart rate or blood pressure changes (cardioactive effects) with the injection of this drug (Lockett 1951). To determine the volume of a 6% solution of sodium pentobarbitone that was injected to provide the anesthetic dose (40 mg/kg), the following formula was used:

body weight of rat * 40mg/kg * 100/ 6000 where the “body weight of rat” was the rat mass in grams; “40 mg/kg” was the required anesthetic dose; “100/6000” was a factor to convert to correct units (ml/kg). After anaesthesia, the animal was immobilized on a small animal operating table.

The trachea was exposed, cannulated with an open cannula so as to allow sufficient airflow to the lungs and an oxygen mask was then placed over the opening of the tracheal tube.

After exposing the jugular vein, a bulldog clamp was placed proximal to the cannulation site, to prevent excessive blood loss. The cannula was inserted into the vein through a small incision and secured. The tubing was then connected to the saline solution side of the 3-way valve.

The final cannulation was that of the femoral artery. The artery was exposed and tied off to cut the blood supply from the leg. A bulldog clamp was then placed proximal to the incision site. After a small incision was made, the cannula was inserted and secured. The incision sites were then covered with saline soaked gauze for the remainder of the experiment.

A temperature probe was inserted into the rectum to monitor the animal's core temperature. Readings of the heart rate and systolic and diastolic blood pressures were recorded with Chart 4 Windows once the readings of heart rate and pressures stabilized.



Figure 3.2 *In vivo* anaesthetized normotensive rat model

3.3 *In vivo* parameters assessed

The purpose of this *in vivo* system was to determine the effects of *Crinum macowanii* in the intact anaesthetized animal both on its own and in combination with cardioactive substances. Measurements of the systolic and diastolic blood pressures, heart rate, and mean arterial pressure were taken, via a cannula, upon the introduction of the plant extract.

3.4. *In vivo* equipment

The equipment that was used for the *in vivo* system included: Three cannulas (tracheal, jugular, and femoral) and syringes, Ascor double syringe pump machine (AP 22), tubing and 3 way valves, a blood pressure transducer (BP Amp ML117; ADInstruments) and computer based data recording system (PowerLab 4/20T; ADInstruments), temperature probe (Thermistor Pod; ADInstruments), sodium pentobarbitone (Sigma-Aldrich) with small needled syringe, saline solution (Adcock Ingram), heparin sodium (Intramed), aqueous extract of *Crinum macowanii*, small animal operating table and tools (Bioscience), thick and thin string, and Kleenex wipers.

3.5. Chemicals used for extraction

The following chemicals were used:

Distilled water and freeze-dried powder of *Crinum macowanii*.

3.6. *In vivo* testing drugs

3.6.1 Control

The control solution for the *in vivo* experiments was a normal saline solution. This was infused for stabilization.

3.6.2 Testing drugs

Various chemicals with known pharmacological action were selected and used in an effort to determine the mechanism of action of *Crinum macowanii*.

Adrenaline (Sigma-Aldrich) is an agonist of both α and β receptors (Willems *et al* 2000). If *Crinum macowanii* exerts its actions through α and β receptors, antagonists of adrenaline will also be antagonistic to *Crinum macowanii*.

Verapamil (Sigma-Aldrich) is a known Ca^{2+} channel blocker (Grossman and Messerli 2004). If *Crinum macowanii* exerts its actions by involving Ca^{2+} channels, verapamil will block its effect.

Atropine (Sigma-Aldrich) is a muscarinic receptor blocker (Ohuchi *et al* 2005). If *Crinum macowanii* exerts its actions using muscarinic receptors, atropine will block its effect.

Atenolol (Sigma-Aldrich) is a β_1 receptor antagonist (Hayashi *et al* 2007). Abolishment of the *Crinum macowanii* effect by atenolol will indicate that it exerts its effects through β_1 receptors.

Prazosin (Sigma-Aldrich) is a α_1 antagonist (Farah *et al* 2006). Abolishment of the *Crinum macowanii* effect by prazosin will indicate that *Crinum* exerts its effects through α_1 receptors.

Reserpine (Sigma-Aldrich) depletes catecholamines (Olivares *et al* 2006). If *Crinum macowanii* exerts its effects by causing the release of catecholamines, reserpine will reduce the effect of *Crinum*.

The crude aqueous extract of *Crinum macowanii* was administered on its own and in combination with the specific chemicals named above where the mode of action was well known in order to investigate the possible mechanism by which the plant extract exerts its effects.

3.7 Data analysis

The statistical methods employed for the evaluation of the data included a comparison of the control group and the drug group (the control group being the known antagonist drugs and the drug group being *Crinum macowanii*).

For both protocols, the percentage change was taken. For the first protocol, which was the single dose analysis, the question asked was whether or not there were significant differences in response at every dose administered. Non-parametric methods (Friedman's test) were used to determine any differences across doses. The calculated difference between the parameter value before administering the drug and the peak value of the parameters after drug administration was taken. If there was evidence of a difference, pairwise comparisons of responses at different dose levels were performed using the Wilcoxon Signed Rank test. Three outcomes were tested (heart rate, systolic blood pressure and diastolic blood pressure) for four to six doses of seven different drugs. Eighty percent of the maximal effect level of the active compound was determined by constructing appropriate dose-response curves. This concentration was used for pre-treatment of the rats in the next phase of the project where the *Crinum macowanii* extract was administered in conjunction with the selected chemicals. The significance level used was a *P* value less than 0,01 and 95 % confidence intervals were given. For comparison purposes, the lowest to highest dose percentage change was noted and the significance of the change was also reported.

In the second protocol, the design of the study was a two-factor study with repeated measures (or repeated observations on the same animal (on one factor)). The first factor was the experimental treatment administered which has two levels. The first level is called between-subjects (different animals are in each treatment group) and the second level is called within-subject factors (the same animal is observed at each dose level). Non-parametric measures (Wilcoxon Rank Sum test) were used to compare the results at each dose separately. The data was entered as a percentage change from the pre-treated values. There were three outcomes tested (heart rate, systolic blood pressure, and diastolic blood pressure) at four to six doses for five different combinations of drugs. Whenever there are a large number of tests, the significance level needs to be more conservative so a P value less than 0,01 was considered to be significant and was used as the threshold value to determine the smallest fixed level at which the null hypothesis could be rejected (determine that data point differences were significant). The two dose response curves were compared and analyzed for significant differences and 95% confidence intervals were employed. The percentage changes between *Crinum macowanii* on its own and *Crinum macowanii* with pre-treatment were noted at the lowest dose and the highest dose (although all doses were compared in the statistics). The significance was reported across all doses.

The first graph displays the actual values at each dose level and is set up in a dose response curve form. The second graph displays all six experiments ($n=6$) in percentage change from pre-treatment values and tracks the responses in parameters at each dose level. With the graphs expressing *Crinum macowanii* and “known” drugs, the pre-treatment values (*Crinum macowanii* combined with pre-treatment drugs) are expressed in blue and the non pre-treatment values (*Crinum macowanii* on its own) are expressed in green. Each concentration was expressed in mg/kg (taking into account the weight of the rat) and was infused for three minutes, giving the concentrations displayed in the x-axis.

3.8 Protocol

3.8.1 Model for drug dosing (Protocol 1 and Protocol 2)

The following protocol was used for the administration of *Crinum macowanii* (the unknown drug) against other drugs to determine *Crinum macowanii* cardioactive effects. The anaesthetized normotensive rat model was employed for the *in vivo* testing of the drugs.

Before the actual protocol was finalized, experiments were performed on the aqueous extract of *Crinum macowanii* to determine the effect of this plant on the cardiovascular system. After this was determined, antagonist drugs were chosen to test against *Crinum macowanii*. This was done to determine which receptor this extract works through.

Five doses of the aqueous extract of *Crinum macowanii* were chosen (100; 10; 1,0; 0,1 and 0,01 mg/kg) for preliminary tests and the lowest concentration was infused first over a three minute time period. The justification behind infusing lower doses of *C. macowanii* to begin the experiment was to gauge the initial response of the rat. The doses were then continually increased until an accurate dose response curve could be measured. The dose response curves were created to determine 80% of the maximal effective dose (reported in Chapter 4). This dose was then the dose infused against the agonist and antagonist drugs.

The following protocol began by testing the various drugs independent of the crude aqueous extract. Due to the dose response method being employed, the drugs of adrenaline, atropine, atenolol, prazosin, verapamil, reserpine and *Crinum macowanii* were each tested alone on the *in vivo* animal and a dose response curve was made for each drug. A dose response curve included infusing the lowest dose of the drug to begin the experiment and gradually increased the dose over time until a toxicity point was reached. The toxic levels of the “known” drugs were determined and 80% was taken for pre-treatment dosages. *Crinum macowanii* was then tested with the pre-treated animals and dose response curves were constructed. All of these results were compared.

After all of the catheters were cannulated, the animal was allowed to stabilize for 20 minutes. The stabilization period included a perfusion of normal saline into the animal at 10 ml/hr.

The dose of the drug administered was dependent on the toxicity of each individual drug but all drugs were infused at the same rate of 10 ml/hr. Only one dose of the chosen drug was administered before another flush and stabilization. After stabilization, another higher dose of the same drug was given. The experiment was continued with alternating periods of flushing and stabilization for higher doses of the chosen drug until a notable toxicity level was achieved.

The first protocol was used to formulate accurate dose response curves for the “known” antagonist drugs in order to establish pre-treatment dosages. The protocol is outlined below.

Stabilize	1 st dose (lowest dose)	Flush	Stabilize	2 nd dose (higher dose)	Flush	Stabilize	3 rd dose (higher dose)	Flush
20 min	3 min.	3 min.	20 min.	3 min.	3 min.	20 min.	3 min.	3 min.

Table 3.1 Protocol for individual dose response curves

(Note: This protocol continued as such until the highest concentration was found.)

The second protocol included the administration of a pre-treatment antagonist drug and then the infusion of *Crinum macowanii*:

Stabilize	Pre-treatment drug	Stabilize	1 st dose <i>Crinum macowanii</i>	Stabilize	2 nd dose <i>Crinum macowanii</i>	Stabilize
20 min.	3 min.	20 min.	3 min	20 min.	3 min.	20 min.

Table 3.2 Protocol for *Crinum macowanii* dose response curves with pre-treatment

(Note: This protocol continued until there was no observable change in heart rate and pressures.)

3.9 Ethical considerations

The UWC ethics committee requires that vertebrate animals be ethically treated when used for experimentation. The animals must therefore be accorded rights while the advancement of biological knowledge unfolds in the experiment.

3.9.1 Animal rights

It is important to acknowledge that animals used in the experimental protocol have certain rights afforded to them throughout the process of research. Animal rights while performing research experiments include the following:

- The research performed must be necessary research and must be performed for justifiable reasons. The animal should not be killed for trivial or irrational reasons.
- The experimental protocol must be humane and ensue no suffering during the research process.
- There must be a small number of animals used to yield a large number of results. It is important to reduce the number of animal based research that is unlikely to result in scientific advancement (Dagg 1999).
- The animals must be able to live, reproduce and grow in a comfortable and non-threatening environment.
- Proper facilities should be available for the animals to live with sufficient space and in the presence of other animals of their own kind.

- Animals should be kept from the prospect of disease or injury by continuous care and maintenance by the researcher.

3.9.2 Humaneness

Certain humane principles guided the experimentation process. These included but were not limited to:

- While it was acknowledged that animal research should be replaced where possible, particularly in areas in which the research system would yield the same or similar results to animal based research, it was impossible to do this work without using animals.
- Dagg suggests that a pilot study should be performed prior to larger experiments (Dagg 1999). This would limit the amount of experiments carried out with a protocol that will fail to produce the desired results. The current study was based on previous studies and the number of test animals was minimized.

3.9.3 Significance

This research had a solid basis for why it was undertaken. It was significant in the advancement of science and education.

3.9.4 Personal obligation

I, Kirstin Persson, undertook the task of animal testing with a personal obligation ensuring their welfare and safety. I took personal responsibility for every experiment performed and every animal used.

CHAPTER 4

Results and discussion

This chapter outlines the results obtained from the *in vivo* anaesthetized normotensive rat experiments. The control and test drugs were infused as stated above in the protocol and the results were recorded using Chart 4 Windows software (ADInstruments). Tables and graphs explained any observed effects on the heart rate and blood pressure parameters. The results were discussed.

4.1 Individual dose response curves

The *in vivo* experiments used the anaesthetized normotensive rat model to observe the effects of individual drugs that were intravenously infused.

The parameters observed throughout the experiments included heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Adrenaline (0,05; 0,10; 0,15; 0,20 and 0,25 mg/kg) was infused and an independent dose response curve was created to compare these effects with the unknown crude aqueous extract of *Crinum macowanii*. Adrenaline was chosen because in past studies it had been found that both adrenaline and *Crinum macowanii* produced dose dependent increases on blood pressure (Mugabo *et al* 2001). As a result of the limited studies performed on *Crinum macowanii*, the drug was only inferred to act similarly to adrenaline and therefore, the antagonistic drugs that were chosen for pre-treatment were antagonistic drugs of adrenaline as well. *Crinum macowanii* (0,05; 0,10; 0,15; 0,20; 0,25 and 0,30 mg/kg) was infused as the “unknown” drug and an independent dose response curve was created to identify observed effects. Once *Crinum macowanii* was tested on its own, and the results confirmed that it increased the tested parameters, other “known” antagonistic drugs were chosen and tested independently including

atropine (0,25; 0,50; 0,75; 1,0; 1,25; 1,50 mg/kg), atenolol (0,25; 2,50; 5,0; 6,25; 7,50 mg/kg), prazosin (100; 200; 300; 400; 500 µg/kg), reserpine (0,25; 0,35; 0,45; 0,50; 0,65; 0,75 mg/kg), and verapamil (1,5; 3,0; 4,5; 6,0 mg/kg). Independent dose response curves were then created in order to find 80% of the maximal response dose of each control drug. 80% of each maximal response dose was then administered as a pre-treatment followed by IV infusion of *Crinum macowanii*. The experiments were arrested when *Crinum macowanii* ceased to produce changes in the parameters tested with increasing concentrations. Dose response curves were then created for each drug as a pre-treatment followed by IV infusion of *Crinum macowanii*. The experiments performed for each drug individually was six. The experiments performed with pre-treatment drugs equaled six as well. This number was chosen because it was the smallest number of animals that could be used in order for the results to be valid yet it also supported the ethical considerations of the project.

All possible measures were taken to create and promote an environment that was stress free for the animals. A 20-minute stabilization period for each rat was allotted after the catheters were cannulated. This allowed the heart rate and blood pressure of each rat to reach normal baseline values. Although this was instituted, variations were noted when comparing experiment baselines (some baselines were higher and other were lower).

There are different reasons that variations in heart rate and blood pressure baselines occurred (e.g. physiological factors, weight of the animal and amount of anesthesia given, blood loss during cannulation, etc.) but the actual cause cannot be identified.

The following table displays the results of the experiments performed for each pre-treatment drug individually infused in order to find 80% of maximal response dose and the paragraphs following the table further clarify the results. The Wilcoxon Rank Sum test was employed for the results.

DRUG AND DOSE RANGES	EFFECT ON HEART RATE		EFFECT ON BLOOD PRESSURE			
	Difference between lowest and highest effect (% change)	Statistical significance of the effect	SYSTOLIC		DIASTOLIC	
			Difference between lowest and highest effect (% change)	Statistical significance of the effect	Difference between lowest and highest effect (% change)	Statistical significance of the effect
adrenaline (0,05 to 0,25 mg / kg)	Increase. 10,2292 % [95% CI (19, 20)]	Significant. ($P = 0,0075$)	Increase. 53,8760 % [95% CI (43,63)]	Significant. ($P < 0,0001$)	Increase. 61,3477 % [95 % CI (46, 76)]	Significant. ($P < 0,0001$)
atropine (0,25 to 1,5mg/kg)	Decrease. 6,9377 % [95% CI (-7, 21)]	Not significant. ($P = 0,2854$)	Decrease. 31,3874 % [95%CI (6,56)]	Significant. ($P = 0,0102$)	Decrease. 44,5694 % [95%CI (23,65)]	Significant. ($P = 0,0014$)
atenolol (0,25 to 7,5mg/kg)	Decrease. 46,1676 % [95% CI (33, 59)]	Significant ($P = 0,0002$)	Decrease. 42,0420 % [95% CI (28,55)]	Significant ($P = 0,0002$)	Decrease. 46,8411 % [95% CI (33, 59)]	Significant ($P < 0,0001$)
prazosin (100 to 500 µg /kg)	Decrease. 15,6268 % [95%CI (8,23)]	Significant ($P = 0,023$)	Decrease. 49,2337 % [95%CI (44,53)]	Significant ($P < 0,0001$)	Decrease. 42,2665 % [95%CI (26,57)]	Significant ($P = 0,0006$)
verapamil (1,5 to 6,0 mg/kg)	Decrease. 29,8943 % [95%CI (22,37)]	Significant ($P < 0,0001$)	Decrease. 51,5962 % [95%CI (34,68)]	Significant ($P < 0,0003$)	Decrease. 50,7582 % [95%CI (36,65)]	Significant ($P < 0,0001$)
reserpine (0,25 to 0,75 mg/kg)	Decrease. 37,1523 % [95%CI (18,55)]	Significant. ($P = 0,0028$)	Decrease. 56,3158 % [95%CI (41,70)]	Significant ($P < 0,0001$)	Decrease. 60,7340 % [95%CI (49,71)]	Significant ($P < 0,0001$)

Table 4.1 Dose response curve findings for pre-treatment drugs.

The maximal response occurred at different doses for each “known” drug. This dose (the dose that gave the maximal response) was multiplied by 80 % (0,80) and the resulting dose was used as pre-treatment doses for each “known” drug (i.e. Atropine: the maximal response dose was found to be 1,50 mg/kg ($1,50 \text{ mg/kg} * 0,80 = 1,20 \text{ mg/kg}$). 1,2 mg/kg was used for pre-treatment. The dose-response curve results indicated that the following doses were to be used in combination with *Crinum macowanii* to elucidate the effects on the heart rate and blood pressure: adrenaline: 0,20 mg/kg, atropine: 1,2 mg/kg, atenolol: 6,0 mg/kg, prazosin: 400 µg/kg, verapamil: 4,8 mg/kg, and reserpine: 0,6 mg/kg.

4.1.1 Effect of adrenaline

4.1.1.1 Effect on heart rate (HR)

Figures 4.1 and 4.2 display the effect of adrenaline on heart rate administered in the dose range of 0,05 to 0,25 mg/kg for three minutes. Increased doses of adrenaline caused significant ($P < 0,01$) dose dependent increases on the heart rate. The lowest dose of 0,05 mg/kg to the highest dose of 0,25 mg/kg showed an increase of 10,2292 % [95% CI (0.19, 20)]. The rats died immediately after the infusion of 0,25 mg/kg.

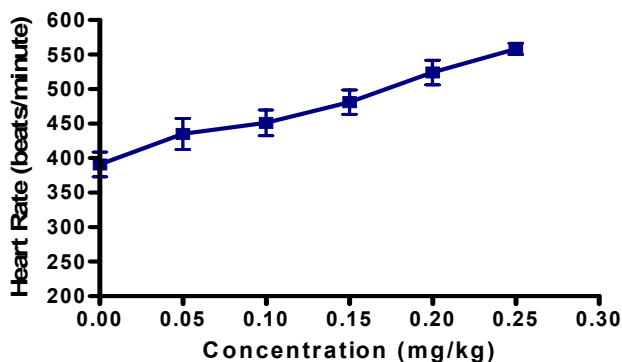


Figure 4.1 Effect of adrenaline on heart rate; Mean \pm SEM, n=6 (dose response curve)

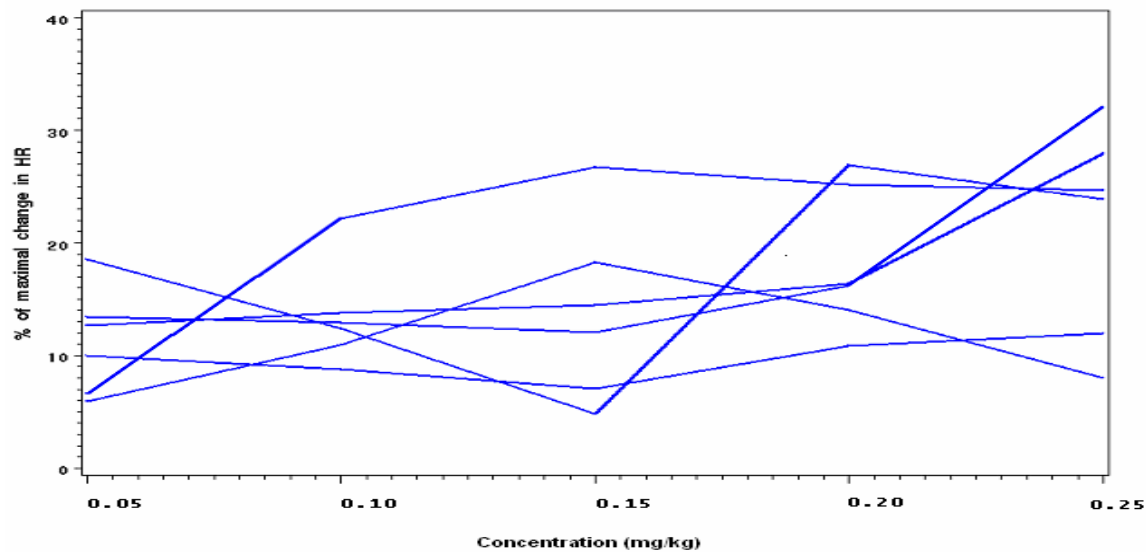


Figure 4.2 Effect of adrenaline on heart rate of each individual rat in dataset (percentage change graph)

4.1.1.2 Effect on systolic blood pressure (SBP)

The effect of adrenaline on systolic blood pressure is pictured in figures 4.3 and 4.4. Adrenaline produced significant ($P < 0,0001$) dose dependent increases in systolic blood pressure from the lowest to the highest dose. The change from 0,05 mg/kg to 0,20 mg/kg was 53,8760 % [95% CI (43,63)]. The rats died immediately after the infusion of 0,25 mg/kg.

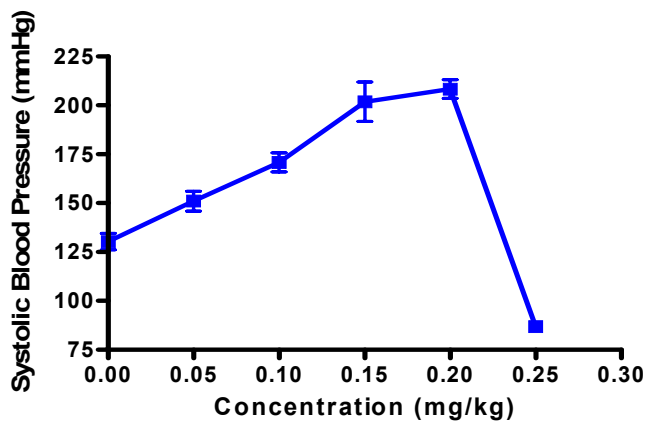


Figure 4.3 Effect of adrenaline on systolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

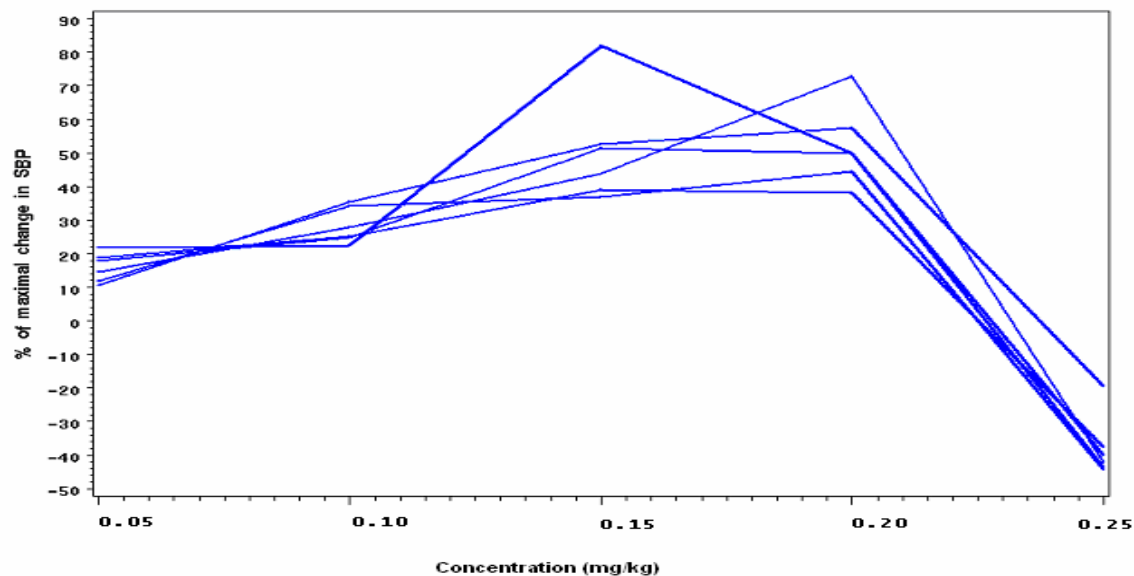


Figure 4.4 Effect of adrenaline on systolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.1.3 Effect on diastolic blood pressure (DBP)

Figures 4.5 and 4.6 show the effect of adrenaline on diastolic blood pressure. Adrenaline produced significant ($P < 0,0001$) dose dependent increases at all doses from the lowest (0,05 mg/kg) to the highest dose (0,25 mg/kg). The increase from 0,05 mg/kg to 0,20 mg/kg was 61,3477 % [95 % CI (46, 76)]. The rats died immediately after the infusion of 0,25 mg/kg.

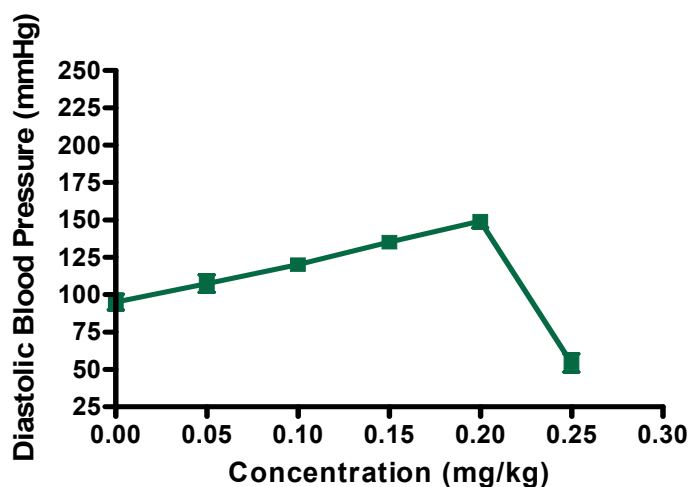


Figure 4.5 Effect of adrenaline on diastolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

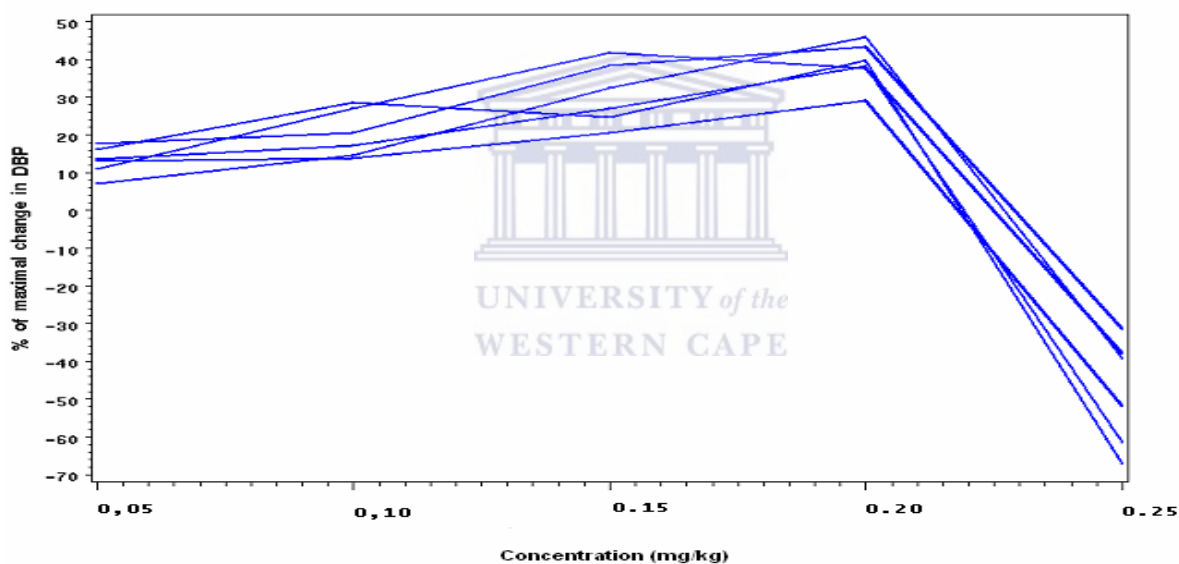


Figure 4.6 Effect of adrenaline on diastolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.2 Effect of *Crinum macowanii*

4.1.2.1 Effect on heart rate (HR)

Crinum macowanii produced significant ($P = 0,0011$) dose dependent increases in heart rate at all doses within the ranges of 0,05 to 0,30 mg/kg as pictured in figures 4.7 and 4.8. There was an

11,6637 % [95%CI (6,17)] difference between the lowest dose of 0,05 mg/kg and the highest dose of 0,30 mg/kg. The rats died immediately after the *C. macowanii* dose of 0,30 mg/kg was infused.

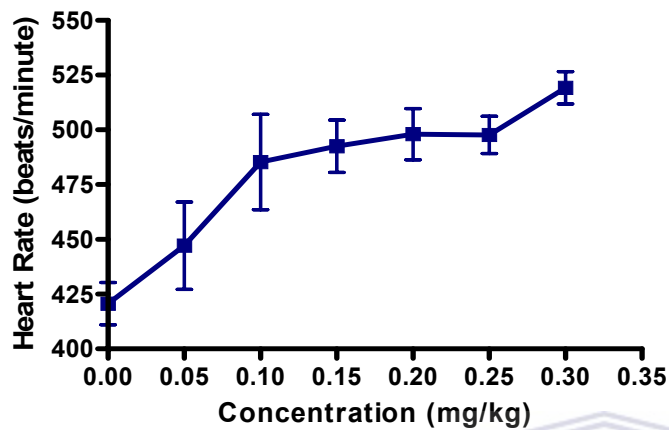


Figure 4.7 Effect of *Crinum macowanii* on heart rate; Mean \pm SEM, n=6 (dose response curve)

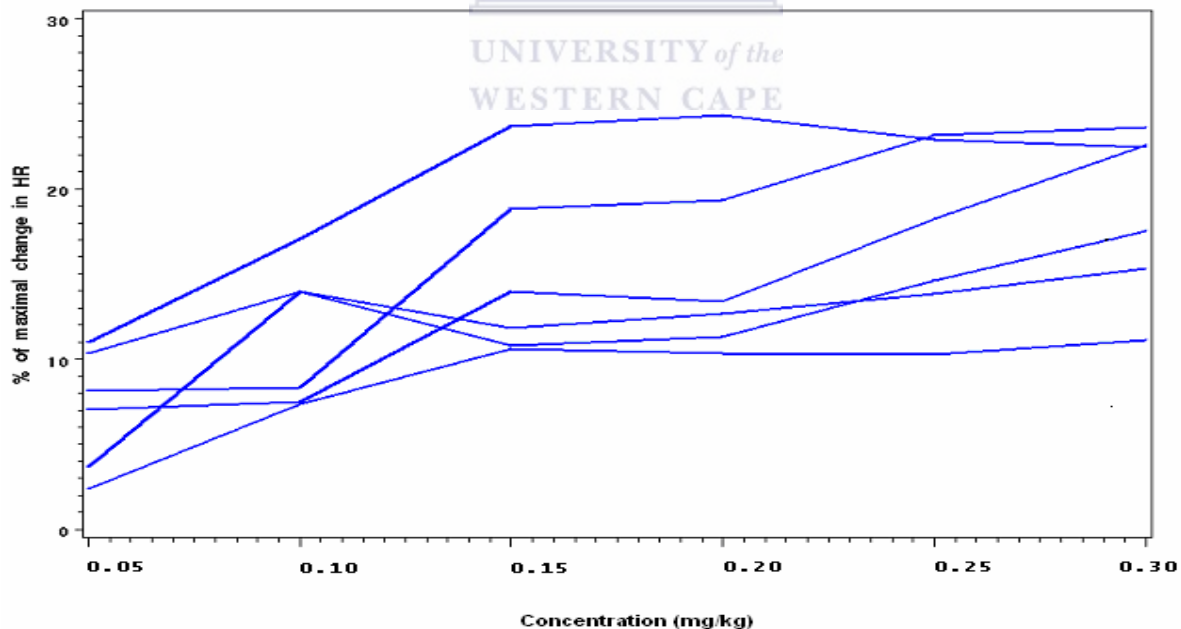


Figure 4.8 Effect of *Crinum macowanii* on heart rate of each individual rat in dataset (percentage change graph)

4.1.2.2 Effect on systolic blood pressure (SBP)

Crinum macowanii produced significant ($P < 0,0001$) dose dependent increases in systolic blood pressure at all doses. The lowest dose of 0,05 mg/kg to the highest dose of 0,30 mg/kg produced a 66,9383 % [95% CI (59,74)] change. At low and intermediate dosages of 0,05 mg/kg to 0,20 mg/kg *Crinum macowanii* increased the systolic blood pressure. At high dosages of 0,25 mg/kg and 0,30 mg/kg, the crude aqueous extract decreased systolic blood pressure. The rats died immediately after the dose of 0,30 mg/kg (Figures 4.9 and 4.10) was infused.

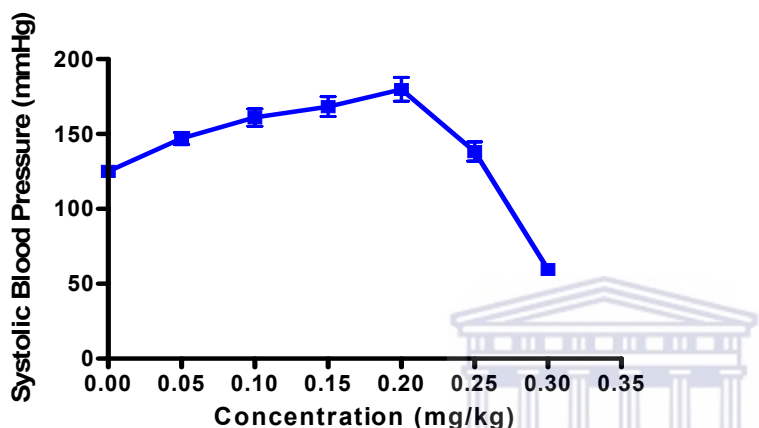


Figure 4.9 Effect of *Crinum macowanii* on systolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

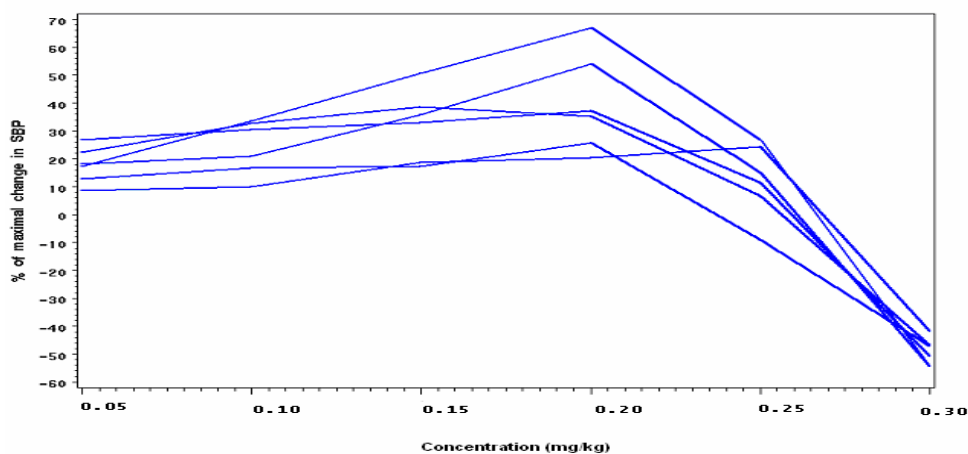


Figure 4.10 Effect of *Crinum macowanii* on systolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.2.3 Effect on diastolic blood pressure (DBP)

Crinum macowanii produced significant ($P < 0,0001$) dose dependent effects on diastolic blood pressure at all doses. From the lowest dose of 0,05 mg/kg to the highest dose of 0,30 mg/kg the percentage change was 86,1631% [95% CI (76,96)]. At low and intermediate dosages of 0,05 mg/kg to 0,20 mg/kg *Crinum macowanii* increased the diastolic blood pressure. At high dosages of 0,25 mg/kg and 0,30 mg/kg, the crude aqueous extract decreased diastolic blood pressure. The rats died immediately after the infusion of 0,30 mg/kg (Figures 4.11 and 4.12).

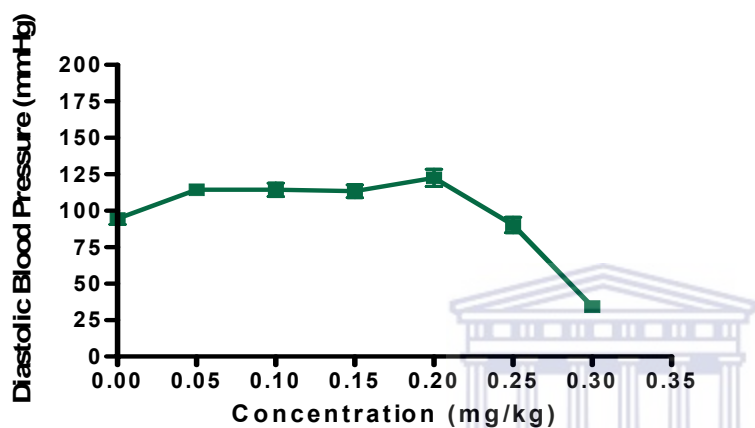


Figure 4.11 Effect of *Crinum macowanii* on diastolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

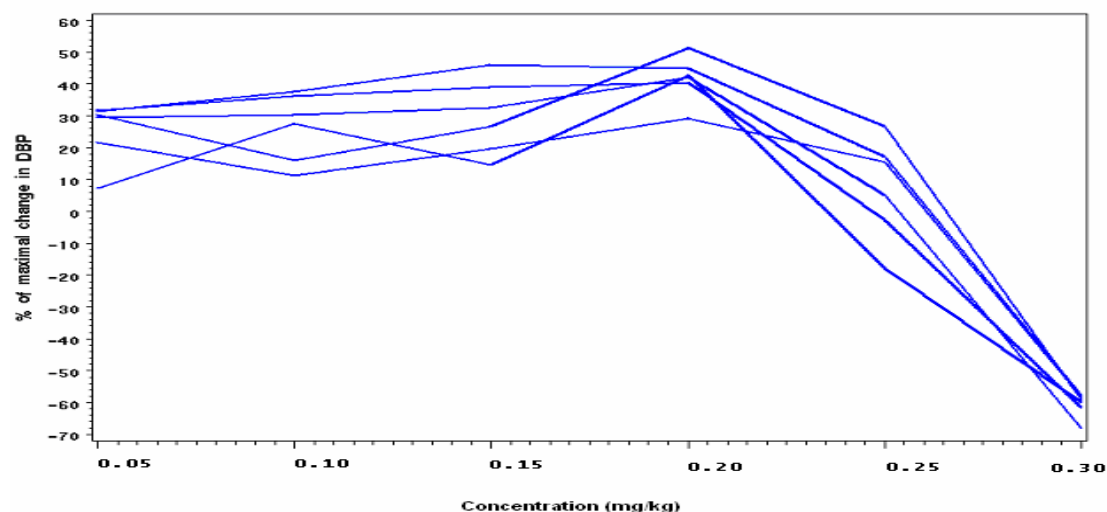


Figure 4.12 Effect of *Crinum macowanii* on diastolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.3 Effect of atropine

4.1.3.1 Effect on heart rate (HR)

Figures 4.13 and 4.14 displays the dose dependent decrease of atropine on heart rate. The difference between the lowest dose of 0,25 mg/kg compared to the highest dose of 1,5 mg/kg was 6,9377 % [95% CI (-7, 21)]. Although atropine lowered the heart rate, it did not do so significantly ($P = 0,2854$) from the lowest to the highest dose.

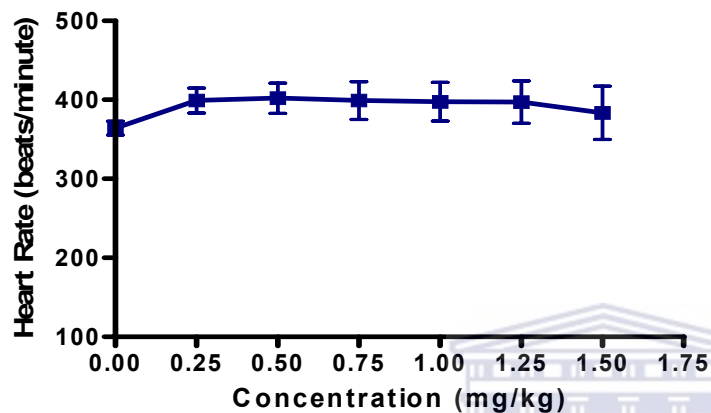


Figure 4.13 Effect of atropine on heart rate; Mean \pm SEM, n=6 (dose response curve)

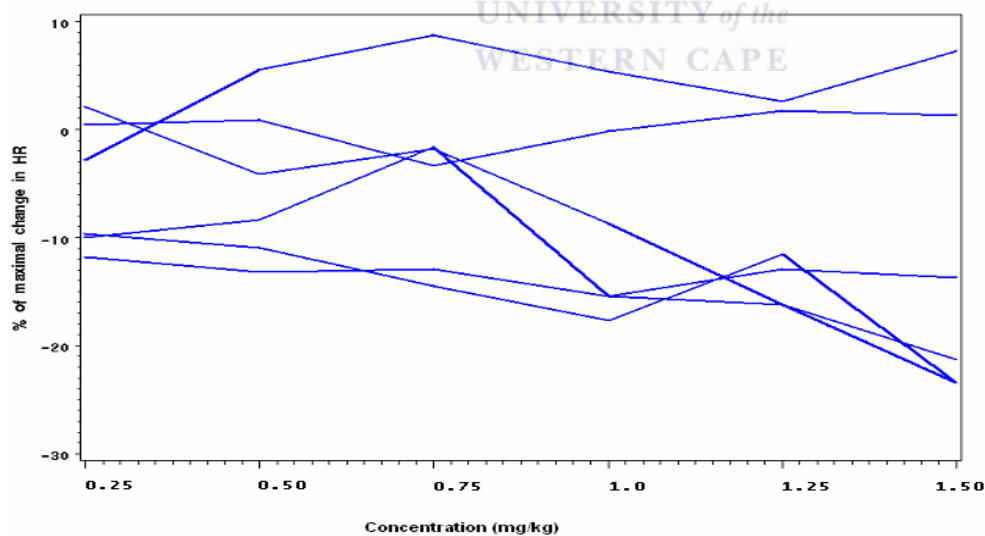


Figure 4.14 Effect of atropine on heart rate of each individual rat in dataset (percentage change graph)

4.1.3.2 Effect on systolic blood pressure (SBP)

Atropine produced a dose dependent decrease in systolic blood pressure as expressed in figures 4.15 and 4.16. From the lowest dose of 0,25 mg/kg to the highest dose of 1,5 mg/kg a percentage change of 31,3874 % [95% CI (6,56)] was observed. Results found that atropine lowered the systolic blood pressure significantly ($P = 0,0102$) when the lowest dose was compared to the highest dose.

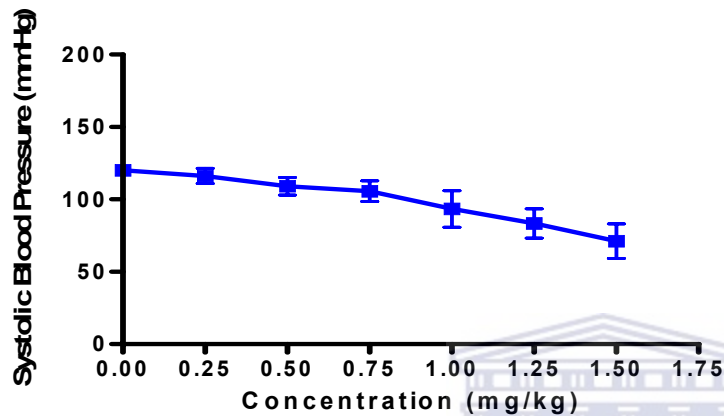


Figure 4.15 Effect of atropine on systolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

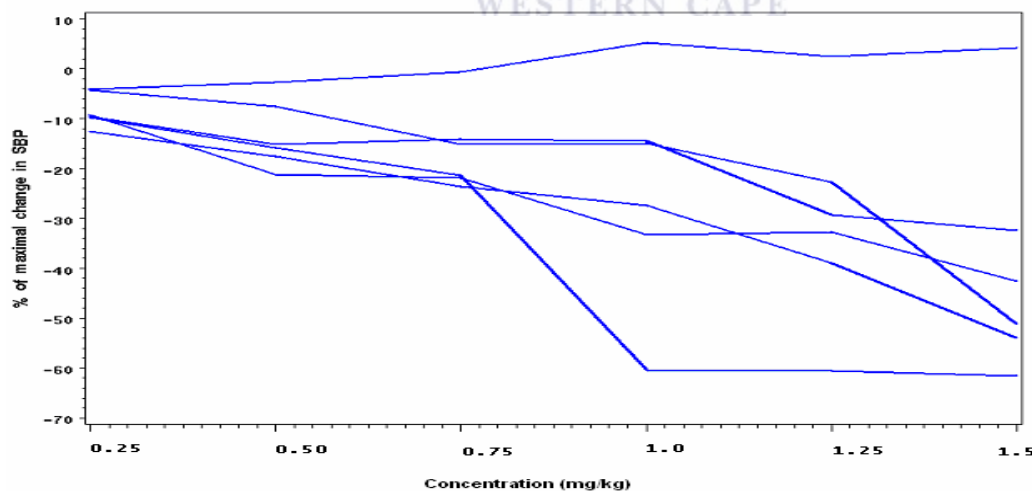


Figure 4.16 Effect of atropine on systolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.3.3 Effect on diastolic blood pressure (DBP)

A significant ($P = 0,0014$) dose dependent decrease in diastolic blood pressure was found when the lowest dose of atropine was compared to the highest dose (Figures 4.17 and 4.18). The difference between the lowest dose of 0,25 mg/kg and the highest dose of 1,5 mg/kg was 44,5694 % [95% CI (23,65)].

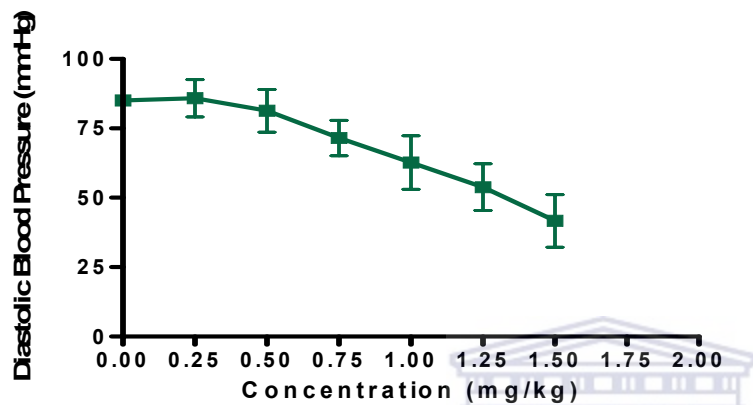


Figure 4.17 Effect of atropine on diastolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

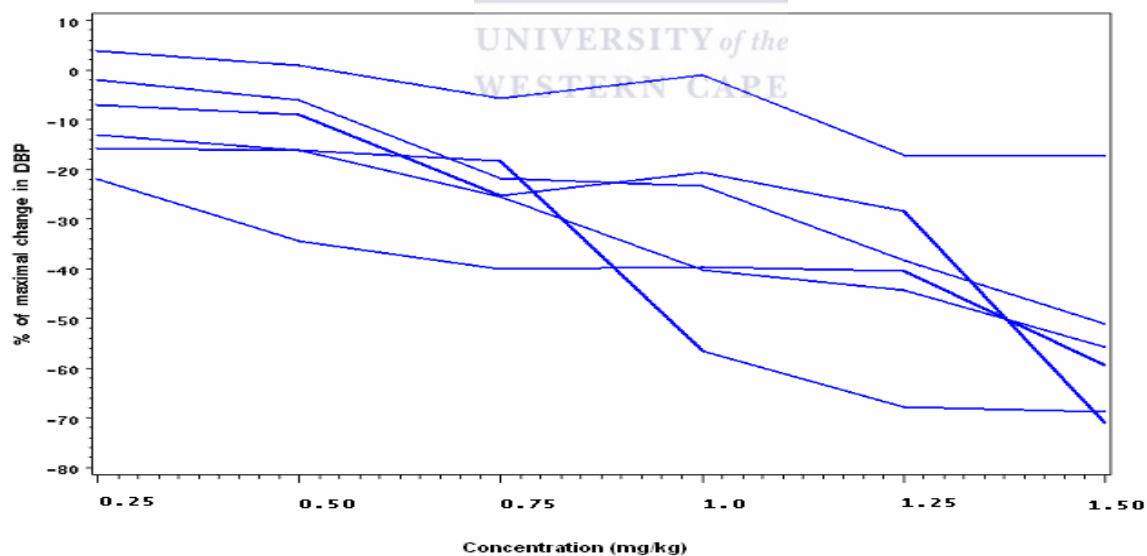


Figure 4.18 Effect of atropine on diastolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.4 Effect of atenolol

4.1.4.1 Effect on heart rate (HR)

Results indicated that atenolol produced a highly significant ($P = 0,0002$) dose dependent decrease in heart rate when the lowest dose was compared with the highest dose. Figures 4.19 and 4.20 express these effects. The change between the lowest dose of 0,25 mg/kg and the highest dose of 7,5 mg/kg was 46,1676 % [95% CI (33, 59)].

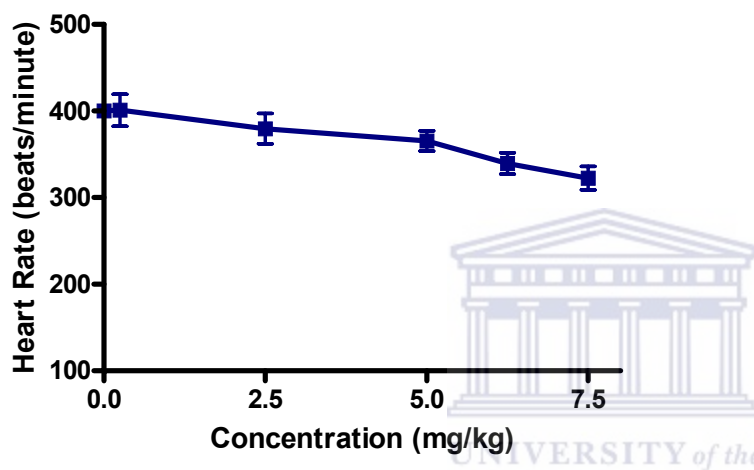


Figure 4.19 Effect of atenolol on heart rate; Mean \pm SEM, n=6 (dose response curve)

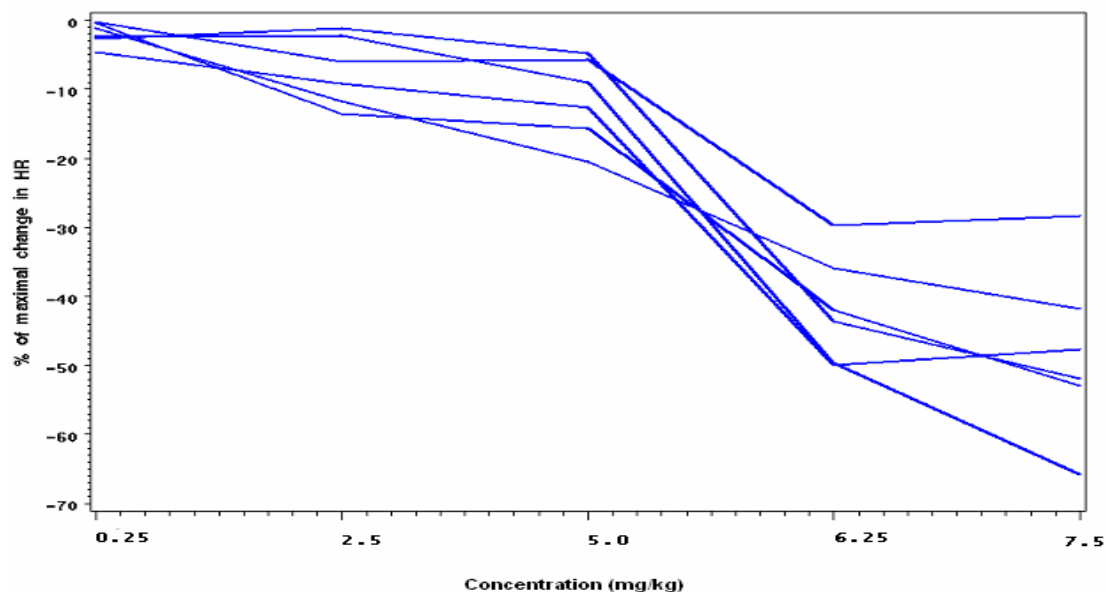


Figure 4.20 Effect of atenolol on heart rate of each individual rat in dataset (percentage change graph)

4.1.4.2 Effect on systolic blood pressure (SBP)

It was found that atenolol produced a significant ($P = 0,0002$) dose dependent decrease in systolic blood pressure with increased doses, when the lowest and highest dosages were compared (Figures 4.21 and 4.22). The difference between the lowest dose of 0,25 mg/kg and the highest dose of 7,5 mg/kg was 42,0420 % [95% CI (28,55)].

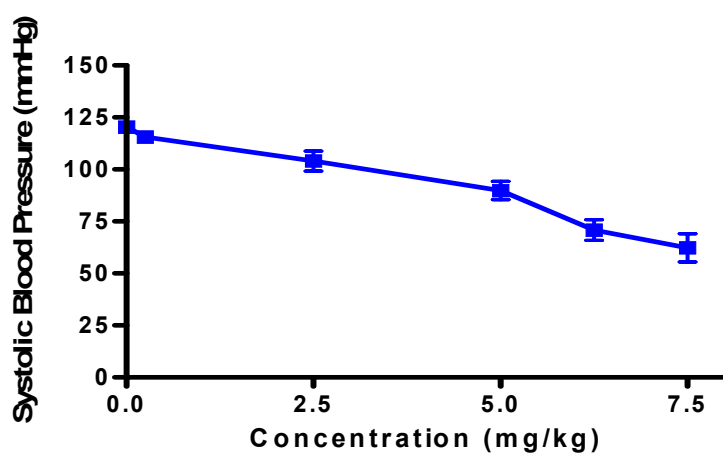
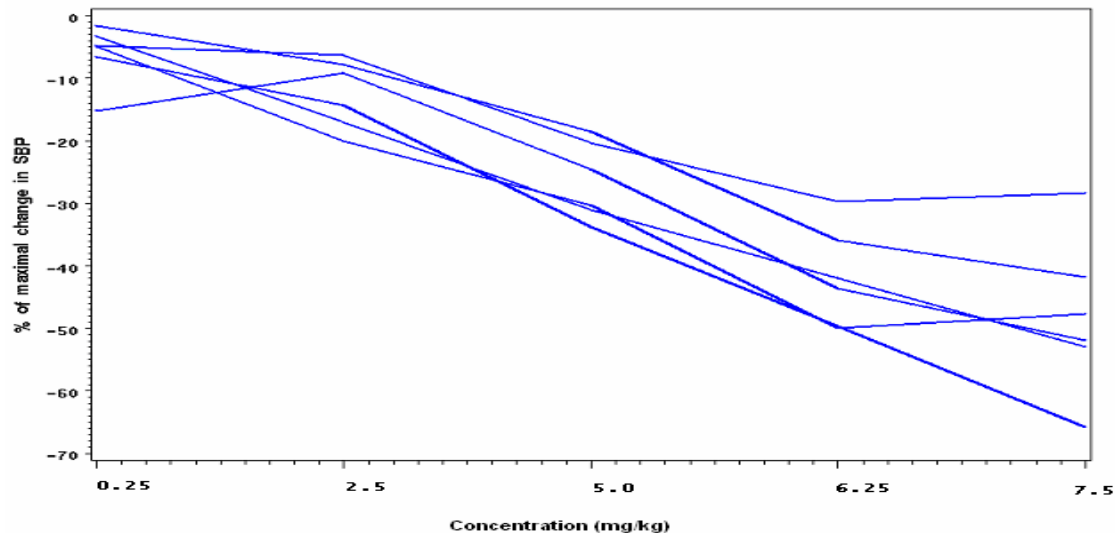


Figure 4.21 Effect of atenolol on systolic blood pressure; Mean \pm SEM, n=6 (dose response curve)



Figures 4.22 Effect of atenolol on systolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.4.3 Effect on diastolic blood pressure (DBP)

A significant ($P < 0,0001$) dose dependent decrease in diastolic blood pressure was observed with increased doses of atenolol. The lowest dose (0,25 mg/kg) to the highest dose (7,5 mg/kg) produced a 46,8411 % [95% CI (33, 59)] change in diastolic pressure (Figures 4.23 and 4.24).

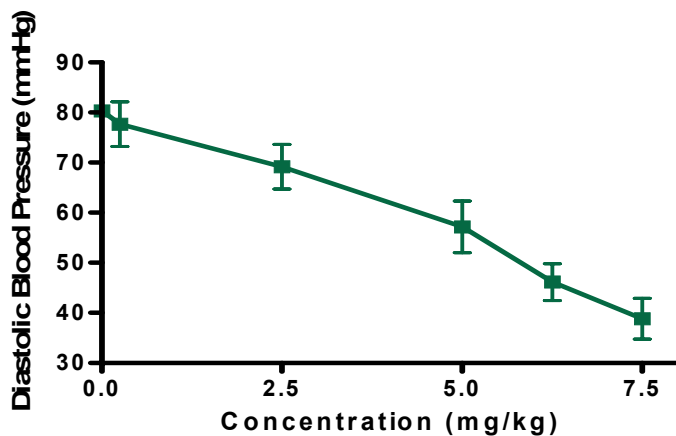


Figure 4.23 Effect of atenolol on diastolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

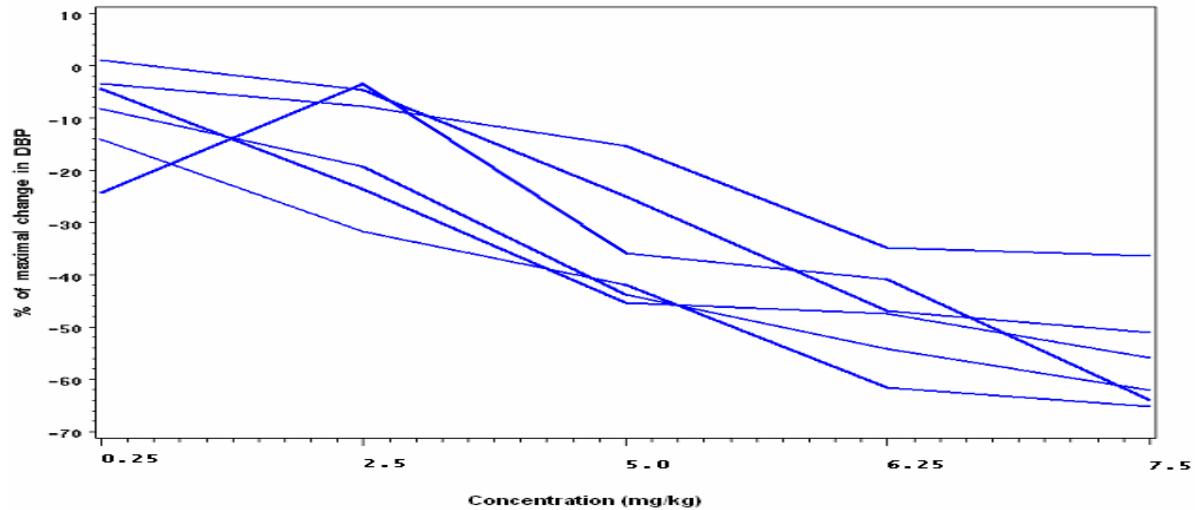


Figure 4.24 Effect of atenolol on diastolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.5 Effect of prazosin

4.1.5.1 Effect on heart rate (HR)

Prazosin produced a dose dependent decrease in heart rate with a difference of 15,6268 % [95% CI (8,23)] between the lowest dose of 100 $\mu\text{g/kg}$, and the highest dose of 500 $\mu\text{g/kg}$. The change was found to be significant ($P = 0,023$) when the lowest and highest dosages were compared (Figures 4.25 and 4.26).

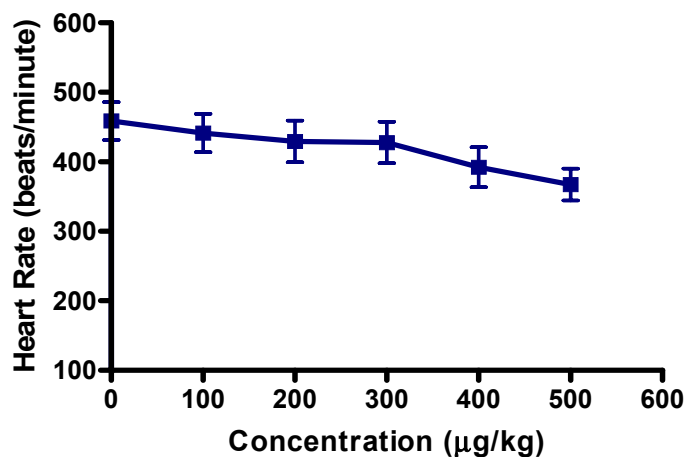


Figure 4.25 Effect of prazosin on heart rate; Mean \pm SEM, $n=6$ (dose response curve)

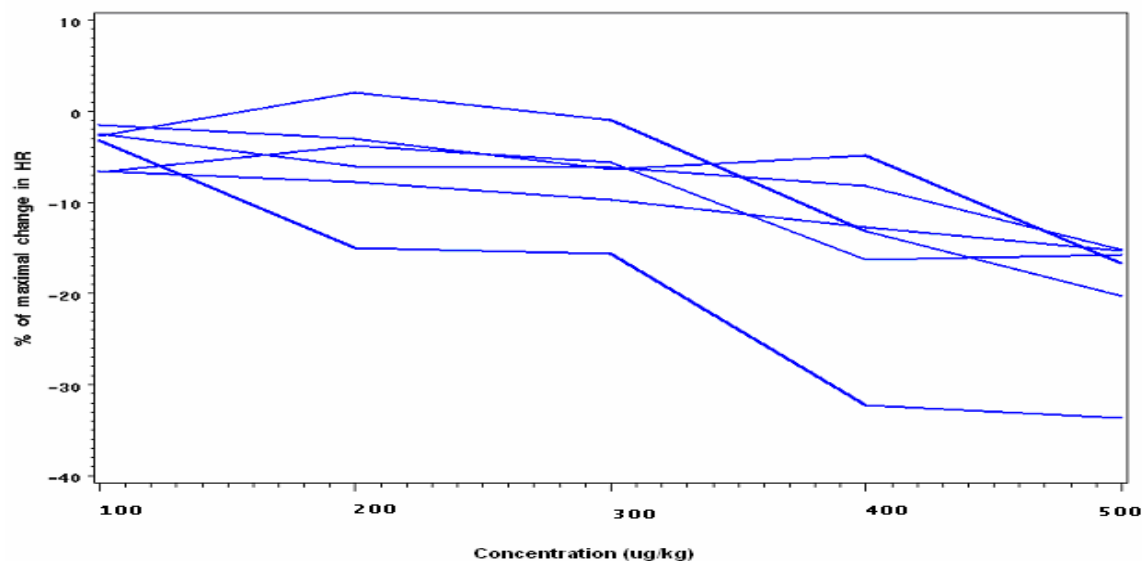


Figure 4.26 Effect of prazosin on heart rate of each individual rat in dataset (percentage change graph)

4.1.5.2 Effect on systolic blood pressure (SBP)

Prazosin was found to cause significant ($P < 0,0001$) dose dependent decreases in systolic blood pressure when the lowest dose was compared to the highest dose. The change between the lowest dose of 100 $\mu\text{g/kg}$ and the highest dose of 500 $\mu\text{g/kg}$ was 49,2337 % [95% CI (44,53)] (Figures 4.27 and 4.28).

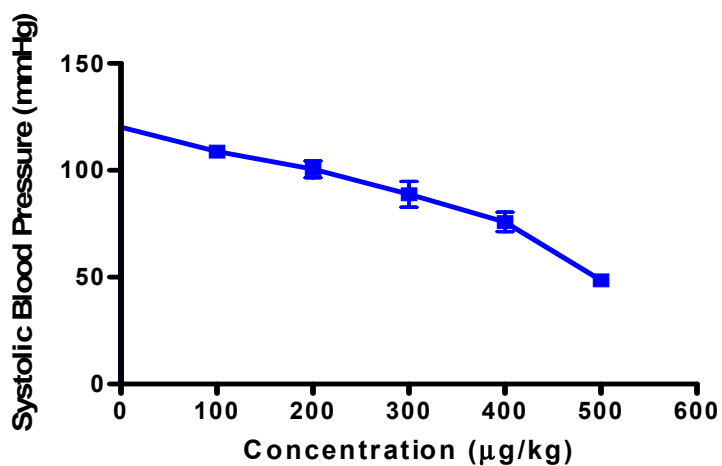


Figure 4.27 Effect of prazosin on systolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

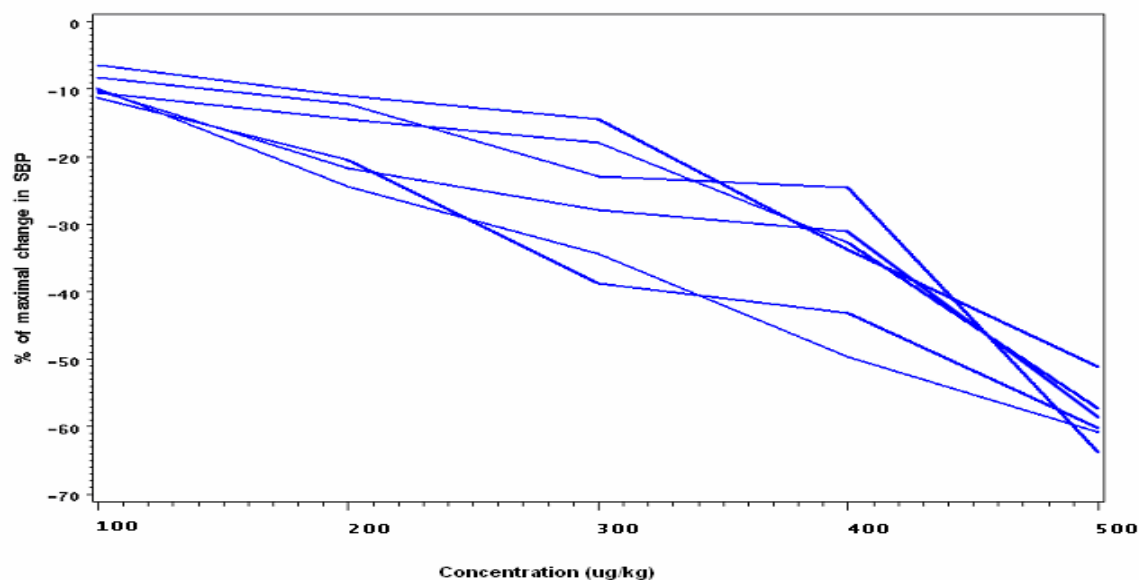


Figure 4.28 Effect of prazosin on systolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.5.3 Effect on diastolic blood pressure (DBP)

Prazosin was found to produce significant ($P = 0,0006$) dose dependent decreases on diastolic blood pressure when the lowest dose was compared to the highest dose (Figures 4.29 and 4.30). The difference between the lowest dose of 100 $\mu\text{g/kg}$ and the highest dose of 500 $\mu\text{g/kg}$ was a change of 42,2665 % [95%CI(26,57)].

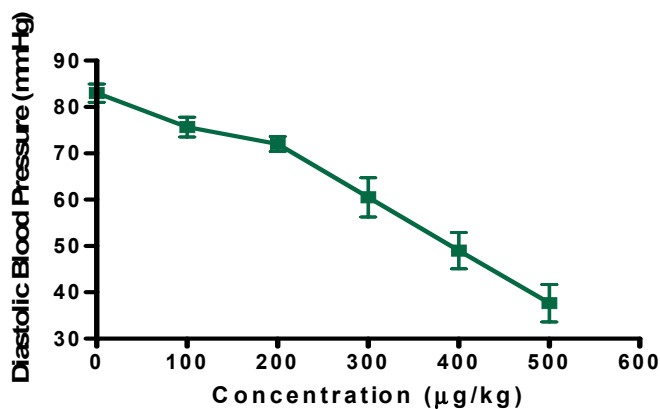


Figure 4.29 Effect of prazosin on diastolic blood pressure; Mean +/- SEM, n=6 (dose response curve)

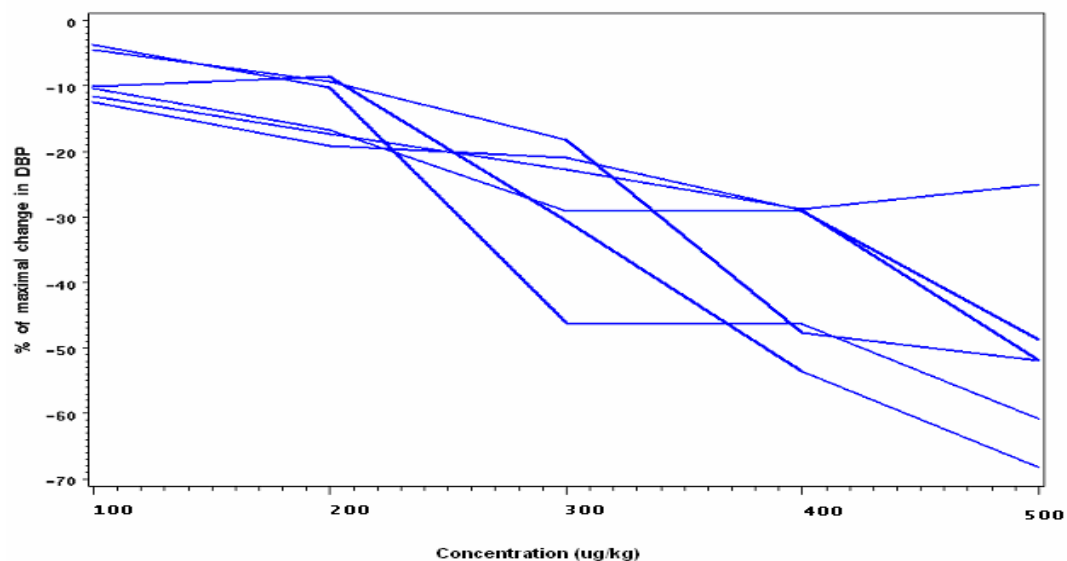


Figure 4.30 Effect of prazosin on diastolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.6 Effect of verapamil

4.1.6.1 Effect on heart rate (HR)

Verapamil produced a dose dependent decrease in heart rate as the dose increased. The change between the lowest dose of 1,5 mg/kg and the highest dose of 6,0 mg/kg was significant ($P < 0,0001$). The percentage change of the lowest dose and the highest dose was 29,8943 % [95% CI (22,37)] (Figures 4.31 and 4.32).

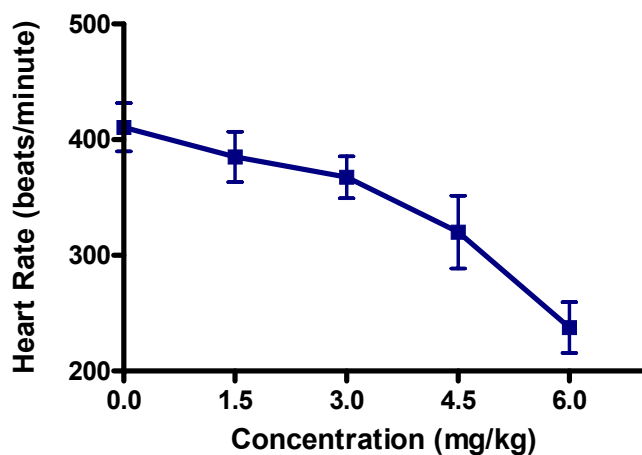


Figure 4.31 Effect of verapamil on heart rate; Mean \pm SEM, n=6 (dose response curve)

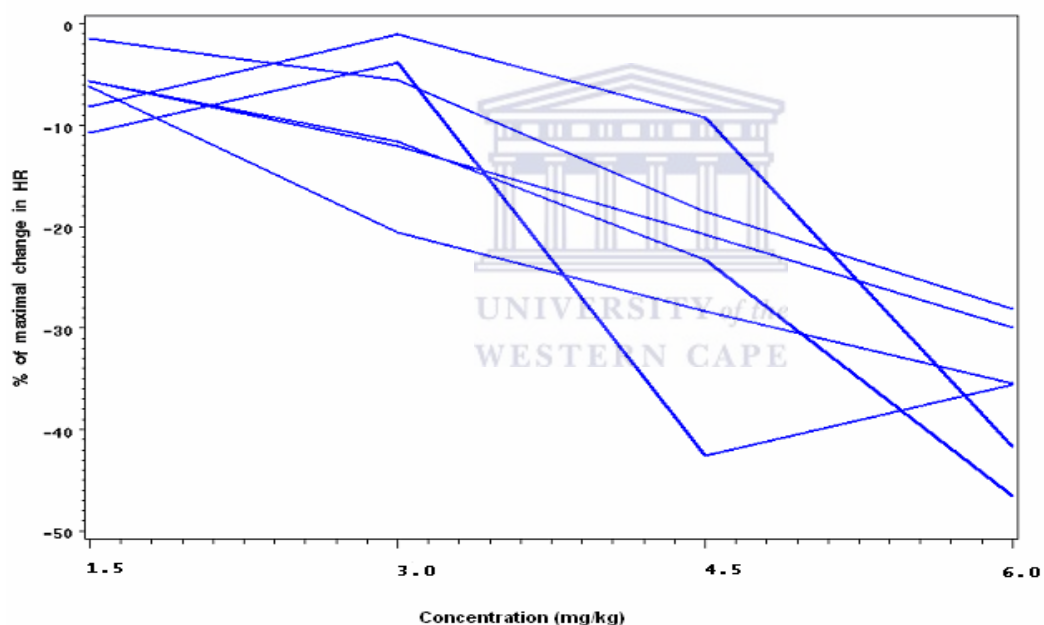


Figure 4.32 Effect of verapamil on heart rate of each individual rat in dataset (percentage change graph)

4.1.6.2 Effect on systolic blood pressure (SBP)

Verapamil produced a dose dependent decrease on systolic blood pressure with increasing doses. The difference between the lowest dose of 1,5 mg/kg and the highest dose of 6,0 mg/kg decreased the pressure by 51,5962 % [95% CI (34,68)]. The overall decrease between the lowest dose and the highest dose was significant ($P < 0,0003$) (Figures 4.33 and 4.34).

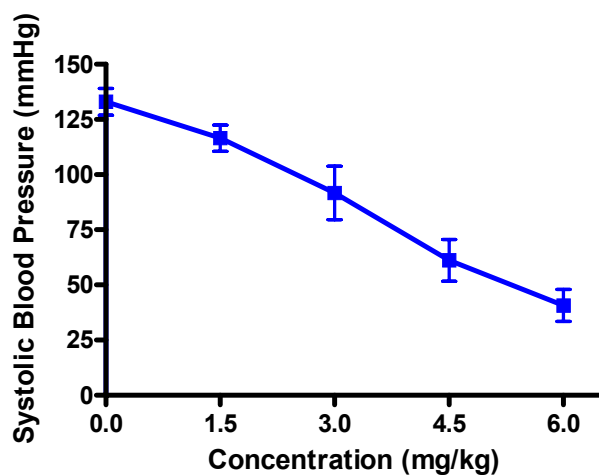


Figure 4.33 Effect of verapamil on systolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

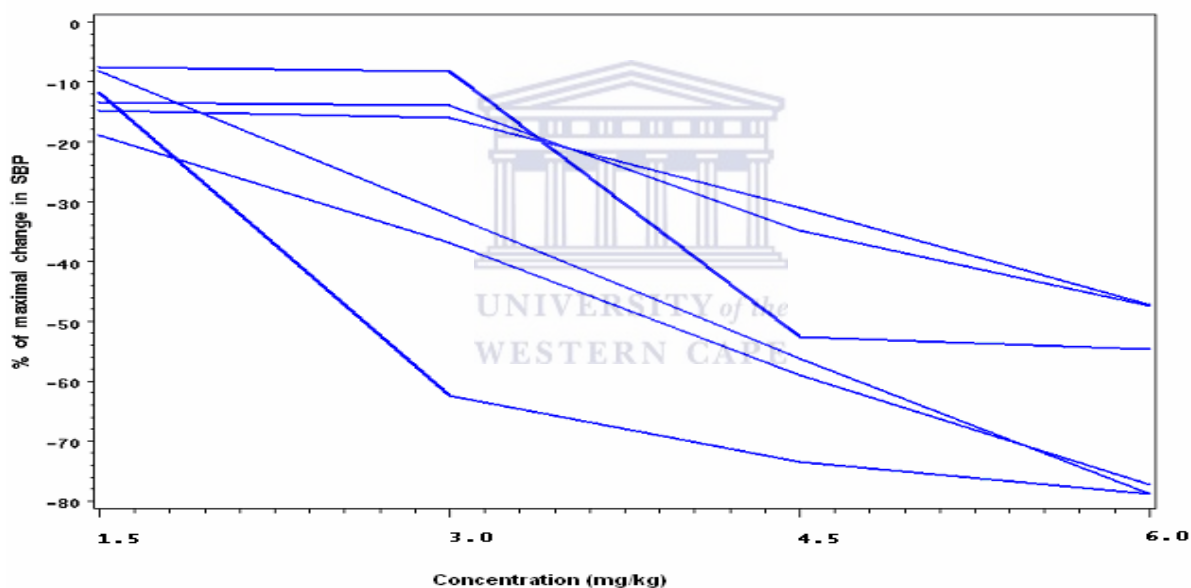


Figure 4.34 Effect of verapamil on systolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.6.3 Effect on diastolic blood pressure (DBP)

Verapamil produced a decrease on diastolic blood pressure as the concentration increased as seen in figures 4.35 and 4.36. The difference between the lowest dose of 1,5 mg/kg and the highest

dose of 6,0 mg/kg caused a 50,7582 % [95% CI (36,65)] decrease in pressure. The change was significant ($P < 0,0001$).

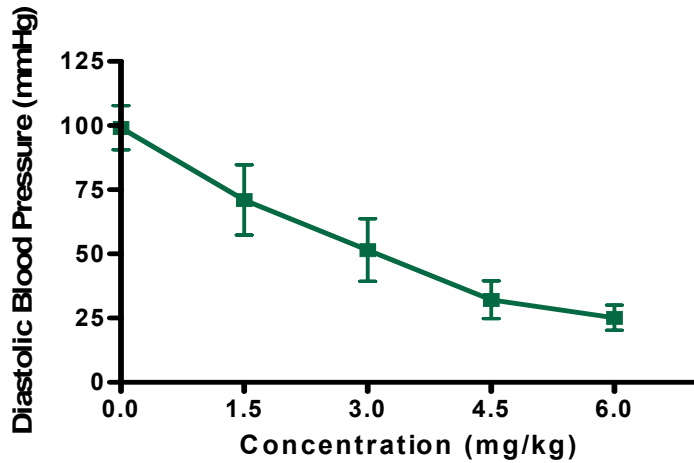


Figure 4.35 Effect of verapamil on diastolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

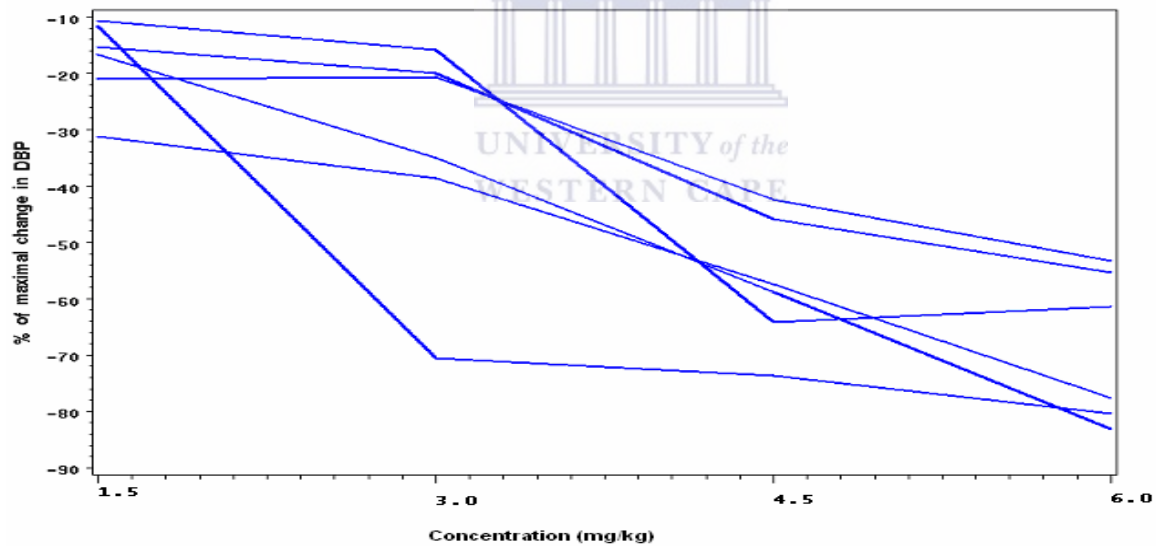


Figure 4.36 Effect of verapamil on diastolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.7 Effect of reserpine

4.1.7.1 Effect on heart rate (HR)

Reserpine significantly ($P = 0,0028$) decreased the heart rate at all doses as the dosage increased from the lowest dose to the highest dose. The difference between the lowest dose of 0,25 mg/kg and the highest dose of 0,75 mg/kg was 37,1523 % [95% CI (18,55)] (Figures 4.37 and 4.38).

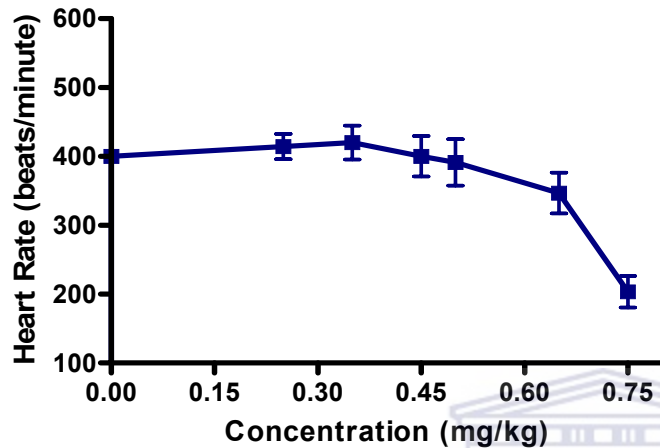


Figure 4.37 Effect of reserpine on heart rate; Mean \pm SEM, n=6 (dose response curve)

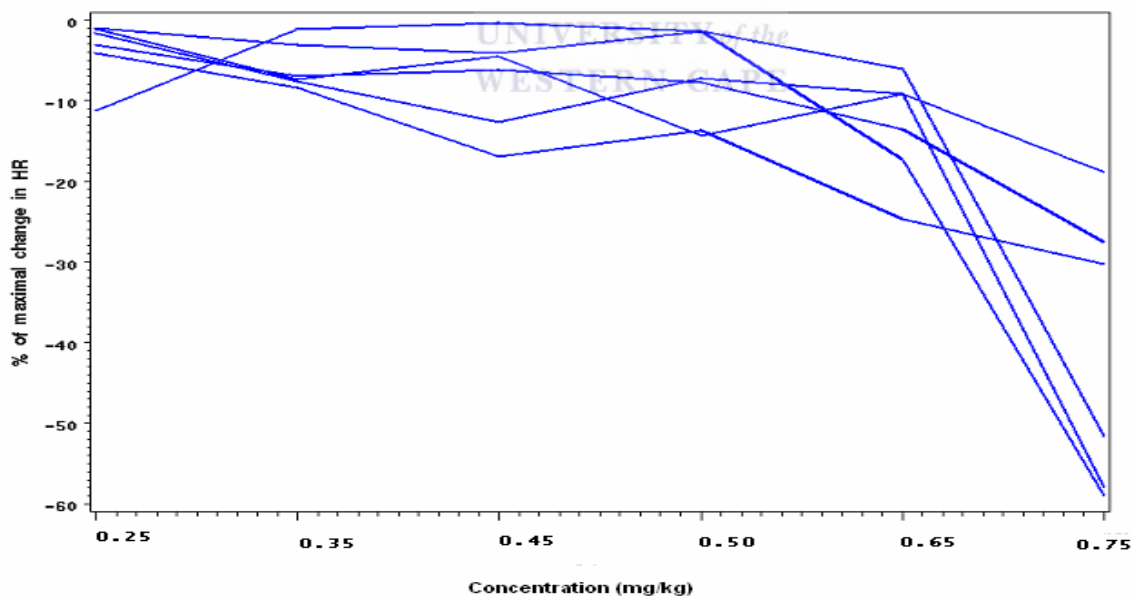


Figure 4.38 Effect of reserpine on heart rate of each individual rat in dataset (percentage change graph)

4.1.7.2 Effect on systolic blood pressure (SBP)

Reserpine produced a significant ($P < 0,0001$) dose dependent decrease on systolic blood pressure as dosages increased. The difference between the lowest dose of 0,25 mg/kg and the highest dose of 0,75 mg/kg was 56,3158 % [95% CI (41,70)]. The change between the lowest dose and the highest doses was significant ($P < 0,0001$) (Figures 4.39 and 4.40).

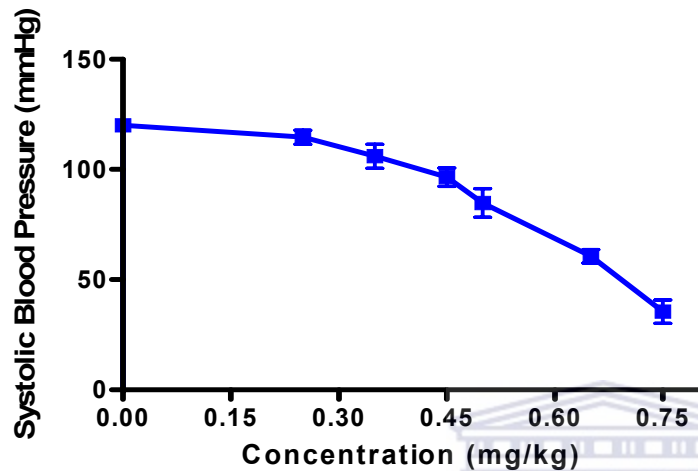


Figure 4.39 Effect of reserpine on systolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

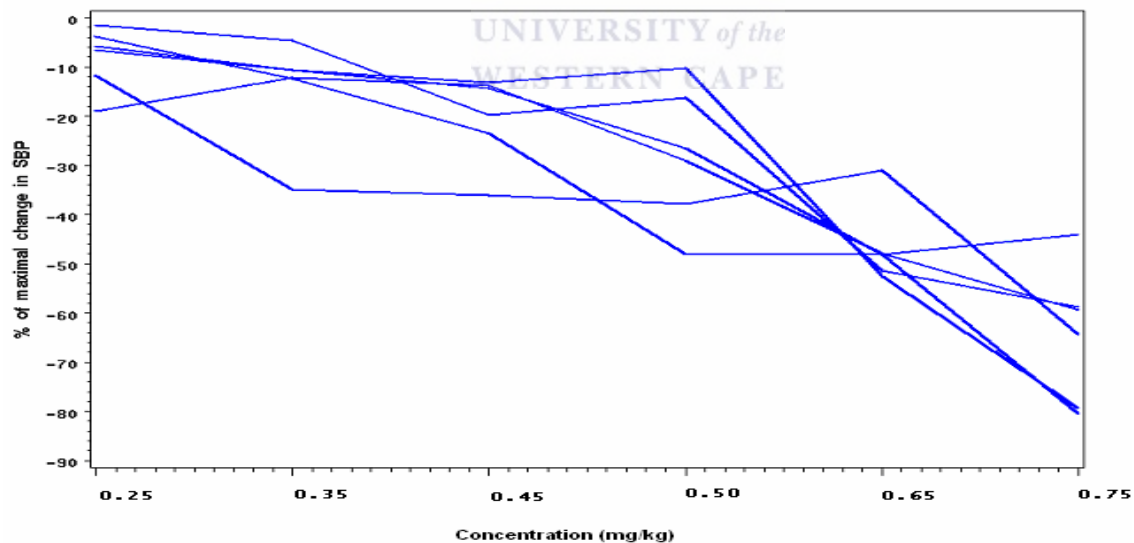


Figure 4.40 Effect of reserpine on systolic blood pressure of each individual rat in dataset (percentage change graph)

4.1.7.3 Effect on diastolic blood pressure (DBP)

There was a dose dependent decrease in diastolic blood pressure for all doses with the introduction of reserpine (Figures 4.41 and 4.42). The change between the lowest dose of 0,25 mg/kg and the highest dose of 0,75 mg/kg was 60,7340 % [95% CI (49,71)]. The change was significant ($P < 0,0001$).

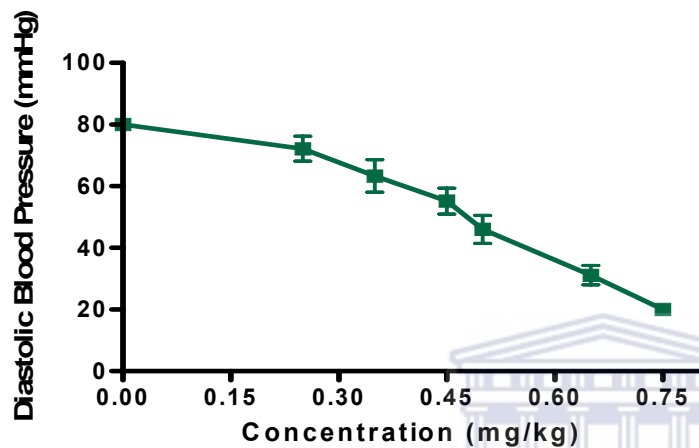


Figure 4.41 Effect of reserpine on diastolic blood pressure; Mean \pm SEM, n=6 (dose response curve)

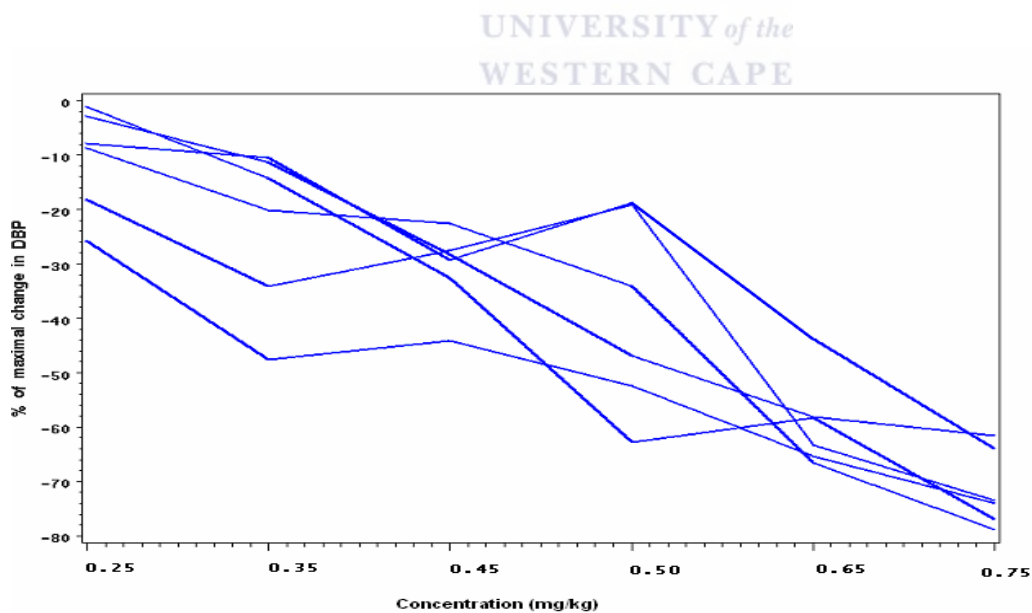


Figure 4.42 Effect of reserpine on diastolic blood pressure of each individual rat in dataset (percentage change graph)

4.2 Effect of *Crinum macowanii* combined with control drugs

4.2.1 Effect of *Crinum macowanii* when pre-treated with atropine

4.2.1.1 Effect on heart rate

Rats pre-treated with atropine (1,2 mg/kg) and then given *Crinum macowanii* produced similar changes in heart rate when compared with rats that were administered *Crinum macowanii* alone. The lowest dose of *Crinum macowanii* (0,05 mg/kg) produced a 2,5873 % [95% CI (-11,16)] increase in heart rate when compared with *Crinum macowanii* on its own. The highest dose of *Crinum* (0,25 mg/kg) produced an increase of 14,2976 % [95% CI (-5,34)] when compared to rats treated with *Crinum macowanii* on its own. All doses produced similar changes ($P > 0,01$) (Figures 4.43 and 4.44).

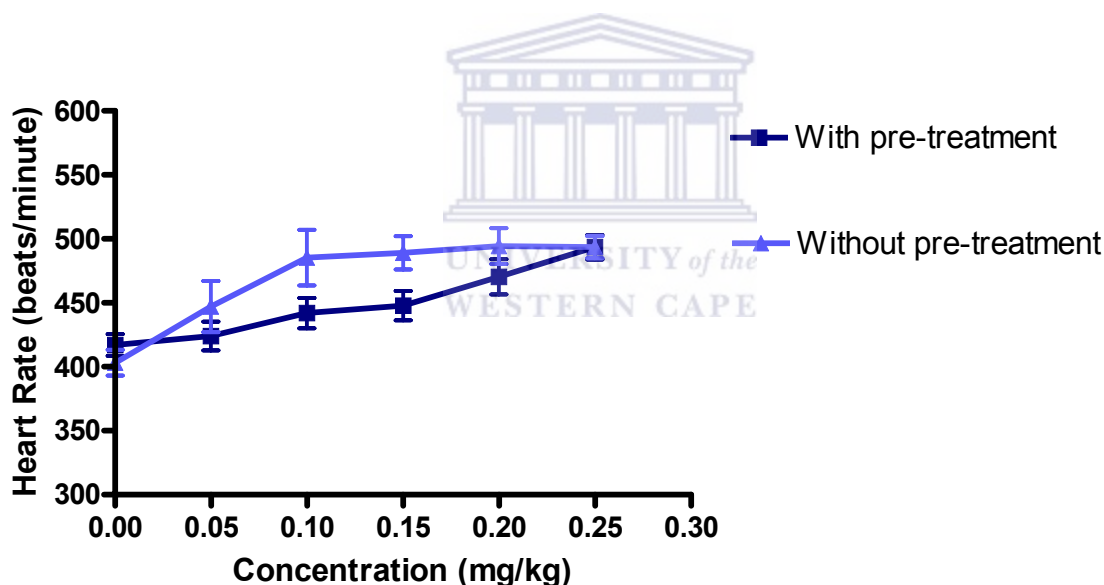


Figure 4.43 Effect of *Crinum macowanii* on heart rate in rats pre-treated with atropine; Mean \pm SEM, n=6 (dose response curve)

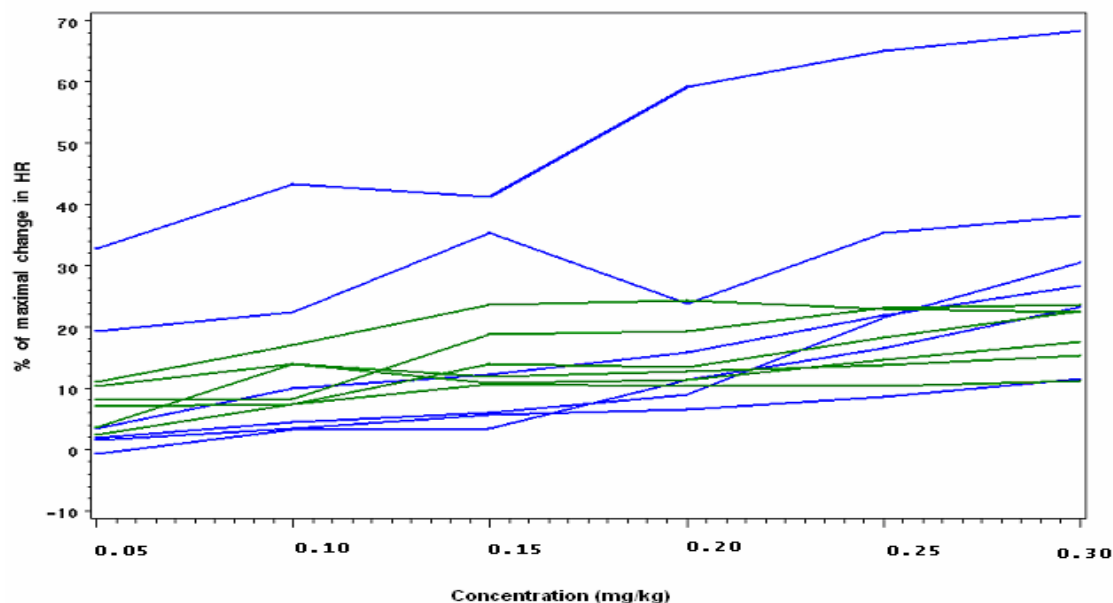


Figure 4.44 Effect of *Crinum macowanii* on heart rate in each individual rat pre-treated with atropine (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.1.2 Effect on systolic blood pressure (SBP)

Rats pre-treated with atropine (1,2 mg/kg) and then administered *Crinum macowanii* produced similar changes in systolic blood pressure when compared with rats that were administered *Crinum macowanii* on its own as seen in figures 4.45 and 4.46. At the lowest dose of 0,05 mg/kg the change of $-3,7880\%$ [95% CI (-15,8)] was observed and at the highest dose of 0,25 mg/kg an $18,7286\%$ [95% CI (-18,55)] change was seen when compared with *Crinum macowanii* infused on its own. The overall changes were similar at all doses ($P > 0,01$).

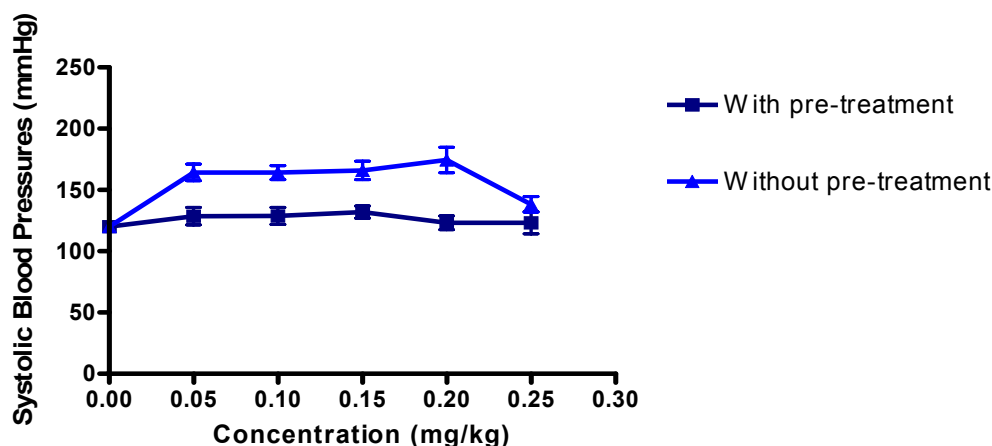


Figure 4.45 Effect of *Crinum macowanii* on systolic blood pressure in rats pre-treated with atropine; Mean \pm SEM, n=6 (dose response curve)

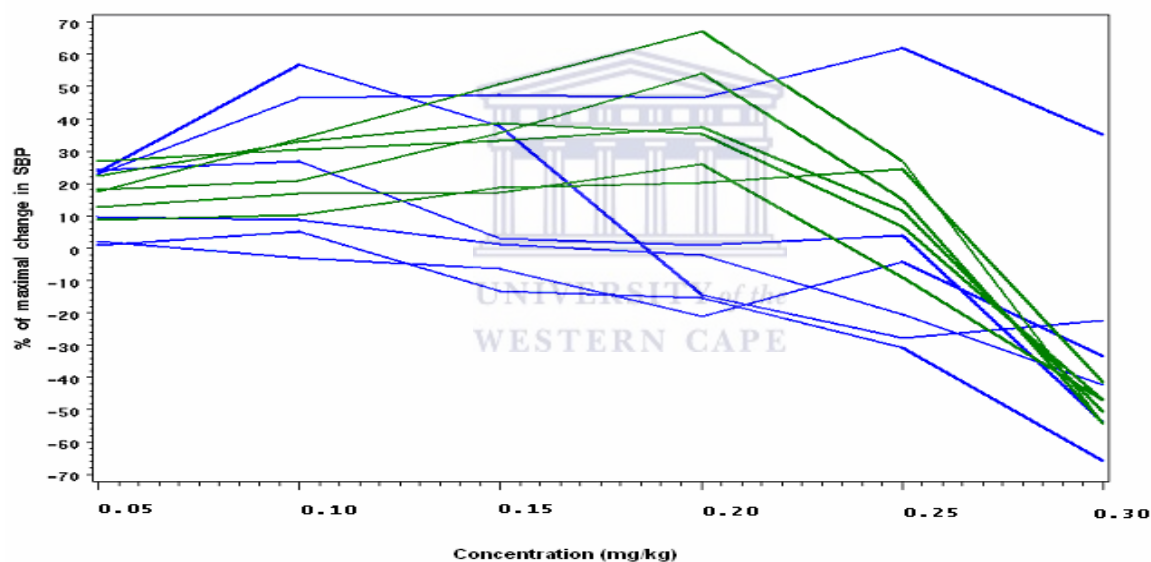


Figure 4.46 Effect of *Crinum macowanii* on systolic blood pressure in each individual rat pre-treated with atropine (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.1.3 Effect on diastolic blood pressure (DBP)

Rats pre-treated with atropine (1,2 mg/kg) and then administered *Crinum macowanii* exerted similar effects on diastolic blood pressure when compared with rats administered *Crinum macowanii* on its own. The change at the lowest dose of 0,05 mg/kg was $-8,7184\%$ [95% CI (-

34,16)] and at the highest dose of 0,25 mg/ kg, 26,8469 % [95% CI (-3,57)] was seen when both doses were compared with *Crinum macowanii* on its own. The changes trended similarly overall ($P > 0,01$) (Figures 4.47 and 4.48).

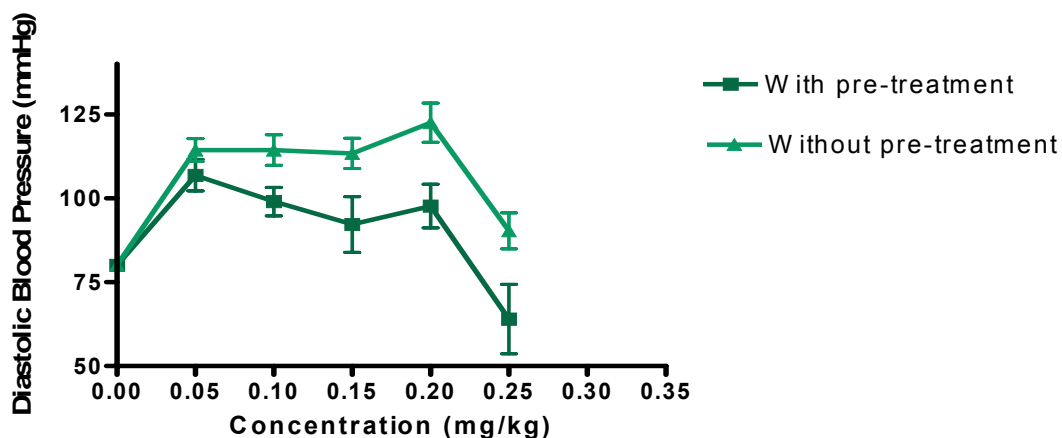


Figure 4.47 Effect of *Crinum macowanii* on diastolic blood pressure in rats pre-treated with atropine; Mean \pm SEM, n=6 (dose response curve)

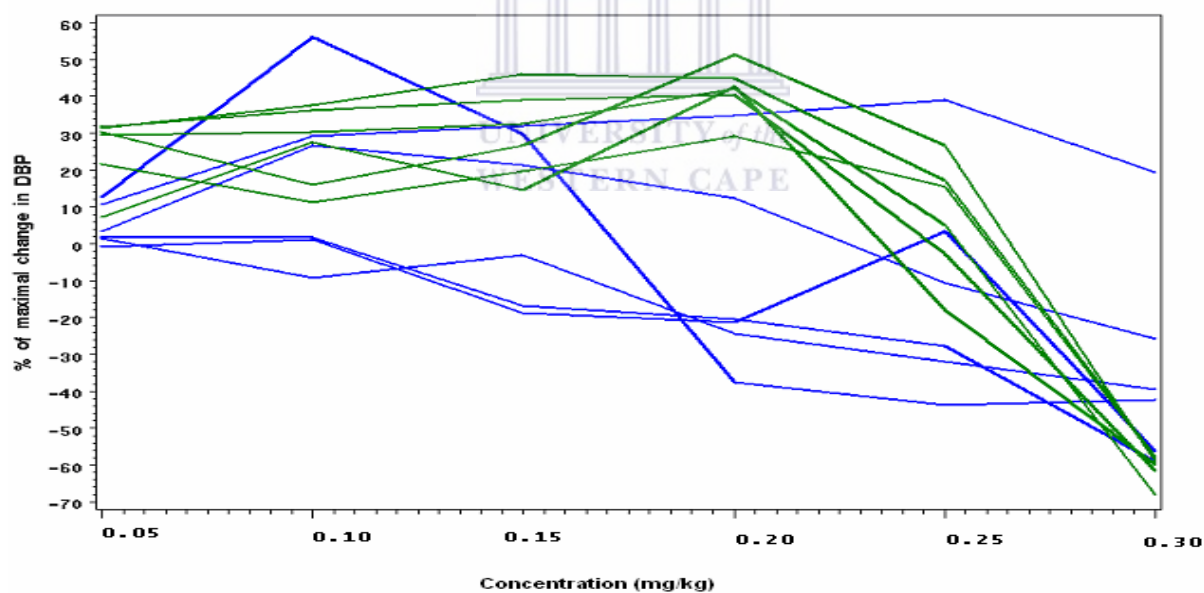


Figure 4.48 Effect of *Crinum macowanii* on diastolic blood pressure in each individual rat pre-treated with atropine (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.2. Effect of *Crinum macowanii* when pre-treated with atenolol

4.2.2.1 Effect on heart rate (HR)

Rats pre-treated with atenolol (6,0 mg/kg) produced a similar effect on heart rate when compared with *Crinum macowanii* administered on its own as seen in figures 4.49 and 4.50. The lowest dose of *Crinum macowanii* (0,05 mg/kg) produced a non-significant ($P = 0,699$) increase in heart rate that was 0,0636 % [95% CI (-7,7)] and at the highest dose of *Crinum macowanii* (0,25 mg/kg) there was also a non-significant ($P = 0,699$) increase of 1,7650 % [95% CI (-9,12)] when compared with *Crinum* administered on its own. All doses produced similar effects ($P > 0,01$).

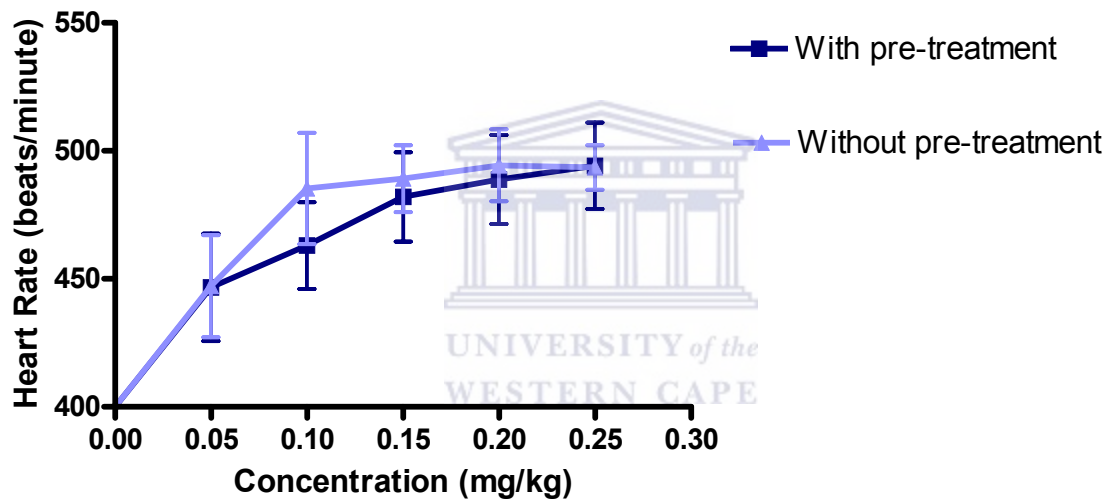


Figure 4.49 Effect of *Crinum macowanii* on heart rate when pre-treated with atenolol; Mean \pm SEM, n=6 (dose response curve)

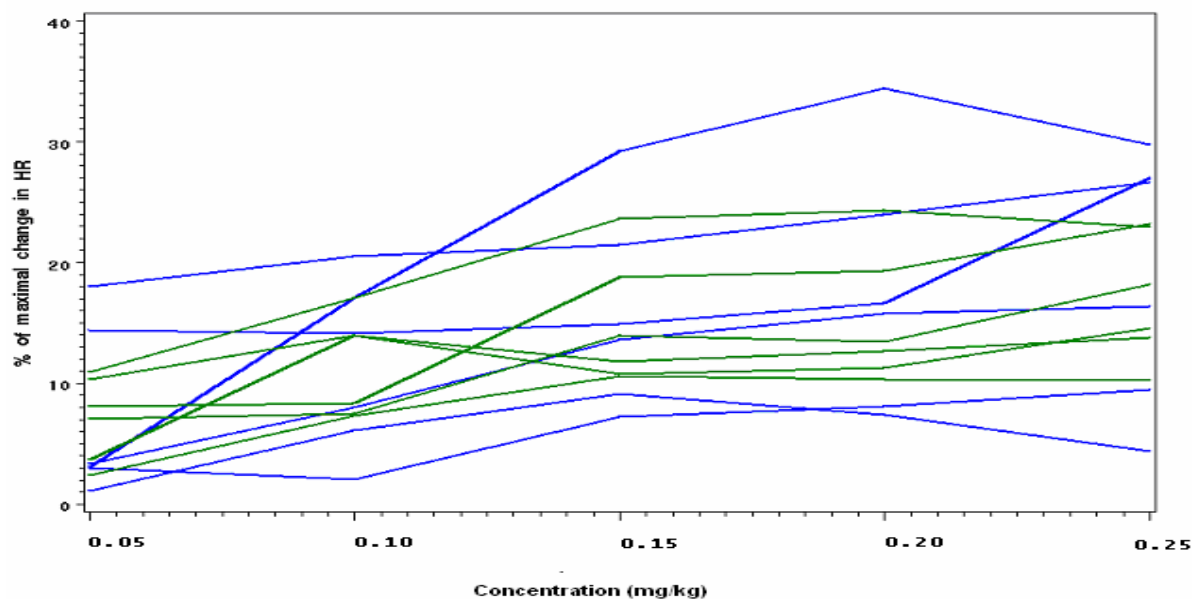


Figure 4.50 Effect of *Crinum macowanii* on heart rate in each individual rat pre-treated with atenolol (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.2.2 Effect on systolic blood pressure (SBP)

When rats pre-treated with atenolol (6,0 mg/kg) were compared with rats administered *Crinum macowanii* on its own, the same changes were seen. The lowest dose of *Crinum macowanii* (0,05 mg/kg) produced a similar ($P = 0,900$) change in blood pressure by 2,1895 % [95% CI (-8,12)] and the highest dose of *Crinum macowanii* (0,25 mg/kg) produced a similar ($P = 0,937$) change the pressure by 0,0050 % [95% CI (-14,14)] (Figures 4.51 and 4.52). All doses produced similar changes ($P > 0.01$).

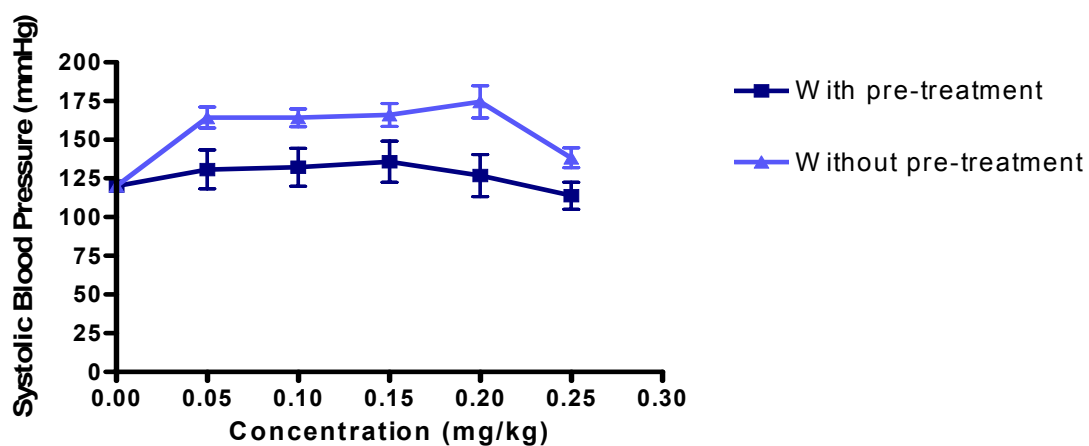


Figure 4.51 Effect of *Crinum macowanii* on systolic blood pressure in rats pre-treated with atenolol; Mean \pm SEM, n=6 (dose response curve)

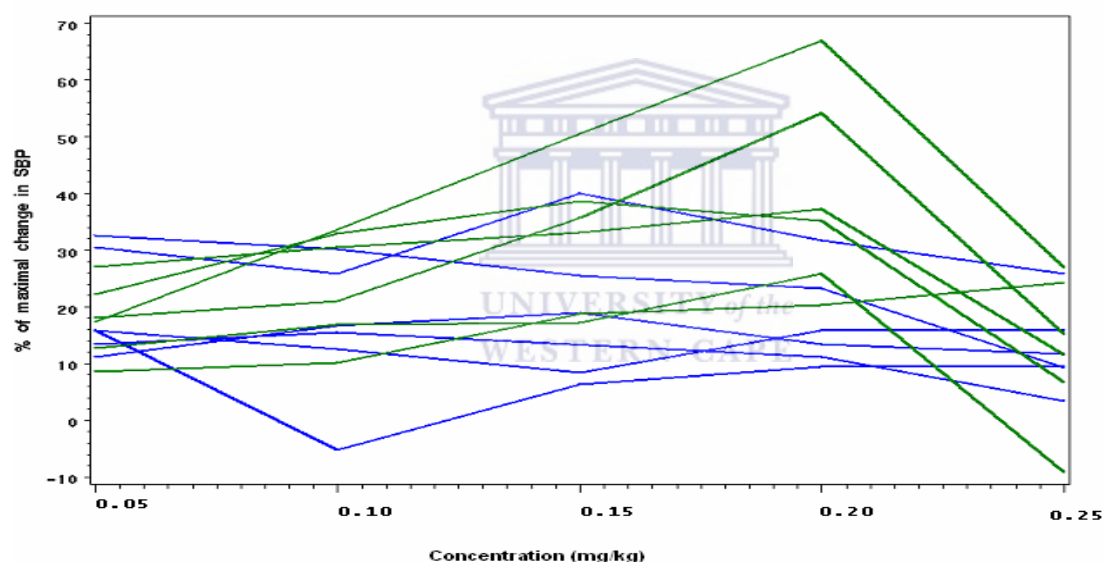


Figure 4.52 Effect of *Crinum macowanii* on systolic blood pressure in each individual rat pre-treated with atenolol (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.2.3. Effect on diastolic blood pressure (DBP)

Rats pre-treated with atenolol (6,0 mg/kg) produced changes in diastolic blood pressure similar to *Crinum macowanii* administered on its own as seen in figures 4.53 and 4.54. The lowest

Crinum dose of 0,05 mg/kg produced a similar ($P = 0,675$) effect of 6,7846 % [95% CI (7, 20)] and the highest Crinum dose of 0,25 mg/kg produced a similar ($P = 0,974$) 1,7985 % [95% CI (-17, 21)] change. Similar effects were displayed at all doses ($P > 0,01$).

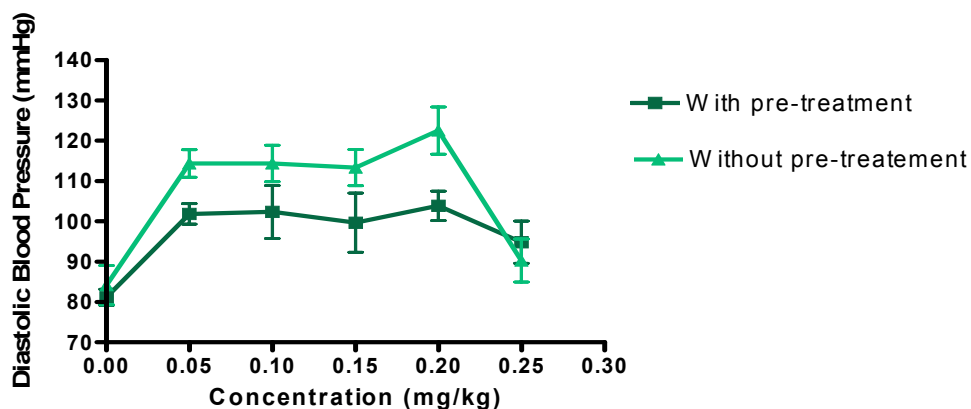


Figure 4.53 Effect of *Crinum macowanii* on diastolic blood pressure when pre-treated with atenolol; Mean \pm SEM, n=6 (dose response curve)

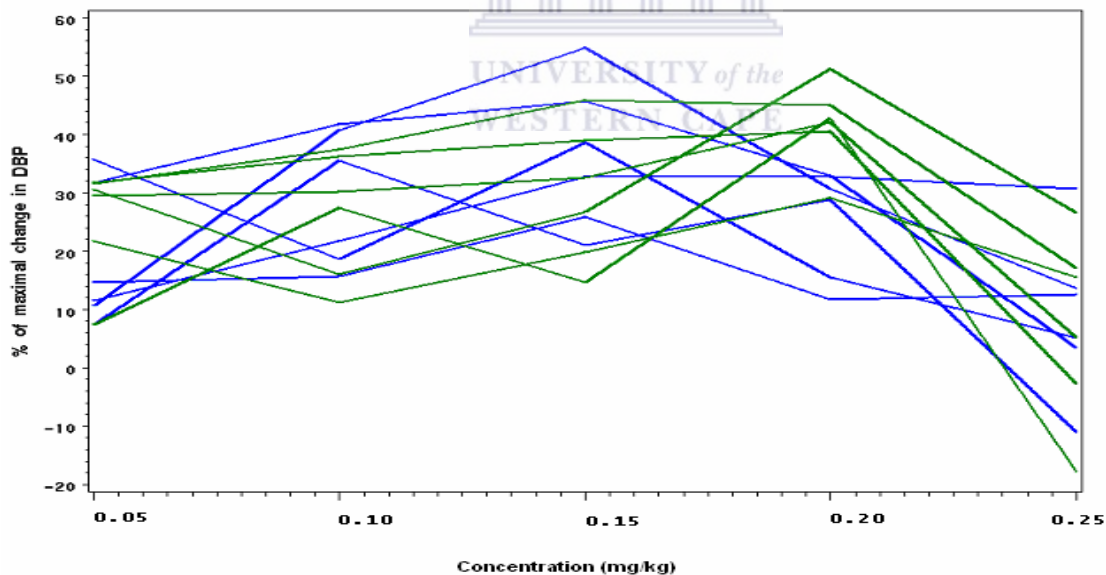


Figure 4.54 Effect of *Crinum macowanii* on diastolic blood pressure in each individual rat pre-treated with atenolol (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.3 Effect of *Crinum macowanii* when pre-treated with prazosin

4.2.3.1 Effect on heart rate (HR)

When pre-treated with prazosin (400 µg/kg) and then given *Crinum macowanii*, rats displayed a similar increase in heart rate as compared to rats administered *Crinum macowanii* on its own (Figures 4.55 and 4.56). The lowest dose of *Crinum macowanii* 0,05 mg/kg increased the heart rate by 0,8233 % [95% CI (-6,5)] and produced a similar ($P = 0,588$) change compared with *Crinum* administered on its own. As well, the highest dose of 0,20 mg/kg increased the heart rate similarly ($P = 0,589$) by 3,2866 % [95% CI (-14,7)]. *Crinum macowanii* ceased to increase the heart rate after the highest dose of 0,2 mg/kg was infused. The overall percentage change was the same for all doses ($P > 0,01$).

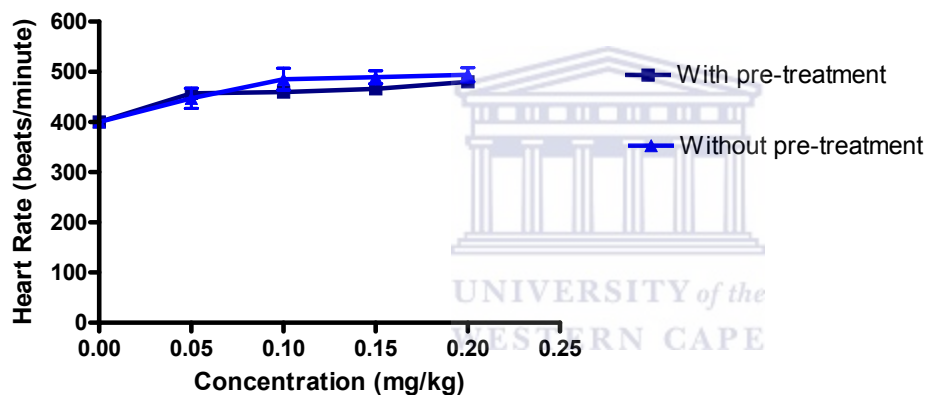


Figure 4.55 Effect of *Crinum macowanii* on heart rate with rats pre-treated with prazosin; Mean \pm SEM, n=6 (dose response curve)

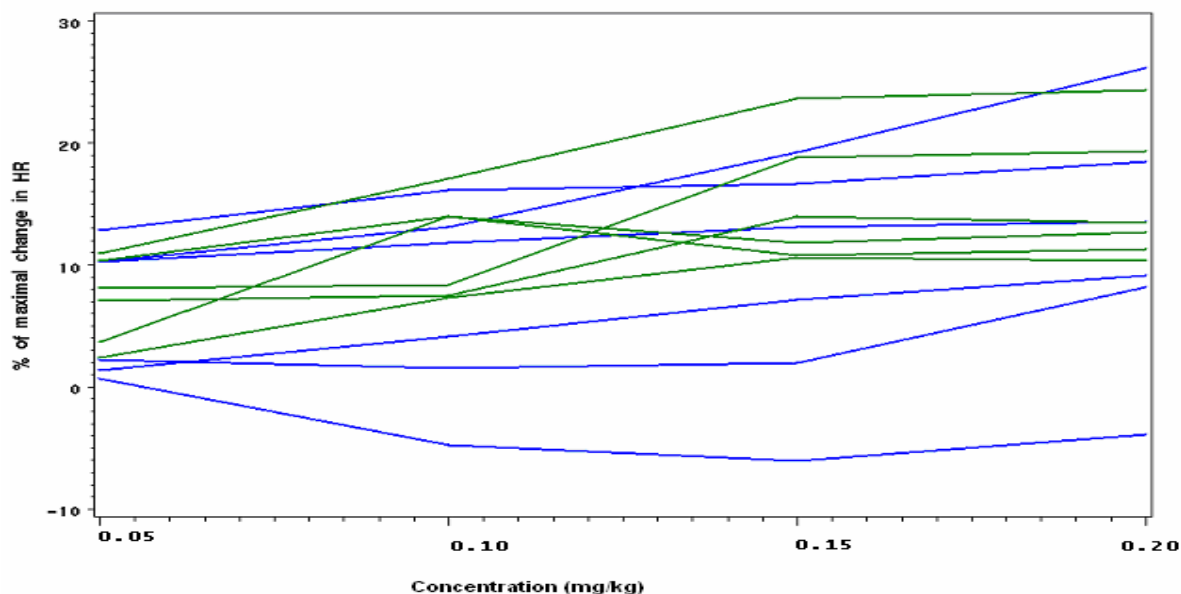


Figure 4.56 Effect of *Crinum macowanii* on heart rate in each individual rat pre-treated with prazosin (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.3.2 Effect on systolic blood pressure (SBP)

Crinum macowanii produced a dose dependent increase in systolic blood pressure when pre-treated with prazosin (400 µg/kg) similar to *Crinum macowanii* administered on its own. At the lowest dose of 0,05 mg/kg the noted similar ($P = 0,309$) change was 4,2532 % [95% CI (-13,4)] and at the highest dose of 0,20 mg/kg the change was 6,5990 % [95% (-26,13)]. The percentage changes for all doses were the same ($P > 0,01$) (Figures 4.57 and 4.58).

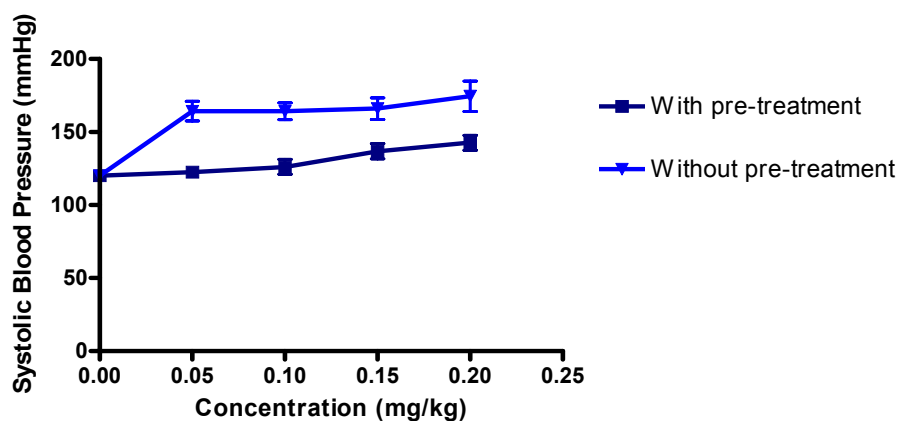


Figure 4.57 Effect of *Crinum macowanii* on systolic blood pressure in rats pre-treated with prazosin; Mean \pm SEM, n=6 (dose response curve)

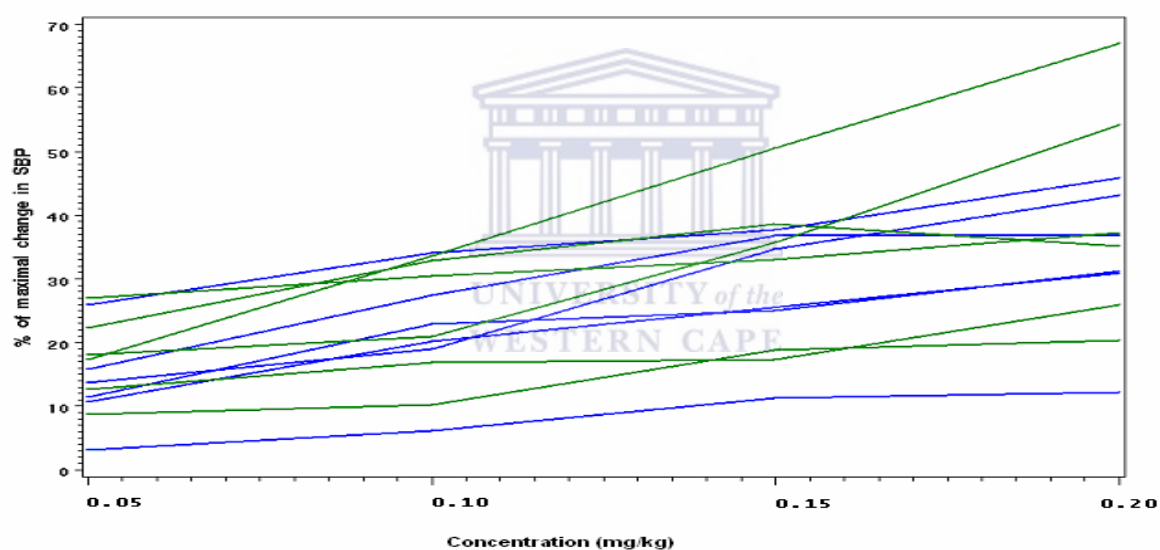


Figure 4.58 Effect of *Crinum macowanii* on systolic blood pressure in each individual rat pre-treated with prazosin (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.3.3 Effect on diastolic blood pressure (DBP)

There was a noted dose dependent increase in diastolic blood pressure in rats pre-treated with prazosin (400 μ g/kg) and then administered *Crinum macowanii*. The change was similar,

however, compared to the administration of *Crinum macowanii* on its own. The lowest dose of 0,05 mg/kg similarly ($P = 0,132$) increased the pressure by 11,7400 % [95% CI (1, 24)] and the highest dose of 0,20 mg/kg increased the diastolic blood pressure similarly ($P = 0,064$) by 17,7640 % [95% CI (2,32)]. The percentages changes for each dose were the same ($P > 0,01$) (Figures 4.59 and 4.60).

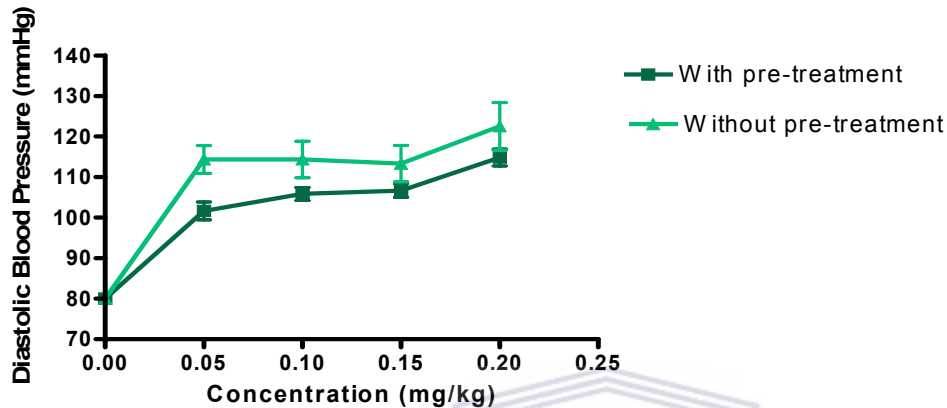


Figure 4.59 Effect of *Crinum macowanii* on diastolic blood pressure in rats pre-treated with prazosin; Mean \pm SEM, n=6 (dose response curve)

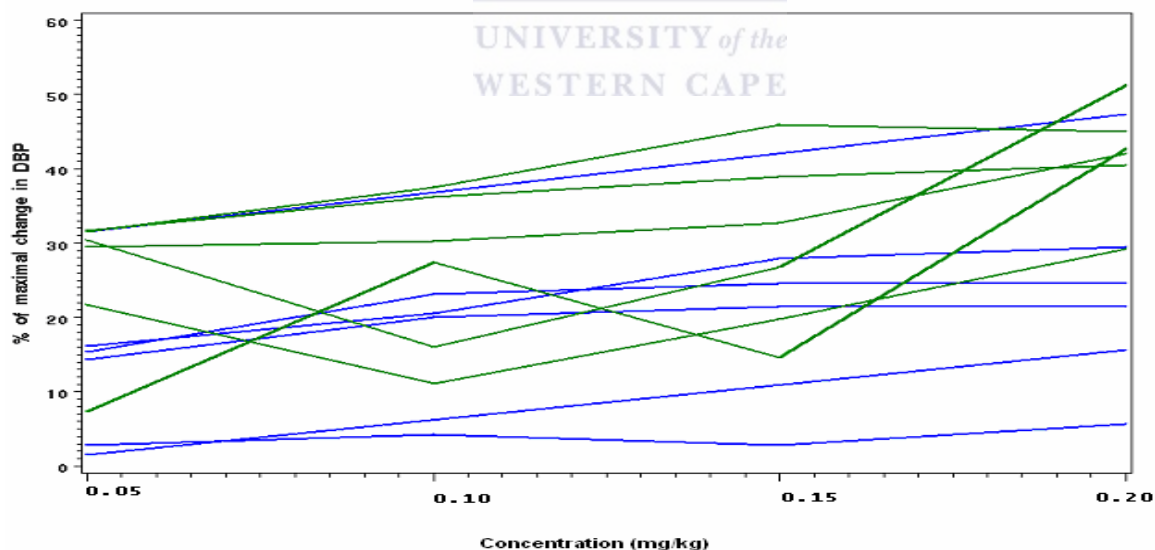


Figure 4.60 Effect of *Crinum macowanii* on diastolic blood pressure in each individual rat pre-treated with prazosin (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.4 Effect of *Crinum macowanii* when pre-treated with verapamil

4.2.4.1 Effect on heart rate (HR)

Crinum macowanii exhibited similar effects on the heart rate response in rats that were pre-treated with verapamil (4,8 mg/kg) and then administered *Crinum macowanii* compared with rats given *Crinum macowanii* on its own. At the lowest dose of 0,05 mg/kg the change in heart rate was similar ($P = 0,699$) at 0,6944 % [95% CI (-9,7)] and the highest dose of 0,20 mg/kg showed a similar ($P = 0,309$) change of 4,2058 % [95% CI (-8,16)]. The percentage changes for each dose showed a similar trend ($P > 0,01$) (Figures 4.61 and 4.62).

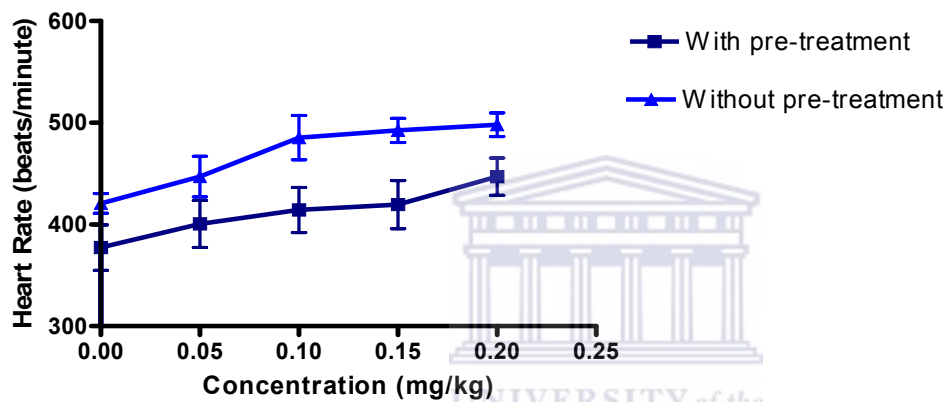


Figure 4.61 Effect of *Crinum macowanii* on heart rate with rats pre-treated with verapamil; Mean \pm SEM, n=6 (dose response curve)

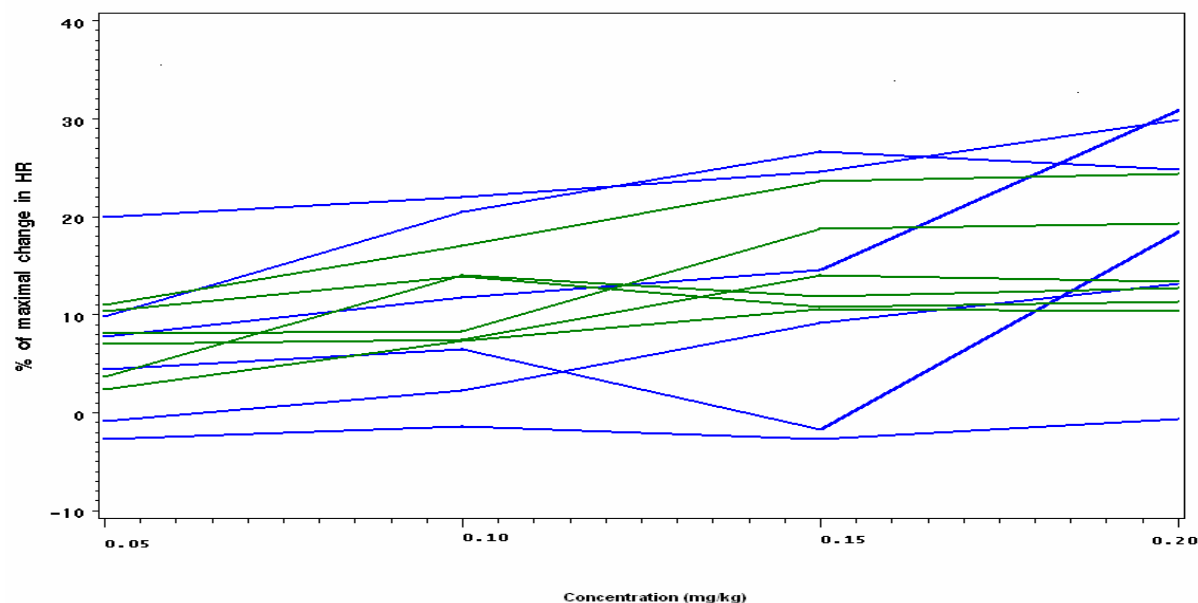


Figure 4.62 Effect of *Crinum macowanii* on heart rate in each individual rat pre-treated with verapamil (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.4.2 Effect on systolic blood pressure (SBP)

Crinum macowanii had significant dose dependent effects on systolic blood pressure when pre-treated with verapamil (4,8 mg/kg) and then administered *Crinum macowanii* compared to *Crinum macowanii* administered on its own. The lowest dose of *Crinum macowanii* (0,05 mg/kg) produced a significant ($P = 0,002$) change of 15,0201 % [95% CI (8,21)] and the highest dose of *Crinum macowanii* (0,20 mg/kg) significantly ($P = 0,002$) increased the systolic blood pressure by 27,3361 % [95% CI (9,45)]. The percentage changes differed significantly ($P < 0,01$) when the pre-treated rats were compared with *Crinum macowanii* administered on its own (Figures 4.63 and 4.64).

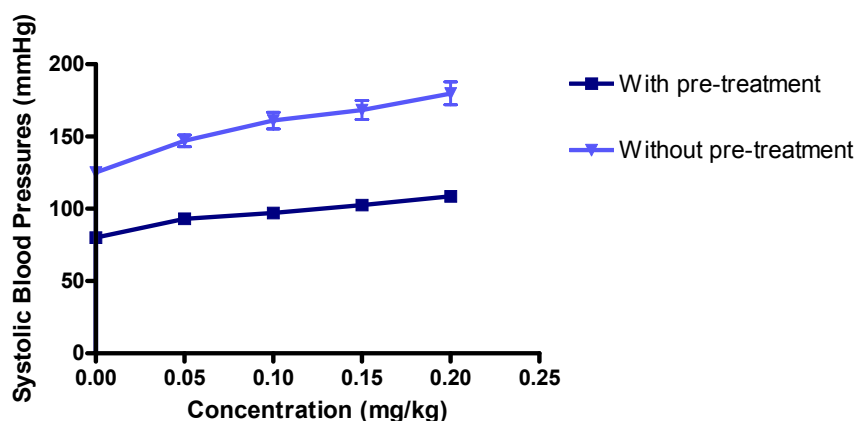


Figure 4.63 Effect of *Crinum macowanii* on systolic blood pressure when pre-treated with verapamil; Mean \pm SEM, n=6 (dose response curve)

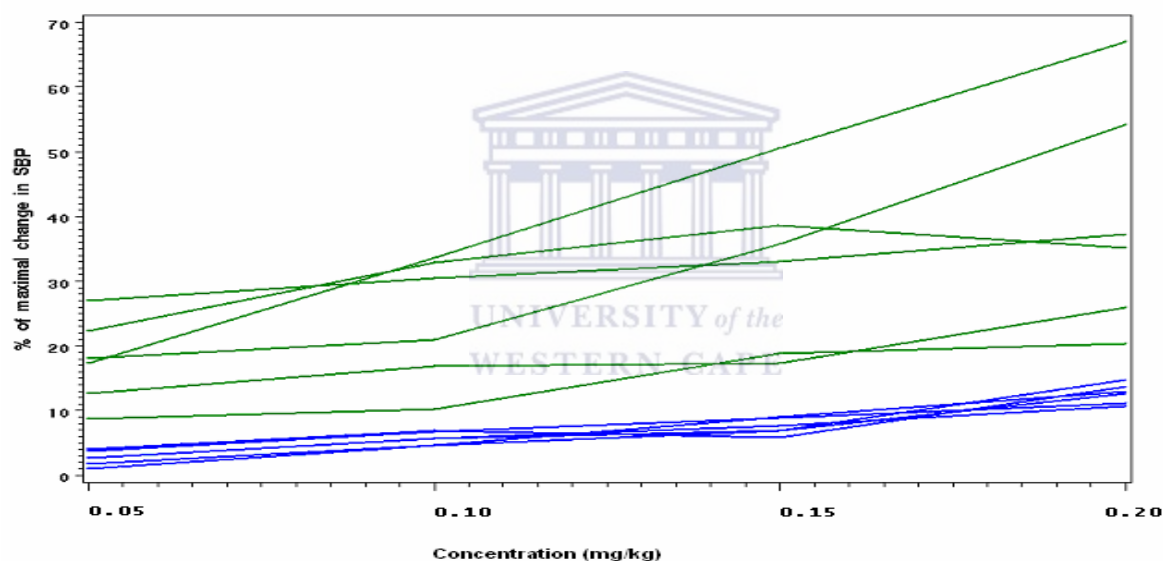


Figure 4.64 Effect of *Crinum macowanii* on systolic blood pressure in each individual rat pre-treated with verapamil (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.4.3 Effect on diastolic blood pressure (DBP)

Crinum macowanii exhibited significantly different effects on diastolic blood pressure with pre-treatment of verapamil as compared to *Crinum macowanii* administered on its own (4,8 mg/kg) (Figures 4.65 and 4.66). The lowest dose of *Crinum macowanii* (0,05 mg/kg) changed the

pressure significantly ($P = 0,002$) by 22,3847 % [95% CI (12,32)] and the highest dose of *Crinum macowanii* (0,20 mg/kg) changed pressures significantly ($P = 0,002$) by 23,9224 % [95% CI (16,31)]. The change at every dose was significant ($P < 0,01$).

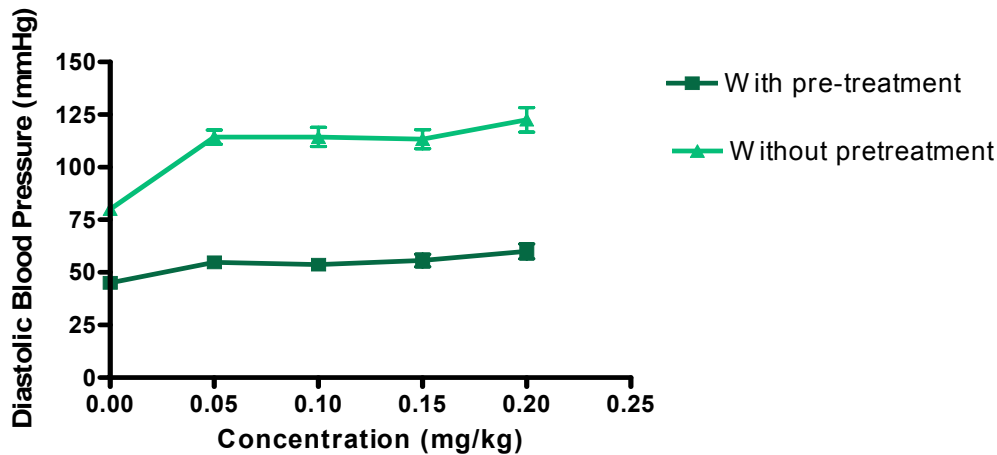


Figure 4.65 Effect of *Crinum macowanii* on diastolic blood pressure in rats pre-treated with verapamil; Mean \pm SEM, n=6 (dose response curve)

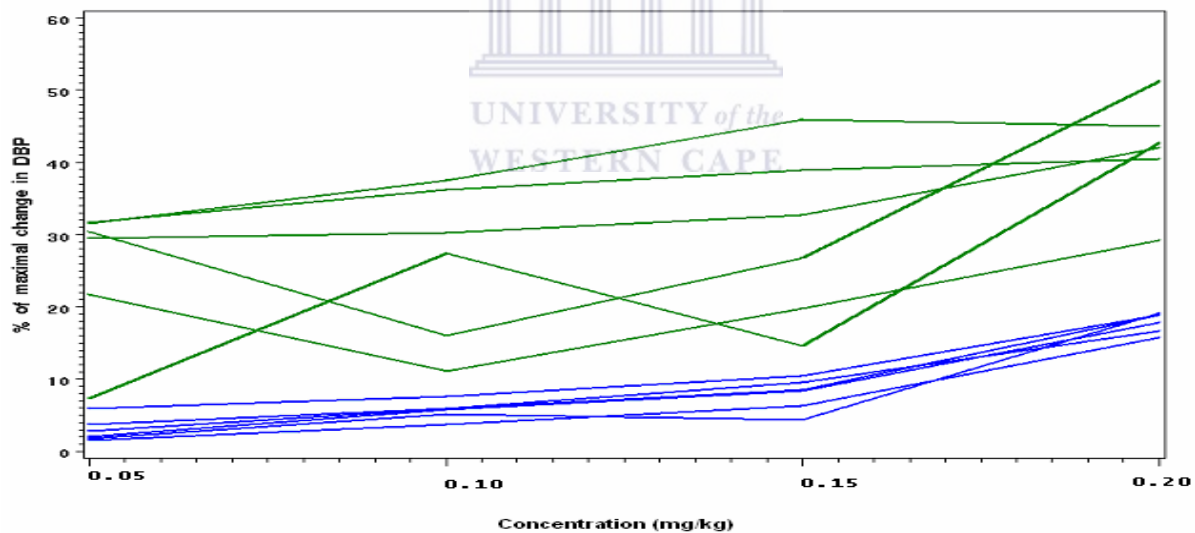


Figure 4.66 Effect of *Crinum macowanii* on diastolic blood pressure in each individual rat pre-treated with verapamil (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.5 Effect of *Crinum macowanii* when pre-treated with reserpine

4.2.5.1 Effect on heart rate (HR)

There was a similar change in heart rate response when rats were treated with *Crinum macowanii* after pre-treatment with reserpine (0,6 mg/kg) and the heart rate response with *Crinum macowanii* on its own (Figures 4.67 and 4.68). The lowest dose of 0,05 mg/kg exhibited a dose dependent increase with a similar ($P = 0,937$) change of 1,3502 % [95% CI (-5,8)]. The highest dose of 0,25 mg/kg increased the heart rate similarly ($P = 0,393$) by 4,1700 % [95% CI (-4,13)]. There were similar changes for all doses ($P > 0,01$).

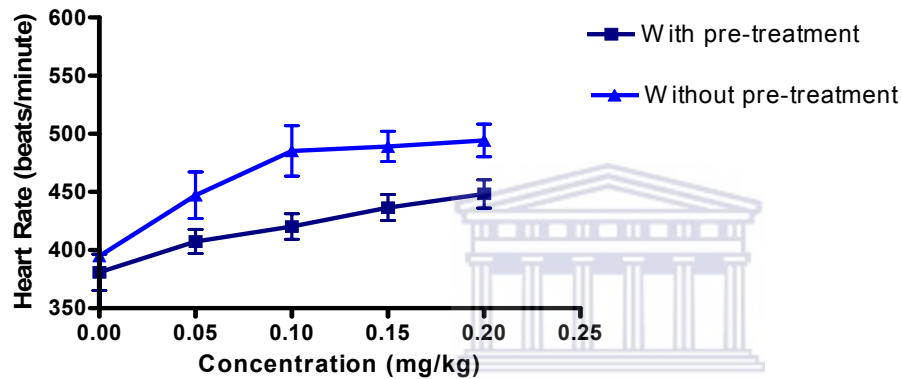


Figure 4.67 Effect of *Crinum macowanii* on heart rate when pre-treating rats with reserpine; Mean \pm SEM, n=6 (dose response curve)

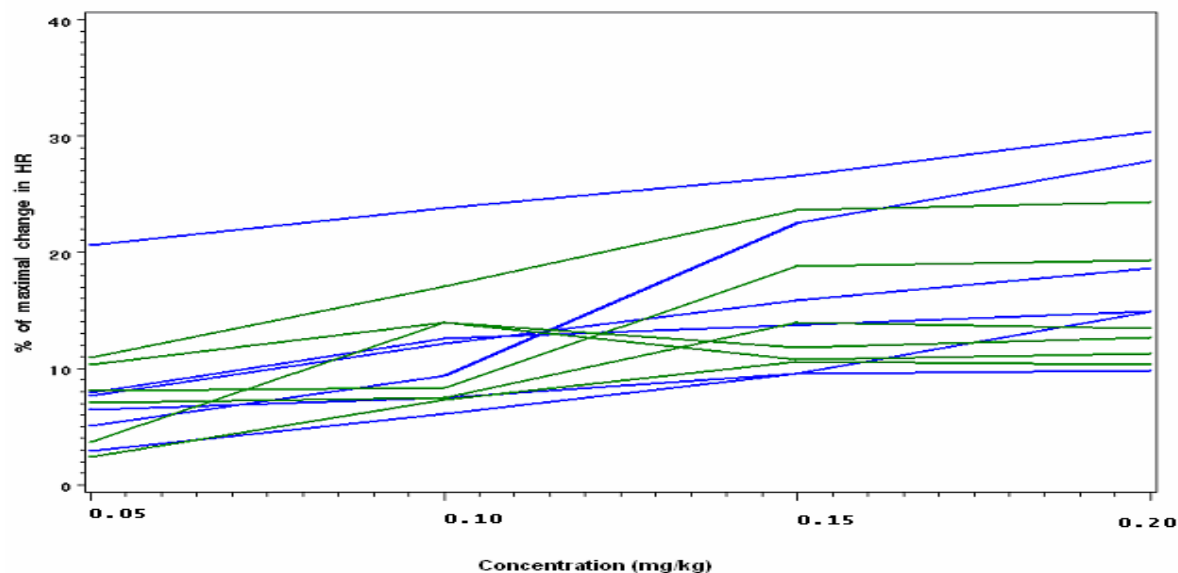


Figure 4.68 Effect of *Crinum macowanii* on heart rate in each individual rat pre-treated with reserpine (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.5.2 Effect on systolic blood pressure (SBP)

The response to *Crinum macowanii* in rats pre-treated with reserpine (0,6 mg/kg) showed the same changes in systolic blood pressure when compared to the dose response curve of *Crinum macowanii* on its own. The lowest dose of 0,05 mg/kg increased systolic blood pressure similarly ($P = 0,240$) by 5,1150 % [95% CI (2,12)] and the highest dose of 0,20 mg/kg increased pressures similarly ($P = 0,484$) by 5,0895 % [95% CI (-13,23)]. The percentage changes for all doses were the same ($P > 0,01$) (Figures 4.69 and 4.70).

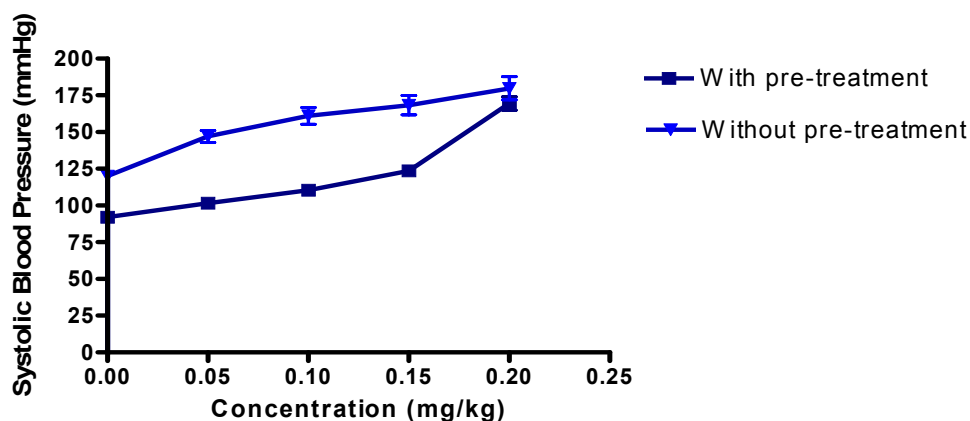


Figure 4.69 Effect of *Crinum macowanii* on systolic blood pressure when pre-treated with reserpine; Mean \pm SEM, n=6 (dose response curve)

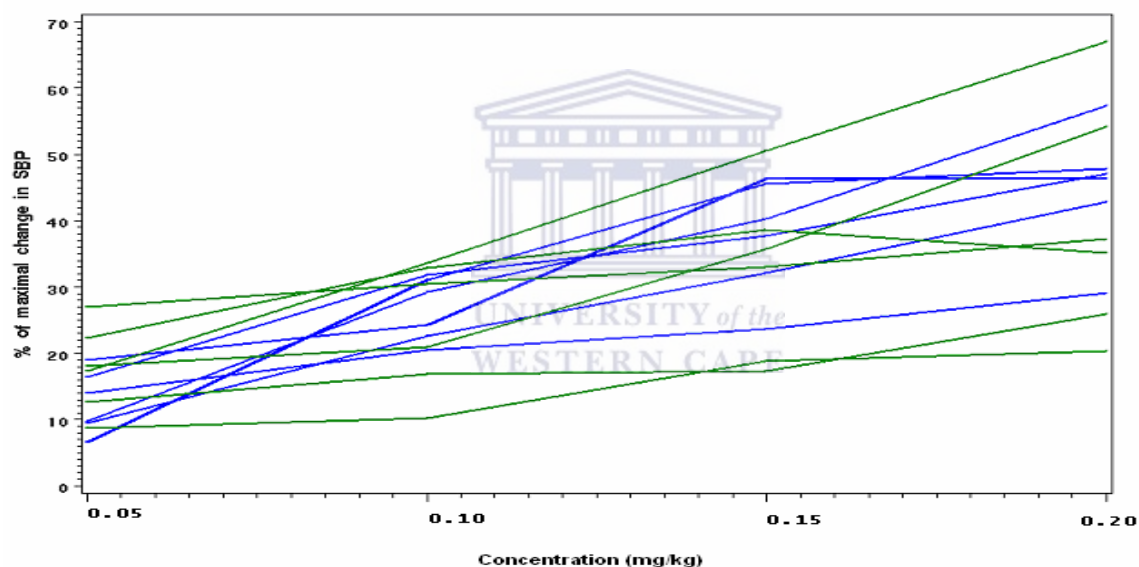


Figure 4.70 Effect of *Crinum macowanii* on systolic blood pressure in each individual rat pre-treated with reserpine (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.5.3 Effect on diastolic blood pressure (DBP)

Administration of *Crinum macowanii* after pre-treatment with reserpine (0,6 mg/kg) produced the same dose dependent increase in diastolic pressure when compared against the response of the increasing doses of *Crinum macowanii* administered on its own. The lowest dose of 0,05

mg/kg produced a similar ($P = 0,041$) increase of 13,9619 % [95 % CI (3,24)] and the highest dose of 0,20 mg/kg a similar ($P = 0,393$) change of 3,6796 % [95% CI (-7,14)] when compared to the same dosages of *Crinum macowanii* on its own. The percentage changes were similar for all doses ($P > 0,01$) (Figures 4.71 and 4.72).

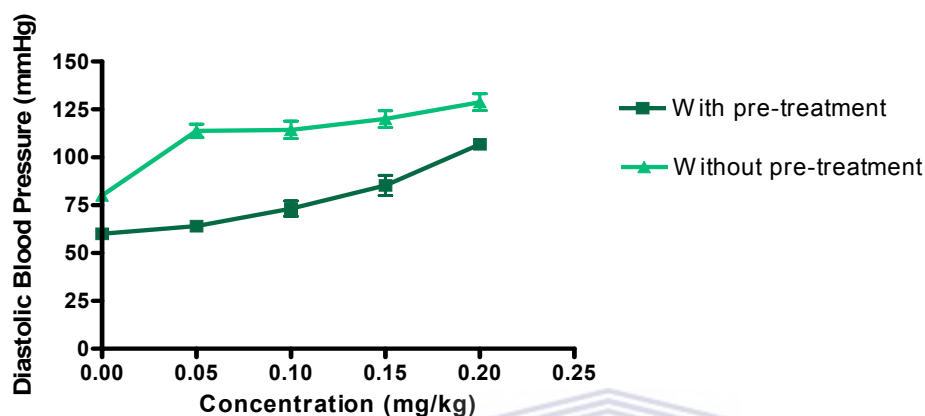


Figure 4.71 Effect of *Crinum macowanii* on diastolic blood pressure with rats pre-treated with reserpine; Mean \pm SEM, n=6 (dose response curve)

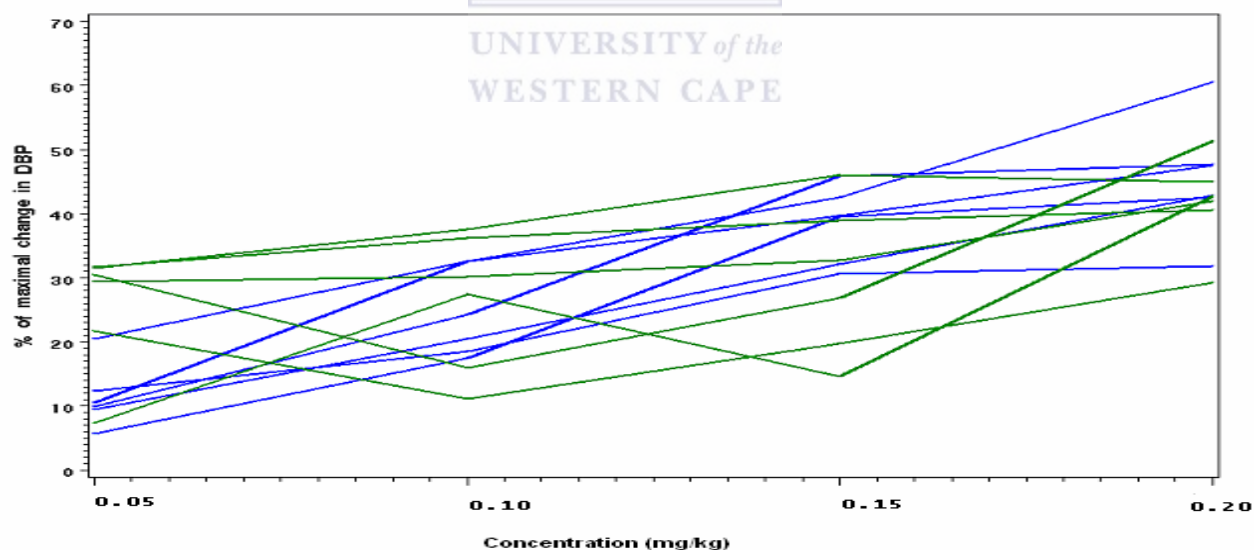


Figure 4.72 Effect of *Crinum macowanii* on diastolic blood pressure in each individual rat pre-treated with reserpine (percentage change graph with pre-treatment values outlined in blue and control values outlined in green)

4.2.6 Effect of *Crinum macowanii* when pre-treated with adrenaline

The pre-treatment of 80 % of the maximal response dose of adrenaline was administered to the rat and the rat died before the administration of the testing drug (*Crinum macowanii*) because of the increase in heart rate, systolic and diastolic pressures. The 80% dose was chosen in the protocol because it was the highest threshold that could be used to completely block the specific receptor and still allow the rat to survive throughout the experiment. Adrenaline is an agonistic drug to *Crinum macowanii* (both increase the parameters tested) and it therefore had drastic effects at the high percentage dose level. Adrenaline was still infused as a pre-treatment to observe its effects but no graphs were made as data points could not be taken.

4.3 Discussion

4.3.1 Adrenaline

Adrenaline is an adrenergic agonist that works through α and β receptors (Willems *et al* 2000). There was an increase in heart rate from 0,05 mg/kg to 0,25 mg/kg. The rat died at 0,25 mg/kg. Regularly, adrenaline's α -adrenoreceptors produce vasoconstriction and the β -adrenoreceptors produce vasodilation of the blood vessels (Willems *et al* 2000). Vasoconstriction and vasodilation are both influenced by systemic vascular resistance. In this study, at both low and sub-lethal high doses adrenaline increased the heart rate. This occurred because it increased the cardiac output by increasing the rate and force of heart contractions (Tortora and Grabowski 2000). At low doses the drug brought about vasoconstriction of the arterioles and veins through α mediated receptors. At very high doses, however, this drug lowered peripheral resistance and blood pressures through the β -adrenoreceptors until death occurred (Holgate and O'Conner 1958). Adrenaline was never effectively used as a pre-treatment for *Crinum macowanii* as they both give agonistic effects, and the 80% maximal response dose (0,20 mg/kg) caused parameters to drastically increase to non-testable levels. 80% of the maximal response was still applied to adrenaline, and not a lower percentage, in order to follow the uniform protocol applied to all other pre-treatment drugs.

4.3.2 *Crinum macowanii*

Crinum macowanii is a cardiovascular agonist of heart rate that has positive chronotropic effects on the heart and increases both the systolic and diastolic blood pressures. At all doses *Crinum macowanii* increased the heart rate. At low dosages of 0,05 mg/kg to 0,20 mg/kg *Crinum macowanii* increased the systolic and diastolic blood pressures and at high dosages of 0,25 to 0,30 mg/kg the crude extract decreased both the systolic and diastolic pressures.

4.3.3 Atropine

Atropine is an anticholinergic drug that competitively inhibits the effect of acetylcholine on muscarinic receptors in the parasympathetic system (Ohuchi *et al* 2005). The negative cardiovascular effects of atropine occur at low doses, therefore, a significant change can be seen on blood pressures when the drug is administered on its own. When administered on its own in higher doses, the heart rate increased by blocking the effect of the muscarinic receptors on the SA nodal pacemaker. There was also increased conduction in the bundle of His. In therapeutic doses, there is no significant effect on the peripheral blood vessels but there is marked vasodilatation at toxic doses (Ohuchi *et al* 2005). Heart rate was minimally affected by low increasing doses of atropine and the heart rate changes overall were non-significant. Atropine treated rats displayed non-significant dose dependent decreases in heart rate and systolic and diastolic pressures when compared to animals that were not pre-treated with atropine.

Cardiac output (CO) and peripheral resistance (PV) determine blood pressure (BP) or better stated, $BP = CO * PR$ (Opie 1998). Cardiac output is also equal to heart rate times stroke volume or $CO = HR * SV$. As has been stated earlier in Chapter 2, an increase in heart rate and/or stroke volume can cause an increase in cardiac output if the other factor does not decrease at the same time. An increase in cardiac output will lead to an increase in blood pressure if the peripheral resistance remains constant (Tortora and Grabowski 2000). This may be the phenomenon seen with *Crinum macowanii* when pre-treated with atropine.

A congenital heart disease study showed that atropine gives a dose dependent bi-phasic effect in humans. The negative chronotropic response to low doses of atropine is believed to be caused through the muscarinic receptors of the central parasympathetic system (Ohuchi *et al* 2005).

Crinum macowanii gave a non-significant dose dependent increase in heart rate with the pre-treatment of atropine when compared to *Crinum macowanii* on its own. The effects of atropine did not decrease the *Crinum macowanii* effects significantly. The non-significant decreases in the effects of *Crinum macowanii* on both systolic and diastolic pressures, with rats pre-treated with Atropine, shows that the traditional plant may not work to increase parameters through these receptors (Figures 4.43- 4.48).

4.3.4 Atenolol

Atenolol is a β_1 selective antagonist (Hayashi *et al* 2007) and lowers the heart rate and blood pressures by competitively inhibiting the effects of noradrenaline on β_1 adrenoceptors in the heart (Hayashi *et al* 2007).

Atenolol slows down the heart rate and reduces the strength of contractions. This reduces the oxygen demand and the volume of blood needed to perfuse the heart muscle (Hayashi *et al* 2007). This drug is often used for treating hypertension because it increases the diameter of the blood vessels, which allows blood to flow under less pressure (Hayashi *et al* 2007).

Pre-treating with atenolol leads to a β_1 receptor blockade and the antagonism of any β_1 mediated effects of *Crinum macowanii* (Hayashi *et al* 2007). Rats that were pre-treated with atenolol showed a dose dependent decrease that was significant on all parameters at all doses. When atenolol was used as a pre-treatment, *Crinum macowanii* administration resulted in non-significant increases in heart rate, systolic and diastolic blood pressures as compared to individual dose response curves of *Crinum macowanii* (Figures 4.49-4.54). This non-significant change may be due to the fact that *Crinum macowanii* does not increase the heart rate and blood pressures through the β_1 receptors.

4.3.5 Prazosin

Prazosin is a competitive antagonist of α_1 adrenoceptors in the vascular smooth muscles (Farah *et al*, 2006). These receptors are responsible for the vasoconstrictive action of norepinephrine, which raises the blood pressure. This vasoconstriction often happens during what is termed the fight-or-flight response, which is induced by endogenous catecholamines in the body. The catecholamine agonists of α_1 receptors tend to be norepinephrine (mentioned before) and phenylephrine (Farah *et al* 2006). Prazosin is an antagonist drug that blocks vasoconstriction. Prazosin lowers the blood pressure by vasodilating the blood vessels, which in turn, lowers the heart rate (Farah *et al* 2006). Prazosin, because of its selectivity of α_1 receptors, does not have the reflex tachycardia caused through the sympathetic baroreflex response, which increases cardiac output and in turn, increases heart rate. It, in actuality, has a minimal effect on cardiac function, which is one of the benefits of using this drug as a control against *Crinum macowanii* (Farah *et al* 2006).

Prazosin showed significant dose dependent decreases in heart rate as well as in systolic and diastolic pressures at all doses when infused individually. When pre-treated with prazosin, *Crinum macowanii* administration showed a non-significant difference in the increase in heart rate and pressures as compared to *Crinum* on its own (Figures 4.55-4.60). This may mean that *Crinum macowanii* does not increase these parameters through α_1 receptors, as there was no significant change between the pre-treatment and individual dose response curves.

4.3.6 Verapamil

Verapamil is a calcium ion inhibitor or calcium ion antagonist (Grossman and Messerli 2004). It blocks calcium channels in the heart and blood vessels. In the heart, it exhibits a negative inotropic effect. It causes vasodilation in peripheral blood vessels and coronary arteries. The anti-hypertensive effects of verapamil are believed to be related to its specific cellular action of selectively inhibiting transmembrane calcium influx in cardiac muscles and arteries (Grossman and Messerli 2004). More specifically, this means that verapamil blocks the movement of calcium into the muscle cells of the coronary arteries (which supply the heart) and other arteries

throughout the body (Grossman and Messerli 2004).

In myocytes, calcium influx has an effect on intracellular signaling and ultimately, on contraction (Porter, Makuck and Rivkees 2002). The first step in this process begins with action potentials opening voltage gated L-type channels to allow small amounts of calcium into the cell (Porter, Makuck and Rivkees 2002). These so called “sparks” trigger calcium to be released from the sarcoplasmic reticulum. This larger influx of calcium activates the contractile apparatus and the myosin cross-bridges cause binding to multiple cytosolic calcium buffers. The most well known buffer is the thin filament protein troponin C (Bers 2000). When calcium binds to troponin C it activates the myofilaments which in turn, contract the muscle (Porter, Makuck and Rivkees 2002). About 600 nmol/L of calcium is needed to activate myofilaments and produce a contraction (Bers 2000). Increased myofilament calcium sensitivity at longer sarcomere lengths is an essential component of Starlings law because the increased diastolic filling results in a stronger contraction in the ventricles (Bers 2000). For relaxation, the amount of calcium declines and calcium breaks away from troponin C, which deactivates the contractile machinery and causes diastole (Bers 2000).

Smooth muscle relaxation in the arteries will increase the diameter of the arteries, which in turn, causes a decrease in blood pressure and peripheral vascular resistance. This relaxation also reduces the pressure against which the heart must pump. The heart as a result, works less and requires less oxygen-carrying blood to perform its functions (Grossman and Messerli 2004).

Calcium functions not only for contraction and relaxation of muscle but also acts as a regulator of calcium release and uptake. The function of calcium dependent protein kinases (CaMK II), and other kinases and phosphatases can indirectly control the release and reuptake of calcium (Porter, Makuck and Rivkees 2002).

Verapamil exhibited a dose dependent non-significant decrease in heart rate but a significant decrease in systolic and diastolic blood pressures at all doses. When pre-treated with verapamil,

Crinum macowanii displayed non-significant differences in heart rate and significant differences in systolic and diastolic pressures when compared to the individual dose response curves of *Crinum macowanii* (Figures 4.61-4.66). If calcium ion flux was inhibited by the effect of verapamil, *Crinum macowanii* exerted significantly decreased effects on systolic and diastolic blood pressures as compared to *Crinum* alone, there is therefore a possibility that *Crinum macowanii* may work through calcium channels to increase the blood pressures.

4.3.7 Reserpine

Reserpine is an anti-hypertensive drug that has been labeled a postganglionic sympathetic nerve terminal blocker (Olivares *et al* 2006). This means that it controls nerve impulses along certain nerve pathways in both central and peripheral neurons. Acetylcholine is the chemical transmitter in both pre and postganglionic synapses (Cheng *et al* 2007). The synthesis of acetylcholine occurs in the cytoplasm of the nerve endings and is kept in vesicles at the presynaptic terminal (Cheng *et al* 2007). Acetylcholine binds to acetylcholine receptors on skeletal muscles and opens sodium channels in the membrane. Sodium ions then enter the muscle cell and stimulate muscle contraction. While acetylcholine induces contractions in the skeletal muscle, it also induces a decrease in contractions in the cardiac muscle (Cheng *et al* 2007). The action potential arrives from the presynaptic terminal and causes a calcium ion influx and also releases the contents of hundreds of vesicles into the synaptic cleft (Cheng *et al* 2007). Acetylcholine binds to specific receptors on the postsynaptic membrane and increases the membrane permeability to sodium, potassium, and calcium ions and induces a postsynaptic potential (Cheng *et al* 2007).

Reserpine works as an antagonist drug because it depletes catecholamines in every neuronal or non-neuronal cell by irreversibly binding to storage vesicles containing dopamine, norepinephrine, and serotonin (Olivares *et al* 2006). This means that reserpine binds tightly to these catecholamine storage vesicles eventually destroying them so that these vesicles cannot take up or store catecholamines. A pharmacological sympothectomy occurs with the treatment of reserpine and as a result, there are depletions in amines in the central nervous system as well as in the peripheral adrenergic neuron. Reserpine operates as a negative chronotropic and negative

inotropic agent by reducing the release of norepinephrine in the heart thereby reducing cardiac output. As well, reserpine's effect on biogenic amines causes the reduction of sympathetic activity in the blood vessels, which relaxes capacitance vessels and lowers peripheral vascular resistance (Olivares *et al* 2006). Both cardiac output and peripheral vascular resistance are decreased during long-term therapy with reserpine.

Reserpine caused a non-significant dose dependent decrease on heart rate and pressures with increasing doses. When reserpine was used as a pre-treatment, *Crinum macowanii* exhibited non-significantly different increases in heart rate and systolic and diastolic pressures compared to the dose response curves of *Crinum macowanii* on its own (Figures 4.67-4.72). This means that *Crinum macowanii* may not work via the release of neurotransmitters from the pre-synaptic storage vesicles.



CHAPTER 5

5.1 Summary and Conclusions

This study showed the *in vivo* effects of the crude aqueous extract of *Crinum macowanii* on normotensive rats. The first protocol involved infusing independent drug doses and creating dose response curves for adrenaline, *Crinum macowanii*, atropine, atenolol, prazosin, verapamil, and reserpine.

Adrenaline showed dose dependent positive chronotropic effects on heart rate and increased systolic and diastolic pressures significantly. At low and intermediate (0,05 mg/kg- 0,20 mg/kg) doses adrenaline increased blood pressures and at higher doses (0,25 mg/kg) adrenaline decreased blood pressures. *Crinum macowanii* also showed positive chronotropic effects on heart rate and increases in systolic and diastolic blood pressures at low and intermediate doses (0,05 mg/kg -0,20 mg/kg). At higher doses (0,25 mg/kg and 0,30 mg/kg) *Crinum macowanii* decreased blood pressures.

When pre-treated with atropine, *Crinum macowanii* exhibited similar changes between heart rates compared to individual dose responses of *C. macowanii*. Pre-treating with atropine also showed similar changes in both systolic and diastolic blood pressures. One explanation of this could be the phenomenon of the interaction between heart rate, cardiac output stroke volume and peripheral resistance. Atropine is a biphasic drug that works through muscarinic receptors (Mugabo *et al* 2001).

Atenolol showed similar dose dependent decreases in heart rate and systolic and diastolic blood pressures. When rats were pre-treated with atenolol, *Crinum macowanii* exhibited similar changes in all parameters. Atenolol is a β_1 antagonist (Hayashi *et al* 2007), which means that the β_1 receptors were blocked with the administration of the pre-treatment drug. Due to the similarities between the dose response curves of *Crinum macowanii* with the pre-treatment of

atenolol and *Crinum macowanii* alone, it can be concluded that *Crinum macowanii* may not work through these receptors.

Similar dose dependent decreases were seen in prazosin when administered on its own. Prazosin is an α -adrenergic blocker, which means it blocks the α_1 receptors (Farah *et al* 2006). When prazosin was used for pre-treatment, similar changes were seen in all parameters between rats pre-treated with prazosin and then administered *Crinum macowanii* and *Crinum macowanii* alone. This may mean that *Crinum macowanii* does not work through α_1 receptors.

Verapamil displayed similar dose dependent decreases in heart rate with increasing concentrations. When used as a pre-treatment, verapamil had significant effects on the increase that was observed in systolic and diastolic pressures with the administration of *Crinum macowanii*. Since verapamil works through calcium channels, it is thought that *Crinum macowanii* may work through these channels to increase systolic and diastolic pressures.

Reserpine is an anti-hypertensive drug that depletes catecholamines by binding itself to the storage site (Olivares *et al* 2006). When infused alone, reserpine caused a significant decrease on all parameters with increasing doses. When reserpine was used as a pre-treatment for *Crinum macowanii* the same decreases in heart rate and blood pressures when compared to *Crinum macowanii* alone were seen. Catecholamines, therefore, may not play a role in the positive chronotropic effects that *Crinum macowanii* gives.

From the results obtained it can be concluded that:

- a) *Crinum macowanii* is a cardioactive plant. The crude aqueous extract of *Crinum macowanii* modified the cardiovascular parameters of the anaesthetized normotensive male Wistar rat.
- b) The effect of the crude aqueous extract gave a dose dependent increase in heart rate at all doses (0,05-0,30 mg/kg) and an increase for systolic and diastolic pressures at lower and intermediate doses (0,05-0,20 mg/kg) but was lethal at higher doses (0,25-

- 0,30 mg/kg).
- c) When used with pre-treated drugs *Crinum macowanii* gave similar increases in heart rate and systolic and diastolic effects with atropine. It can, therefore, be inferred that *Crinum macowanii* does not work via muscarinic receptors.
 - d) *Crinum macowanii* displayed similar effects on all parameters when used with atenolol and prazosin. This may mean that the crude aqueous extract is not mediated by adrenergic receptors.
 - e) *Crinum macowanii* displayed similar effects on heart rate and significantly different effects on blood pressures when pre-treated with verapamil. When pre-treated with reserpine, similar effects were observed in all parameters. This may mean that *Crinum macowanii* does not work with catecholamines to bring about the changes in parameters but may possibly function by a mechanism involving calcium ion transport.

5.2 Recommendations

Although this study adds to the knowledge of the pharmacological effects of *Crinum macowanii*, it leaves a lot of questions yet unanswered on the effects of this traditional plant. More *in vivo* studies are needed to understand the dose dependent effects of *Crinum macowanii* on heart rate and blood pressure. Pithing rats for this *in vivo* study would add a lot of information to this project. If the rat was pithed, the CNS system would shut down completely and drug effects may be more clearly seen without the interference of catecholamines. This will give a more definite confirmation of the mechanism of action of *Crinum macowanii*.

REFERENCES

- American Heritage Stedman's Medical Dictionary, 2nd ed. 2004. Houghton Mifflin Company.
- Archer, R.H. and Condry, C. 1999b. *Crinum macowanii*. Flowering Plants of Africa 56: 30-35.
- Bers, Donald M. 2000. Calcium fluxes involved in control of cardiac myocyte contraction. *Circulation Research*, 87: 275.
- Bryant, A.T. 1966. Zulu medicine and medicine-men. Struik, Cape Town.
- Bugajski, A.J., Zurowski, P. Thor, and Michalska, Gadek A. 2007. Effect of subdiaphragmatic vagotomy and cholinergic agents in the hypothalamic-pituitary-adrenal axis activity. *Journal of Physiology and Pharmacology*, 58 (2): 335-347.
- Cameron, Oliver G., Gunsher, Sharon, and Hariharan, M. 1990. Venous Plasma Epinephrine Levels and the Symptoms of Stress. *Psychosomatic Medicine*, 52: 411-424.
- Cheng, Yuhui, Suen, Jason K., Zoran, Radic, Bond, Stephen D., Holst, Michael J., McCammon, Andrew J. 2007. Continuum stimulations of acetylcholine diffusion with reaction-determined boundaries in neuromuscular junction models. *Biophysical Chemistry*, 127 (3): 129-139.
- Dagg, A.I. 1999. Responsible animal-based research: three flags to consider. *Journal of Applied Animal Welfare Science*, 2(4): 337-346.
- Dawei, W., Yuping L., Wei, L., and Huwei, L. 2004. Separation methods for anti-bacterial and anti-rheumatism agents in plant medicines. *Journal of Chromatography B*, 812(1-2): 101-117.
- Dolphin, Annette C. 2006. A short history of voltage-gated calcium channels. *British Journal of Pharmacology*, 147: S56-S62.
- Fangan, B.M. and Nordal, I. 1993. A comparative analysis of morphology, chloroplast- DNA and distribution within the genus *Crinum* (Amaryllidaceae). *Journal of Biogeography*, 20: 55-61.
- Farah, Vera M.A., Luis, F.Joaquim, and Morris, Mariana. 2006. Stress cardiovascular/autonomic interactions in mice. *Physiology and Behaviour*, 89 (4): 569-575.
- Fennell, C.W. and van Staden, J. 2001. *Crinum* Species in traditional and modern medicine. *Journal of Ethnopharmacology*, 78:15-26.
- Garvey, H.L. 1969. The mechanism of action of verapamil on the sinus and AV nodes. *European Journal of Pharmacology*, 8 (2): 159-166.
- Ghosal, S., Saini, K.S., Razdan, S. 1985. *Crinum* Alkaloids: Their Chemistry and Biology. *Phytochemistry*, 24(10): 2141-2156.

Gilman, AF, Rall, WT, Nies, AD, Taylor, P, Eds. 1990. Goodman and Gilman's The Pharmacologic Basis of Therapeutics, 8th ed. Pergamon Press, New York.

Grossman, Ehud and Messerli, Franz H. 2004. Calcium Antagonists. *Cardiovascular Diseases*, 47 (1): 34-57.

Hall-Craggs, E.C.B.1995. Anatomy as a basis for Clinical Medicine. Williams and Wilkins, London.

Hamilton, A. 1993. Conservation and medical plants: present utilization and future products. *Acta Horticulturae*, 330: 43-49.

Harborne, J.B., Baxter, H. 1993. Phytochemical Dictionary: A Handbook of Bioactive Compounds from plants. Taylor and Francis, London.

Hartman, RD, Liaw, JJ, He, JR, Barraclough, CA. 1992. Effects of reserpine on tyrosine hydroxylase mRNA levels in locus coeruleus and medullary A1 and A2 neurons analyzed by *in situ* hybridization histochemistry and quantitative image analysis methods. *Molecular Brain Research*, 13(3):223-229.

Hayashi, Toshio, Packiasamy, A.R. Juliet, Akita, M.A., Jun, F., Hisako, M.H., Fukatsu, A., Akihisa, I. 2007. β 1 antagonist and β 2 agonist, celiprolol, restores the impaired endothelial dependent and independent responses and decreased TNF α in rat with type II diabetes. *Life Science*, 80(6): 592-599.

Holgate, J.A. and O'Connor, W.J. 1958. The effects of infusions of adrenaline and noradrenaline on the heart rate and arterial blood pressure of conscious dogs. From the Department of Physiology, School of Medicine, University of Leeds.

Kohler, C., Berkowitz, BA and Spector, S. 1975. Antihypertensive drugs and catecholamine metabolism: effects of reserpine and hydralazine on tyrosine hydroxylase activity and norepinephrine concentrations in the spontaneously hypertensive rat. *Journal of Pharmacology and Experimental Therapeutics*, 193 (2): 443-451.

Lee, MR. 2007. Solanaceae IV: Atropa belladonna, deadly nightshade. *The journal of the Royal College of Physicians of Edinburgh*, 37 (1): 77-84.

Lewis, J.R. 1990. Amaryllidaceae alkaloids. *Natural Product Reports* 7: 549-556.

Livius,V.U., Kilo, J., Luscher, T. and Gassmann, M. 2000. Circulation. The handbook of experimental animals. The laboratory rat. Georg J. Krinke, 17: 345-355.

Lockett, Mary F. 1951. The effect of pentobarbitone anesthesia on the heart rate and on the response of the heart rate to sympathomimetic amines in atropinized bitches. *Journal of Physiology*, 112(3-4): 262-272.

Mander, M. 1997. Medical plant marketing and strategies for sustaining the plant supply in the Bushbuckridge area and Mpumalanga Province. Nelspruit: Department of Water Affairs and Forestry.

Marieb, Elaine N., Mallatt, J. 2001. Human Anatomy (3rd ed) Benjamin Cummings, San Francisco.

Martini, Frederic H., Timmons, Michael J., Tallitsch, Robert B. 2003. Human Anatomy (4th Ed.) Prentice Hall, New Jersey: 532-535.

McAllister, R.G. Jr and Kirsten E.B. 1982. The pharmacology of verapamil. IV. Kinetic and dynamic effects after single intravenous and oral doses. *Clinical Pharmacology and therapeutics*, 31 (4): 418-426.

Meyer, B.J., Papendorp, Dirk H. Van, Meij, Hester S., Viljoen, Margaretha. 2002. Human Physiology (3rd ed.) Juta: Landowne, R.S.A.

Meyer, J.J.M., Afalayan, A.J., Taylor, M.B., Engelbrecht, L. 1996. Inhibition of herpes simplex virus type 2 by aqueous extracts from shoots of *Helichrysum queronites* (Astraceae). *Journal of Ethnopharmacology*, 52: 41-43.

Mongillo, Marco, Tocchetti, Carlo G., Terrin, Anna, Lissandron, Valentina, Cheung, York-Fong, Dostmann, Wolfgang R., Pozzan, Tullio, Kass, David A., Nazareno, Paolocci, Houslay, Miles D., Zaccolo, Manuela. Compartmentalized Phosphodiesterase-2 activity blunts β -adrenergic cardiac inotropy via an NO/cGMP-dependent pathway. *Circulation Research*, 98: 226.

Mugabo, Pierre, Davids, F., Mohamed, F., Burger, A.P. and Cyster, L. Cardiovascular effects of *Crinum macowanii* on the isolated perfused rat heart. Poster presented at the International Immunopharmacology Congress at Sun City; 17-21 September 2001.

Nackley, Andrea G., Tan, Kai Soo, Fecko, Karamarie, Flood, Patrick, Datchenko, Luda, and Mairner William. 2007. Catechol- *O*- methyl transferase inhibition increases pain sensitivity through activation of both β_2 and β_3 adrenergic receptors. *Pain*, 128 (3): 199-208.

Nair, J.J., Machocho, A.K., Campbell, W.E., Brun, R., Viladomat, F., Codina, C., and Bastida, J. 2000. Alkaloids from *Crinum Macowanii*. *Journal of Phytochemistry*, 54 (8): 945-950.

Naqvi, N.H. and Blaufox, M.D. 1998. Blood Pressure Measurement: An Illustrated History. Taylor and Francis Publishing, New York.

Njagi, Angela. 2004. The Effects of the Alkaloid present in the Ethyl Acetate: Hexane (4:1) fraction of *Crinum Macowanii* on the isolated perfused rat heart. UWC, Bellville.

Normann, Hans, Snyman, Ina, and Cohen, Morris 1996. Indigenous knowledge and its uses. Human Sciences Research Council Publishers, Pretoria.

Ohuchi, Hideo, Hamamichi, Yuji, Hayashi, Tamaki, Watanabe, Tamao, Osamu, Yamada, Toshikatsu, Yagihara, and Shigeyuki, Echigo 2005. Negative Chronotropic response to low-dose atropine is associated with parasympathetic nerve-mediated cardiovascular response in postoperative patients with congenital heart disease. *International Journal of Cardiology*, 99 (3): 455-462.

Olivares, Ruben Martinez, Villanueva, Ivan, Racotta, Radu, Pinon, Manuel 2006. Depletion and Recovery of catecholamines in several organs of rats treated with reserpine. *Autonomic Neuroscience: Basic and Clinical*, 128: 64-69.

Opie, Lionel H. 1998. The Heart: Physiology, from cell to circulation. Lippincott, Williams and Wilkens, Philadelphia.

Osawa, S., Heasley, L.E., Dhanasekaran, N., Gupta, S.K., Woon, C.W., Berlot, C., and Johnson, G.L. 1990. Mutation of the Gs protein alpha subunit NH2 terminus relieves an attenuator function, resulting in constitutive adenylyl cyclase stimulation. *Molecular Cell Biology*, 10 (6): 2931-2940.

Padley, James R., Overstreet, David H., Pilowsky, Paul M., Goodchild, Ann K. 2005. Impaired cardiac and sympathetic autonomic control in rats differing in acetylcholine receptor sensitivity. *American Journal of Physiology*, 289: H1985-H1992.

Pedersen, M.E., Cockcroft, J.R. 2007. The Vasodilatory Beta-blockers. *Current Hypertension Report*, 9 (4): 269-277.

Pham, L.H., Dopke, W., Wagner, J., and Mugge, C. 1998. Alkaloids from *Crinum Amabile*. *Phytochemistry*, 48 (20): 371-376.

Porter, George A., Makuck, Ryan, F., Rivkees, Scott A. 2002. Reduction of intracellular calcium levels inhibits Myoblast Differentiation. *Journal of Biological Chemistry*, 277 (32): 28942-28947.

Roberts, M. 1990. Indigenous Healing Plants. Southern Book, Halfway House, Johannesburg.

Seedat, Y.K. 1996. Is the pathogenesis of hypertension different in black patients. *Journal of Human Hypertension* 3, S35-S37.

Silke, B., Hendry, W.G., and Taylor, S.H. 1981. Immediate and sustained haemodynamic effects of prazosin during upright exercise in man. *British Heart Journal*, 46(6): 663-670.

Silverthorn, Dee Unglaub. 2003. Human Physiology (2nd ed). Prentice Hall, New Jersey.

Steinberg, SF. 1999. The molecular basis for distinct β -adrenergic receptor subtype actions in cardiomyocytes. *Circulation Research*, 85: 1101-1111.

Steinman, Alan M., Smerin, Stanley E., and Barchas, Jack D. 1969. Epinephrine metabolism in mammalian brain after intravenous and intraventricular administration. *Science*, 165 (3893): 616-617.

Steyn, K., Gaziano, TA, Bradshaw, D., Laybsher, R., Fourie, J. 2001. South African Demographic and Health Coordination Team. Hypertension in South African adults results from the Demographic and Health Survey, 1998. *Journal of Hypertension*, 19:1717-1725.

Tabuti, J.R.S., Dhillon, S.S., Lye, K.A. 2003. Traditional medicine in Balamoji country, Uganda: its practitioners, users, and viability. *Journal of Ethnopharmacology*, 85: 119-129.

Thibodeau, Gary A. and Patton, Kevin T. 1993. Anatomy and Physiology (2nd ed). Mosby, St. Louis.

Tortora, Gerald J. and Grabowski, Sandra Reynolds 2000. Principles of Anatomy and Physiology (9th ed). John Wiley and Sons, New York.

Veale, D.J.H., Furman, K.I., and Oliver, D.W. 1992. South African traditional herbal medicines used during pregnancy and childbirth. *Journal of Ethnopharmacology*, 36(3): 185.

Wang, Jin, Fu-ming, Shen, Min-wei, Wang, Ding-feng SU. 2006. Effects of nine antihypertensive drugs on blood pressure variability in sinoaortic-denervated rats. *Acta Pharmacologica Sinica*, 27 (8), 1013-1017.

Whelton, Paul K., He, Jiang, and Louis, Gail T. (Eds.) 2003. Lifestyle Modification for the Prevention and Treatment of Hypertension. Marcel Dekker, New York: 1-19.

Wildman, W.C. 1960. Alkaloids of the Amaryllidaceae. In: Manske, R.H.F., Holmes, H.L. (Eds.). The Alkaloids: Chemistry and Physiology, vol. VI. Academic Press, New York.

Willems, Edwin, W. Valdivia, Luis Felipe, San Juan, Eduardo Ramirez, Saxena, Pramod R., Villalon, Carlos M. 2000. Pharmacological identification of the major subtypes of adrenoceptors involved in the canine external carotid vasoconstrictor effects of adrenaline and noradrenaline. *Life Sciences*, 69:143-153.

World Health Organization (WHO). 2002.WHO Traditional Medicine Strategy 2002-2005. World Health Organization, Geneva. WHO/EDM/TRM/2002.1

Wurtman, Richard J., Kopin, Irwin J., Horst, Dale, Fischer, Josef E. 1964. Epinephrine and organ blood flow: effects of hyperthyroidism, cocaine, and denervation. *American Journal of Physiology*, 207 (6): 1247-1250.

Xiao, R-P, Cheng, H. and Zhou, Y-Y. 1999. Recent advances in cardiac β_2 - adrenergic signal transduction. *Circulation Research*, 85: 1092-1100.

