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CARDIOVASCULAR EFFECTS OF *HARPAGOPHYTUM PROCUMBENS* DC [PEDALIACEAE] SECONDARY ROOT AQUEOUS EXTRACT IN SOME MAMMALIAN EXPERIMENTAL ANIMAL MODELS

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Abstract

In an attempt to scientifically appraise the '*healing powers*' and medicinal value of *Harpagophytum procumbens* DC root aqueous extract (HPE), and throw some light on the efficacy and safety of the medicinal plant product, the cardiovascular effects of the herb's root aqueous extract (HPE) have been investigated in some mammalian experimental animal models. The results of this laboratory animal study indicate that relatively low to moderate doses of *H. procumbens* root aqueous extract (HPE, 10–400 mg/kg i. v.) produced dose-dependent hypotensive and cardio-depressant effects on systemic arterial blood pressures and heart rates of pentobarbitone-anaesthetized rats. Relatively low to high concentrations of the plant's extract (HPE, 10–1000 μg/ml) also produced concentration-related biphasic responses in isolated cardiac muscle strips of guinea-pigs and isolated portal veins of rats. Relatively low concentrations of the plant's extract (HPE, 10–100 μg/ml) always produced initial slight, transient and non-significant ($P>0.05$) positive chronotropic responses in isolated spontaneously-beating right atria, but significant ($P<0.05$) positive inotropic responses in isolated electrically-driven left atria of guinea-pigs. However, moderate to high concentrations of the plant's extract (HPE, 400–1000 μg/ml) always induced dose-dependent, significant ($P<0.05$–0.001), secondary longer-lasting, negative chronotropic and inotropic responses of the isolated spontaneously-beating right-, and isolated electrically-driven left-, atrial muscle preparations of guinea-pigs. The plant's extract also produced concentration-related biphasic effects on rat isolated portal vein. Low to high concentrations of the plant's extract (HPE, 10–1000 μg/ml) always produced dose-dependent, initial slight, transient and significant ($P<0.05$–0.001) contractions of the rat isolated portal veins, followed by secondary, longer-lasting, significant ($P<0.05$–0.001) relaxations of the muscle preparations. Although the precise mechanisms of the hypotensive and

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cardio-depressant actions of HPE are unknown, the vasorelaxant action of the plant's extract is speculated to contribute, at least in part, to the hypotensive action of the plant's extract. The results of this laboratory animal study lend pharmacological credence to the suggested folkloric uses of *Harpagophytum procumbens* secondary root in the management and/or control of hypertension and certain cardiac disorders in some communities of South Africa.

Keywords: *Harpagophytum procumbens,* cardiovascular effects.

Introduction

The rich floral biodiversity of South Africa has provided herbal health practitioners and other traditional healers in the country with an impressive pool of '*natural pharmacy*' from which plants are selected as ingredients to prepare herbal remedies for the treatment, management and/or control of an array of human disorders. One of such therapeutically-useful medicinal plants of South Africa is *Harpagophytum procumbens* DC [family: Pedaliaceae]. *Harpagophytum procumbens*, locally known as "*Devil's claw, Grapple plant, Wood spider, or Harpago*", is widely used in South African traditional medicine for the treatment, management and/or control of a plethora of human ailments.

Harpagophytum procumbens DC is a weedy, perennial plant with annual creeping stems spreading from a central, thick, fleshy, tuberous tap-root (Watt and Breyer-Brandwijk, 1962; Henderson and Anderson, 1966; Van Wyk *et al*., 2002). The leaves are greyish-green and are usually irregularly divided into several lobes. The tubular flowers are either yellow and violet, or uniformly dark violet. The fruits have numerous characteristically long arms with sharp, grapple-like hooks (thorns), as well as two straight thorns on the upper surface (Watt and Breyer-Brandwijk, 1962; Van Wyk *et al*., 2002; Van Wyk and Wink, 2004). *Harpagophytum procumbens* is virtually restricted to the Southern part of Africa, occurring mainly in South Africa, Namibia, Botswana and Zimbabwe. The plant is commonly referred to as '*Devil's claw*' because of its claw-like fruits which may cling tenaciously to the foot and other parts of an animal's body and, is thus dispersed in this way (Watt and Breyer-Brandwijk, 1962; Van Wyk *et al*., 2002; Van Wyk and Wink, 2004). The thick, fleshy, tuberous secondary tap-roots of *Harpagophytum procumbens* are usually dried and used in South African traditional medicine. In the form of infusions, decoctions, tinctures, powders and extracts, *H. procumbens* secondary tap root is used for a variety of health conditions. It has an ethnomedical reputation for efficacy in anorexia, indigestion, diabetes mellitus, hypertension, gout, fevers, skin cancer, infectious diseases (including tuberculosis), allergies, osteoarthritis, fibrositis and rheumatism, being particularly effective in small joint diseases (Watt and Breyer-Brandwijk, 1962; Van Wyk *et al*., 2002; Van Wyk and Wink, 2004).

When taken on a regular daily basis, it has a subtle laxative effect. Small doses of the plant's root extract are used for menstrual cramps, while higher doses assist in expelling retained placentas. '*Devil's claw*' is also used *post-partum* as an analgesic and to keep the uterus contracted. The dry, powdered tuberous root of the plant is used directly as a wound dressing, or it is mixed with animal fat or vaseline to make a wound-healing and burn-healing ointment. Commercial creams

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and ointments of *H. procumbens* are applied topically for minor muscular aches and pains, and to painful joints (Watt and Breyer-Brandwijk, 1962; Van Wyk *et al*., 2002; Van Wyk and Wink, 2004). Serum cholesterol and uric acid levels are also reduced by *H. procumbens* preparations (Van Wyk and Gericke, 2000).

Reports on cardiovascular pharmacology of *Harpagophytum procumbens* DC secondary root extracts are sparse in the literature. Circosta *et al*., (1984) reported protective effects of crude methanolic extracts of the secondary roots of *Harpagophytum procumbens* DC in some experimental arrhythmias (induced by aconitine, calcium chloride, chloroform-epinephrine) in rats and rabbits. Similarly, Costa De Pasquale *et al*., (1985) have investigated the effects of pre-treating rats with crude methanolic extracts of *Harpagophytum procumbens* secondary roots, and with harpagoside, on experimental model of hyperkinetic ventricular arrhythmias (HVA), using Langendorff preparations of rat heart. The latter investigators induced HVA in the Langendorff preparations of the rat hearts by ischaemic perfusion and reperfusion at basal conditions of 50 mmHg pressure and 8 ml/min coronary flux. They observed that crude methanolic extracts of *Harpagophytum procumbens* DC secondary roots and harpagoside produced dose-dependent, significant, protective effects on HVA-induced reperfusion. Apart from these early works suggesting cardio-protective effects of *Harpagophytum procumbens* secondary root extracts in some experimental forms of arrhythmias, we are unaware of any other report on the cardiovascular pharmacology of *Harpagophytum procumbens* DC secondary roots extracts.

In an attempt to scientifically appraise the '*healing powers*' and medicinal value of *Harpagophytum procumbens* root aqueous extract (HPE), and throw more light on the safety and efficacy of the herb, we have, in the last few years, focused our research attention on the pharmacological effects of the plant's secondary root extract, using a variety of experimental animal models. Some South African traditional health practitioners have claimed that *H. procumbens* secondary root extract is an effective remedy in the management and/or control of hypertension and certain cardiac disorders. The present study was, therefore, designed to examine the cardiovascular effects of *H. procumbens* secondary root aqueous extract in some mammalian experimental animals, using *in vivo* and *in vitro* techniques.

Materials and Methods

Plant Material

Fresh pieces of *Harpagophytum procumbens* DC secondary roots were purchased from Upington 'Muthi' Market in the Northern Cape Province of South Africa (between November, 2001 and March, 2003). The roots were identified by the staff of the North-West University's Botany Department as the secondary roots of *Harpagophytum procumbens* DC [family: Pedaliaceae]. Voucher specimen of the plant's secondary roots have been deposited in the University's Herbarium.

Preparation of *Harpagophytum procumbens* Root Aqueous Extract

One kilogramme (1 kg) of fresh secondary roots of *H. procumbens* were sliced and air-dried at room temperature. The sliced, air-dried roots of the plant were ground into fine powder in a

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Waring commercial blender. The powder was Soxhlet extracted twice, on each occasion with 2.5 litres of distilled water at room temperature for 24 hours with shaking. The combined aqueous extracts were filtered and concentrated to dryness under reduced pressure at 30±1^{o}C. The resulting aqueous extract was freeze-dried, finally giving 15.56 g [i. e., 1.556% yield] of a light-brown, powdery crude aqueous extract of *Harpagophytum procumbens* secondary root. Aliquot portions of the crude root aqueous extract residue were weighed and dissolved in distilled water for use on each day of our experiment.

Animal Material

Young adult, Wistar rats (*Rattus norvegicus*) of both sexes weighing 250–300 g; and Dunkin-Hartley guinea-pigs (*Cavia porcellus*) weighing 300–450 g; were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light; and were allowed free access to food and water *ad libitum*.

Effects of *Harpagophytum procumbens* **Root Aqueous Extract on Rat Systemic Arterial Blood Pressure and Heart Rate *in vivo*:**

Arterial blood pressures and heart rates of pentobarbitone-anaesthetized rats were measured directly from arterial catheters as described in detail by Ojewole (1976) and Bunag (1984). Pentobarbitone (60 mg/kg i. p.)-anaesthetized male and female, young adult Wistar rats weighing 300–450 g were used. The trachea of each rat was cannulated for artificial respiration, but the animal was still allowed to breathe spontaneously. When necessary, the animals were artificially ventilated with room air, using Palmer positive-pressure ventilation pump (at a rate of 20/min., and stroke volume of 40–60 ml). The right femoral vein of each rat was cannulated for drug administration, and heparin (200 units/kg body weight) was administered intravenously. Additional small doses of the anaesthetic agent (pentobarbitone) were administered intravenously when necessary, during the course of the experiment. Arterial blood pressures (systolic, diastolic and mean blood pressures) and limb lead II electrocardiogram (ECG) were recorded in the absence, and in the presence of HPE (and other drugs used) by means of an Elema-Schonander Mingograph. The electrocardiogram (ECG, limb lead II) and arterial blood pressures were monitored at a fast paper speed of 250 mm/min. Systemic arterial blood pressure of each rat was recorded with a capacitance transducer (Elema-Schonander EMT 35) from a catheter inserted through the left carotid artery (lying in the aortic arch) of the animal. The Elema-Schonander differentiator was calibrated by measuring the slope of the upstroke of the pressure pulse, and both systolic and diastolic blood pressures (expressed in mmHg) were recorded. Mean arterial blood pressure was obtained by electronic integration. Heart rate was calculated by counting either the number of pulses from the arterial blood pressure, or the number of QRS-complex peaks from the ECG record, and expressed in beats/min. Doses of HPE (and other drugs used) were injected intravenously into the animals through the femoral vein catheters in volumes not exceeding 0.3 ml, and washed in with 0.1 ml of distilled water, the vehicle in which HPE (and other drugs used) were dissolved. Distilled water was used as control.

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Effects of *Harpagophytum procumbens* Root Aqueous Extract on Guinea-Pig Isolated Cardiac Muscles

Spontaneously-beating isolated right atria of guinea-pigs

Isolated, spontaneously-beating right atrial muscle strips of guinea-pigs were prepared and set-up under physiological conditions as described in detail earlier by Ojewole (1976). Male and female Dunkin-Hartley guinea-pigs weighing 300–450 g were used. Each of the animals was killed by applying a sharp blow to the back of its head and bled out. The animals' hearts were quickly excised and placed in Petri-dishes containing oxygenated Krebs-Henseleit physiological solution at room temperature. Intact right atria were carefully dissected out free from ventricular, left atrial and connective tissues, and avoiding damage to the pace-maker region. The isolated right atrial muscle strips were then suspended in 30-ml 'Ugo Basile Two-Chambered Organ Baths' (model 4050) containing Krebs-Henseleit physiological solution (of composition, in g/litre: NaCl, 6.92; KCl, 0.34; NaH_2PO_4, 0.15; $NaHCO_3$, 2.1; $MgCl_2$, 0.11; $CaCl_2$, 0.26; and glucose, 1.00) maintained at 32±1^oC and continuously aerated with carbogen (i. e., 5% carbon-dioxide + 95% oxygen gas mixture). Two right atrial muscle preparations (one used as 'control' and the other one used as 'HPE- or drug-treated' preparation) were always set-up to allow for changes in the atrial muscle sensitivity. Each preparation was subjected to a resting tension of 0.75 g, and allowed to equilibrate for 30–45 minutes, or until when the spontaneous contractions of the atrial muscle were stable, before it was challenged with HPE (and other drugs used). Doses of HPE (and other drugs used) were added to the bath-fluid either cumulatively or sequentially (non-cumulatively), and washed out three-to-five times after the maximum responses of the tissues were attained. Concentrations of HPE (and other drugs used) were repeated where appropriate and/or possible, at regular intervals of 20–30 minutes after the last washing. The spontaneous amplitude and rate of contractions, as well as HPE- (and other drugs-) induced responses of the isolated atrial muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers, 2-Channel "Gemini" Recorder, and pen-recording microdynamometers (model 7070). The rate of contractions of the atrial muscle strips in the absence, and in the presence, of HPE (and other drugs used) was estimated at a fast paper speed of 300 mm per minute.

Electrically-driven isolated left atria of guinea-pigs

Isolated, electrically-driven left atrial muscle strips of guinea-pigs were prepared and set-up under physiological conditions as described in detail earlier by Ojewole (1976). Male and female Dunkin-Hartley guinea-pigs weighing 300–450 g were used. Each of the animals was killed by applying a sharp blow to the back of its head and bled out. The animals' hearts were quickly excised and placed in Petri-dishes containing oxygenated Krebs-Henseleit physiological solution at room temperature. Intact left atria were carefully dissected out free from ventricular, right atrial and connective tissues. The left atrial muscle strips were then impaled on thin platinum wire electrodes and suspended in 30-ml 'Ugo Basile Two-Chambered Organ Baths' (model 4050) containing Krebs-Henseleit physiological solution maintained at 32±1^oC and continuously aerated with carbogen (i. e., 5% carbon-dioxide + 95% oxygen gas mixture). Each left atrial

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muscle preparation was driven electrically with square wave pulses of 5 msec duration at a frequency of 3 Hz and with supramaximal voltage of 5–10 volts delivered from SRI stimulators. Two left atrial muscle preparations (one used as 'control' and the other one used as 'HPE- or drug-treated' preparation) were always set up to allow for changes in the atrial muscle sensitivity. The tissues were subjected to a resting tension of 0.75 g, and allowed to equilibrate for 30–45 minutes, or until when the force of contractions of the left atrial preparations were stable, before they were challenged with doses of HPE (or other drugs used). Doses of HPE (and other drugs used) were added to the bath fluid either cumulatively or sequentially (non-cumulatively), and washed out three-to-five times after the maximum responses of the tissues were attained. Concentrations of HPE (and other drugs used) were repeated where appropriate and/or possible, at regular intervals of 20–30 minutes after the last washing. The electrically-induced contractions, as well as HPE- (and other drugs-) induced responses of the isolated atrial muscle preparations were recorded isometrically by means of Ugo Basile force displacement transducers, 2-Channel "Gemini" Recorder, and pen-recording microdynamometers (model 7070). The force of contractions of the atrial muscle strips in the absence, and in the presence, of HPE (and other drugs used) was estimated at a fast paper speed of 300 mm per minute.

Effects of *Harpagophytum procumbens* Root Aqueous Extract on Rat Isolated Portal Vein

The experimental procedure used for the rat isolated portal vein was adopted from that described in detail earlier by Ojewole (1976). Wistar albino rats of both sexes weighing 2500–300 g were used. Each of the animals was killed by applying a sharp blow to the back of its head and bled out. The abdomen of each animal was quickly opened by a midline incision, and the intestines were pulled to the left side of the animal. Portal veins with *in situ* lengths of approximately 20 mm were carefully cleaned free of connective, extraneous and fatty tissues, and then removed. The portal veins were separately suspended in 30-ml 'Ugo Basile Two-Chambered Organ Baths' (model 4050) containing Krebs-Henseleit physiological solution maintained at 36 ± 1^{o}C and continuously aerated with carbogen (i. e., 5% carbon-dioxide + 95% oxygen gas mixture). Two isolated portal vein preparations (one used as 'control' and the other one used as 'HPE- or drug-treated' preparation) were always set up to allow for changes in the venous muscle sensitivity. Each of the isolated venous muscle preparations was allowed to equilibrate for a period of 30–45 minutes under an applied resting tension of 0.5 g, before it was challenged with concentrations of HPE (and other drugs used). Doses of HPE (and other drugs used) were applied to the bath-fluid either cumulatively or sequentially (non-cumulatively), and washed out three-to-five times after the maximum responses of the tissues were attained. Concentrations of HPE (and other drugs used) were repeated where appropriate and/or possible, at regular intervals of 20–30 minutes after the last washing. The amplitude and frequency (rate) of the spontaneous, myogenic contractions, as well as the HPE- (and other drug-) induced responses of the isolated portal veins were recorded isometrically with the aid of Ugo Basile force-displacement transducers, a 2-Channel "Gemini" Recorder, and pen-recording microdynamometers (model 7070). The amplitude and frequency (rate) of contractions of the venous muscle strips in the absence, and in

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the presence, of HPE (and other drugs used) were estimated at a fast paper speed of 300 mm per minute.

Preparations from Reserpinized Guinea-pigs and Rats

Some of the experiments were carried out on isolated atrial muscle preparations and portal veins taken from guinea-pigs and rats pretreated with reserpine (5 mg/kg i. p.) 18–24 hours before use. Satisfactory reserpinization was confirmed by the absence of positive inotropic responses to bath-applied tyramine (2.5–10 μg/ml).

Data Analysis

Data obtained from 'test' group of anaesthetized rats, guinea-pig isolated atrial muscle strips and rat portal vein preparations treated with *Harpagophytum procumbens* root aqueous extract (HPE) and other drugs used, as well as those obtained from distilled water-treated 'control' rats and isolated muscle preparations, were pooled, and expressed as means (±SEM). The difference between the plant extract (HPE)- or drug-treated 'test' means, and distilled water-treated 'control' means, was analyzed statistically. Where appropriate, one-way analysis of variance (ANOVA), or the Student's t-test (Snedecor and Cochrane, 1967), was used to determine the level of significance of the difference between the 'test' and 'control' group data means. Values of $P \leq 0.05$ were taken to imply statistical significance.

Re s u l t s

Rat Systemic Arterial Blood Pressure and Heart Rate *in vivo*

Relatively moderate to high doses of *Harpagophytum procumbens* root aqueous extract (HPE, 10–500 mg/kg i. v.) produced dose-dependent and significant ($P<0.05$–0.001) reductions in the arterial blood pressures and heart rates of pentobarbitone-anaesthetized rats. Figure 1 shows a typical trace, while Table 1 summarizes the results obtained. The decreases in blood pressures and heart rates persisted for 5–60 minutes (depending on the dose administered), after which they gradually returned to baseline values. The cardio-depressant effects of the plant's extract (HPE, 10–500 mg/kg i. v.) were not modified by 18–24 hours pretreatment with atropine (1.5 mg/kg i. p.) or mepyramine (8.0 mg/kg i. p.). At the same dose levels, HPE (10–500 mg/kg i. v.) depressed or abolished the rise in arterial blood pressures and heart rates of the anaesthetized rats induced by noradrenaline or histamine (1 μg/kg i. v.). The plant's extract (HPE, 10–500 mg/kg i. v.) dose-dependently potentiated the depressor effects of acetylcholine (1 μg/kg i. v.) on the cardiovascular system of the anaesthetized rats used.

Spontaneously-beating isolated right atria of guinea-pigs

Relatively low to moderate concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–100 μg/ml) usually induced concentration-dependent, biphasic responses, consisting

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of an initial slight, transient, stimulant but non-significant (P>0.05) positive chronotropic responses, and significant (P<0.05–0.01) increases in the amplitude of contractions of spontaneously-beating right atrial muscle strips. Relatively moderate to high concentrations of the plant's extract (HPE, 200–1000 μg/ml) always produced an initial transient but significant (P<0.05–0.001) increases in the amplitude of contraction, followed by gradual, secondary longer-lasting, significant (P<0.05–0.001) negative chronotropic responses. Figure 2 illustrates a typical trace, while Figure 3 summarizes the results obtained. The negative chronotropic effects of moderate to high concentrations of the plant's extract (HPE, 200–1000 μg/ml) were resistant to exogenous, bath-applied atropine (0.5–2.0 μg/ml). Pretreatment of the guinea-pigs with reserpine (5 mg/kg i. p.) for 18–24 hours, only partially inhibited HPE-induced initial transient stimulant, positive chronotropic responses of the isolated cardiac muscle preparations.

Table 1.
Cardiovascular effects of HPE (50–400 mg/kg i.v.) in pentoobarbitone anaesthetized rats. Values given represent mean changes from controls (±SEM) 7–9 observations.

	Before HPE	After HPE			
		Mean changes from control values			
Cardiovascular parameter	Control	HPE doses (mg/kg i. v.)			
		50	100	200	400
Systolic Blood Pressure (mm Hg)	120±7	-14±4*	-32±5**	-79±6***	-106±8***
Diastolic Blood Pressure (mm Hg)	90±5	-12±3*	-30±4**	-68±5***	-85±7***
Mean Blood Pressure (mm Hg)	100±6	-10±4*	-25±5**	-75±6***	-85±8***
Heart rate (beats/min.)	386±20	-56±18	-180±16*	-240±15**	-312±15***

*P<0.05; **P< 0.01; ***P<0.001 vs control

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Electrically-driven isolated left atria of guinea-pigs

Relatively low to moderate concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–100 μg/ml) usually provoked concentration-related, transient, initial stimulant but significant (P<0.05–0.01) positive inotropic responses. However, relatively moderate to high concentration of the plant's extract (HPE, 200–1000 μg/ml) always produced biphasic effects, consisting of an initial very transient but significant (P<0.05–0.01) positive inotropic response, followed by gradual, secondary longer-lasting, highly significant (P<0.01–0.001) negative inotropic responses. Figure 4 illustrates a typical trace, while Figure 5 summarizes the results obtained.

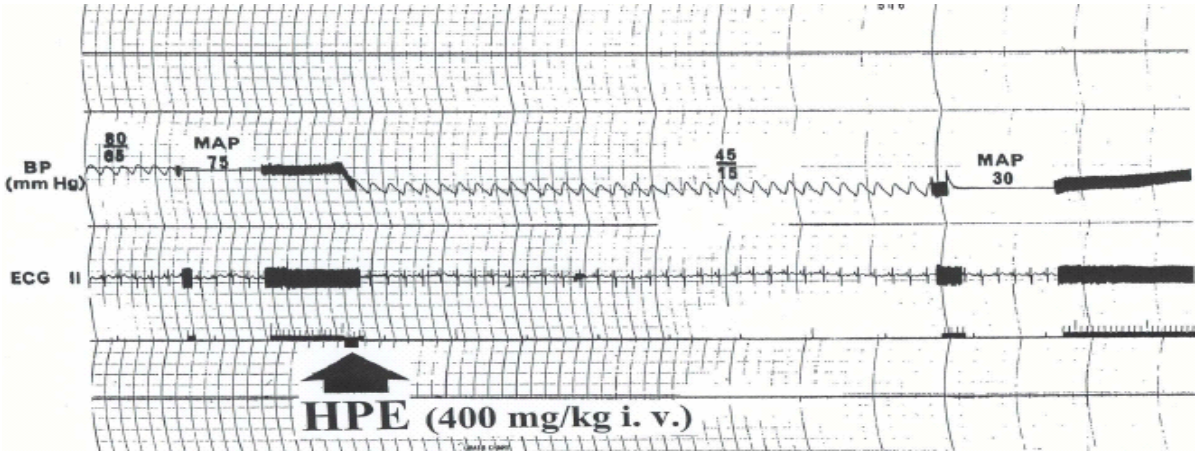

Figure 1. Effects of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 400 mg/kg i. v.) on the arterial blood pressure and heart rate (calculated from ECG limb lead II) of a pentobarbitone-anaesthetized rat. HPE (400 mg/ml) was administered intravenously into the rat at the left-hand-side solid (closed) upright-pointing arrow.

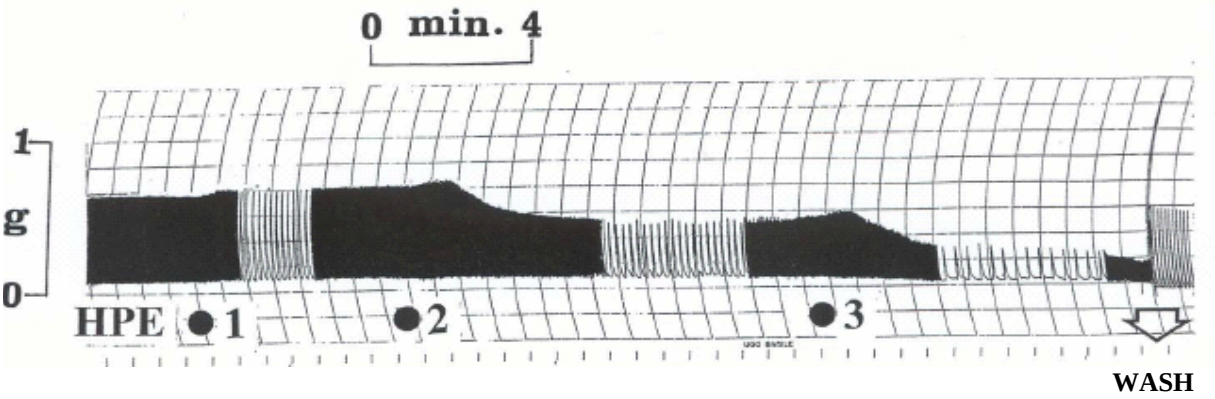

Figure 2. Effects of graded concentrations of *Harpagophytum procumbens* secondary root aqueous extract on isolated, spontaneously-beating right atrial muscle strip of the guinea-pig. HPE 1, 2 and 3 represent 100, 200 and 400 μg/ml of *Harpagophytum procumbens* secondary root aqueous extract cumulatively added to the bath-fluid at the solid, closed (●) dots respectively. HPE was washed out at the open, downward-pointing, right-hand-side arrow.

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The negative inotropic effects of moderate to high concentrations of the plant's extract (HPE, 200–1000 μg/ml) were resistant to exogenous, bath-applied atropine (0.5–2.0 μg/ml).
Pretreatment of the guinea-pigs with reserpine (5 mg/kg i. p.) for 18–24 hours, only partially inhibited HPE-induced initial transient stimulant, positive inotropic responses of the isolated cardiac muscle preparations.

Rat Isolated Portal Vein

Relatively low to high concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–1000 μg/ml) always produced biphasic effects on rat isolated portal veins. The HPE-induced responses of the rat isolated portal veins always consisted of dose-related initial slight, transient stimulant but significant ($P<0.05–0.01$) contractions of the venous muscle preparations, followed by secondary, longer-lasting, significant ($P<0.05–0.001$) relaxations of the muscle strips. During the initial transient, stimulant, contractile phase, the plant extract (HPE, 10–1000 μg/ml) usually increased the contractile frequency, and inhibited the amplitude of the spontaneous, myogenic contractions of the isolated portal vein in a concentration-dependent manner. Moderate to high concentrations of the plant's extract (HPE, 200–1000 μg/ml) always depressed or abolished the amplitude of the spontaneous, myogenic contractions of the portal veins, sharply contracted the muscle strips, and thereafter relaxed the muscle preparations in a concentration-related fashion. Figure 6 shows a typical trace, while Figure 7 summarizes the results obtained.
The possibility that the HPE-induced responses of the guinea-pig isolated atrial strips, and rat isolated portal vein muscle preparations used in this study might involve interaction with Ca^{2+} at the cell membrane was also investigated. The concentration of Ca^{2+} in the bathing Krebs-Henseleit solution was reduced from 0.26 g/litre to 0.13 g/litre, and raised from 0.26 g/litre to 0.52 g/litre respectively. The initial transient, stimulant responses of the isolated muscle strips induced by relatively low concentrations of HPE (10–100 μg/ml) were reduced and/or abolished in the presence of low calcium concentration [Ca^{2+} = 0.13 g/litre] in the bathing Krebs physiological solution. However, the secondary negative, inhibitory responses of the isolated muscle strips produced by moderate to high concentrations of HPE (200–1000 μg/ml) increased as the concentration of the external Ca^{2+} was reduced. Raising the bathing fluid Ca^{2+} concentration from 0.26 g/litre to 0.52 g/litre increased and/or enhanced low HPE (10–100 μg/ml) concentration-induced initial transient, stimulant responses of the isolated muscle preparations. However, the secondary negative, inhibitory responses of the isolated muscle strips induced by moderate to high concentrations of HPE (200–1000 μg/ml) decreased as the Ca^{2+} concentration of the external bathing fluid was increased.

In all cases, washing of the isolated cardiac and venous muscle preparations with fresh, normal Krebs-Henseleit physiological solution 3–5 times usually restored physiological activities of the isolated muscle strips to normal, control values.

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Discussion

Relatively moderate to high doses of *Harpagophytum procumbens* root aqueous extract (HPE) produced dose-related, significant (P<0.05–0.001) decreases in the arterial blood pressures and heart rates of pentobarbitone-anaesthetized rats. These observations are in agreement with the findings of Circosta *et al.*, (1984), who reported that high doses of dried, crude methanolic extract of *Harpagophytum procumbens* secondary root caused dose-dependent, significant reductions in the arterial blood pressures of conscious, normotensive rats. With high doses of *H.*

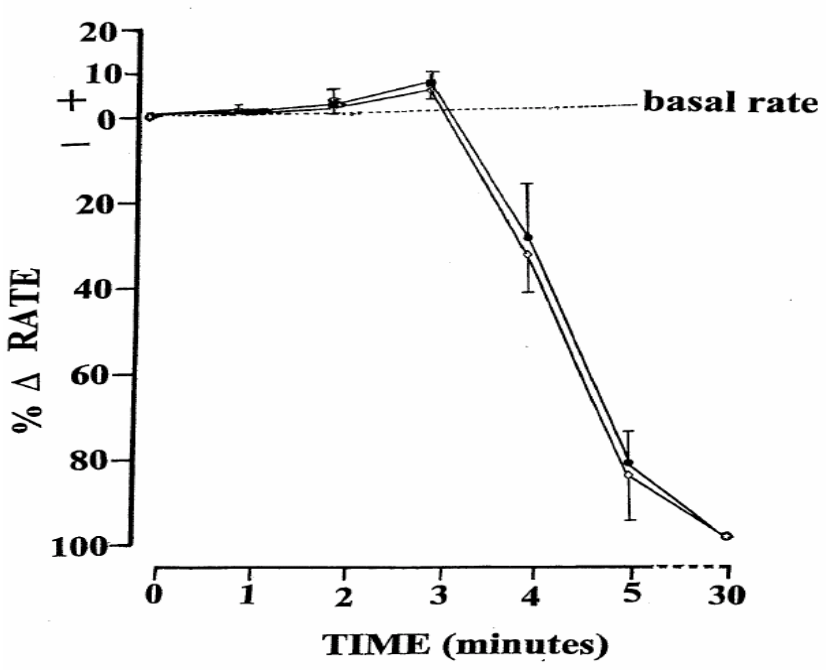

Figure 3. Effects of relatively high concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, ◇—◇ 400 and ■—■ 600 μg/ml) on the rate (frequency) of contractions of isolated, spontaneously-beating right atrial muscle strips of guinea-pigs. Each point represents the mean (±SEM) of 6–8 observations, while the vertical bars denote standard errors of the means.

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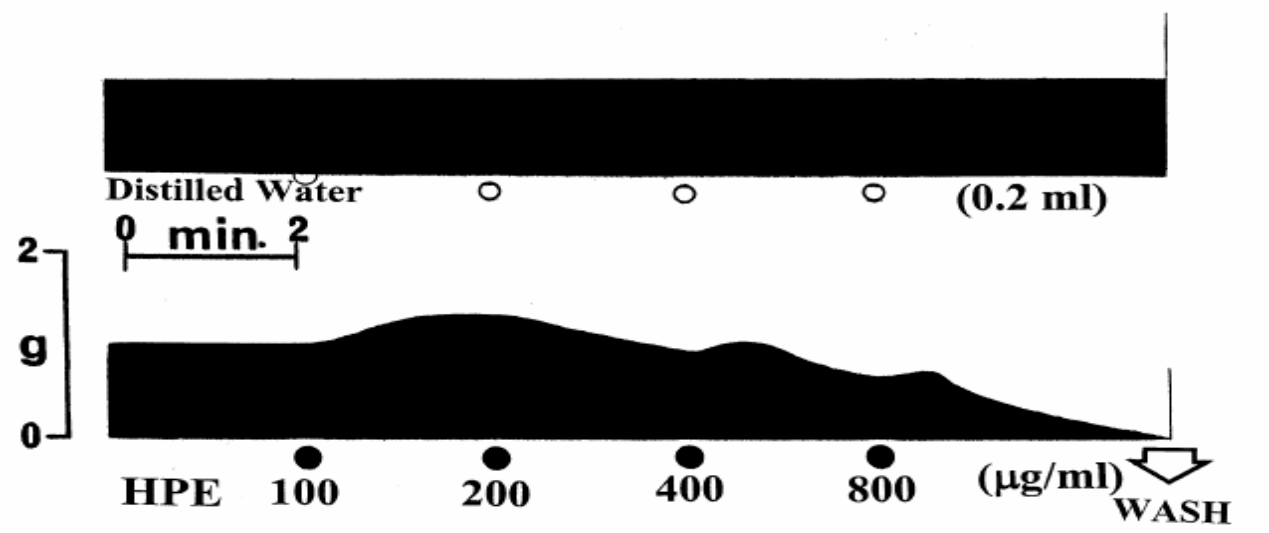

Figure 4. Effects of graded concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 100–800 μg/ml) and distilled water (0.2 ml) on isolated, electrically-driven left atrial muscle strips of guinea-pigs. Graded concentrations of HPE (100–800 μg/ml) were cumulatively added to the bath-fluid at the (solid) closed (●) dots [in the 'drug-treated' lower trace], while 0.2 ml distilled water was also cumulatively added to the bath-fluid at the open (○) dots [in the 'control' upper trace]. HPE was washed out at the open, downward-pointing, right-hand-side arrow.

procumbens dried root methanolic extract, Circosta *et al.*,(1984) also observed concomitant decreases in the heart rates of conscious rats. In spontaneously-beating Langendorff preparations of rabbit hearts, Circosta *et al.*, (1984) found that *Harpagophytum procumbens* dried root crude methanolic extract caused mild decreases in the heart rate with concomitant mild positive inotropic effects at lower doses, but marked negative inotropic effects at higher doses. According to Circosta *et al.*, (1984), the negative chronotropic and positive inotropic effects of harpagoside (the major constituent of *Harpagophytum procumbens* dried root extract) were comparatively higher with respect to that of the extract, whereas harpagide (another constituent of the plant's root extract) only had a slight negative chronotropic effect and a considerable negative inotropic effect. In the present study, however, the cardiac effects of harpagoside and harpagide were neither examined nor compared with those of the crude aqueous extract used. Hence, we are unable to comment appropriately on the latter findings of Circosta *et al.*, (1984). It would appear, however, that both harpagoside and harpagide contribute significantly to the inotropic and chronotropic effects of *Harpagophytum procumbens* dried root extract. Although the precise mechanism of the hypotensive action of the plant's extract is obscure at present, it is speculated that the vaso-relaxant action of the herb's extract may have contributed, at least in part, to its hypotensive effect in the mammalian experimental animals used.

The results of the present study also strongly indicate that *Harpagophytum procumbens* root aqueous extract (HPE) possesses biphasic effects on isolated cardiac muscles of the guinea-pig and rat isolated portal vein. However, the initial transient stimulant effect of the plant's extract is likely to be partially due to its ability to release catecholamines from tissue stores, since the initial stimulant and/or positive chronotropic and positive inotropic responses of the atrial muscle strips to bath-applied low concentrations of the plant's extract were partially inhibited by 18–24

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hours reserpine pretreatment. The precise mechanism of the secondary, cardio-depressant effect of the plant's extract on isolated cardiac muscle preparations is unknown at the moment. However, because the secondary cardio-depressant and venous relaxant effects of the plant's extract (HPE) were resistant to blockade by standard, receptor specific antagonists in all the isolated muscle preparations tested, it is speculated that the secondary, longer-lasting cardio-depressant and veno-relaxant effects of HPE on the muscle preparations may be non-specific in nature. Furthermore, the finding that changes (decrease or increase) in calcium ion concentrations of the bathing physiological solution modified the responses of the isolated tissue preparations used to bath-applied concentrations of *Harpagophytum procumbens* root aqueous extract (HPE), would appear to suggest that HPE affects calcium mobilization and/or sequestration, and possibly also, calcium release from its various tissue stores. Further studies are certainly needed to shed more light on this plausible mechanism of action of HPE.

Harpagophytum procumbens roots have been reported to be rich in sugars, phytosterols, triterpenoids, coumarins, flavonoids and iridoids (Watt and Breyer-Brandwijk, 1962; Van Wyk and Gericke, 2000; Van Wyk *et al*., 2002; Van Wyk and Wink, 2004). Although only a few pharmacological studies on *H. procumbens* root extract have been reported in the biomedical literature, the iridoids harpagoside (a cinnamic acid ester), harpagide and procumbide are speculated to contribute, at least in part, to the cardiovascular properties of the plant's root extract.

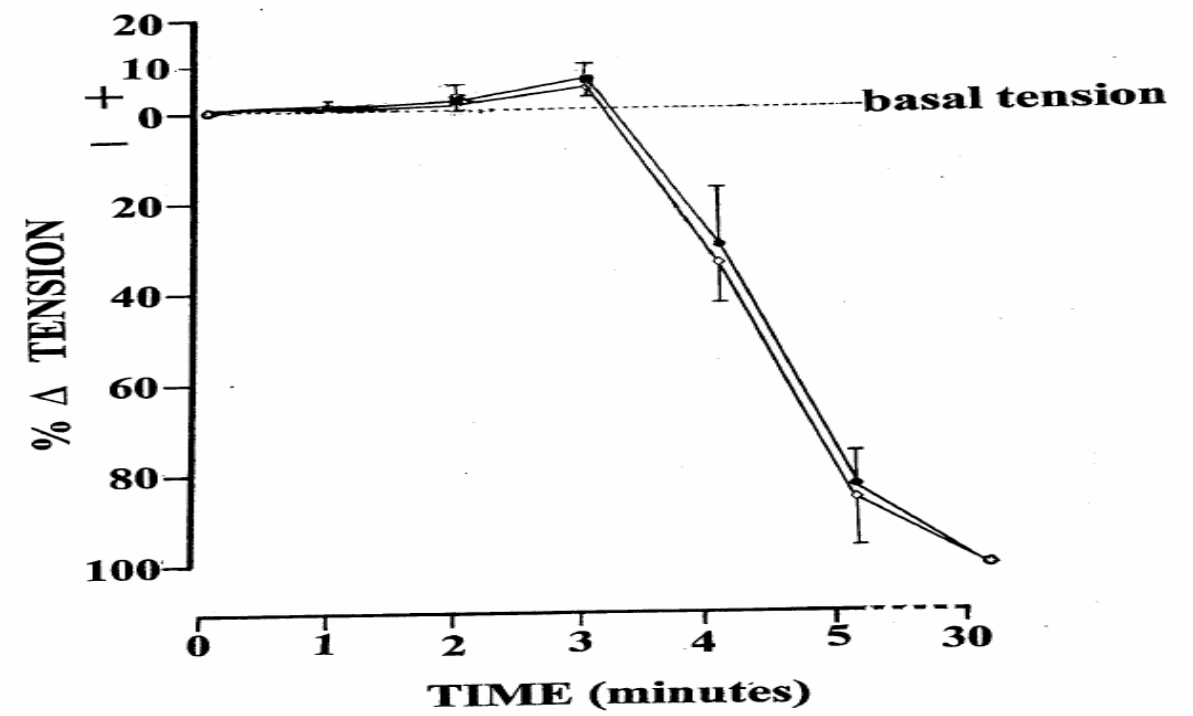

Figure 5. Effects of relatively moderate to high concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, ○—○ 400 and ■—■ 600 μg/ml) on isolated, electrically-driven left atrial muscle preparations of guinea-pigs. Each point represents the mean (±SEM) of 6–9 observations, while the vertical bars represent standard errors of the means.

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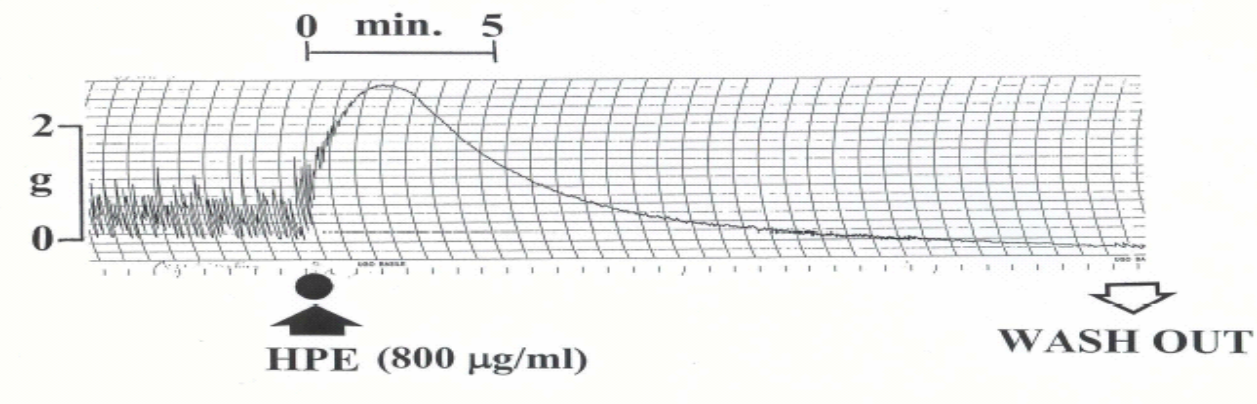

Figure 6. Dual effects of a relatively high concentration of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 800 µg/ml) on the amplitude (force) and frequency (rate) of spontaneous, myogenic contractions of the rat isolated portal vein. HPE (800 µg/ml) was added to the bath-fluid at the left-hand-side (solid) closed dot (●) and upright-pointing arrow, and washed out at the right-hand-side open downward arrow.

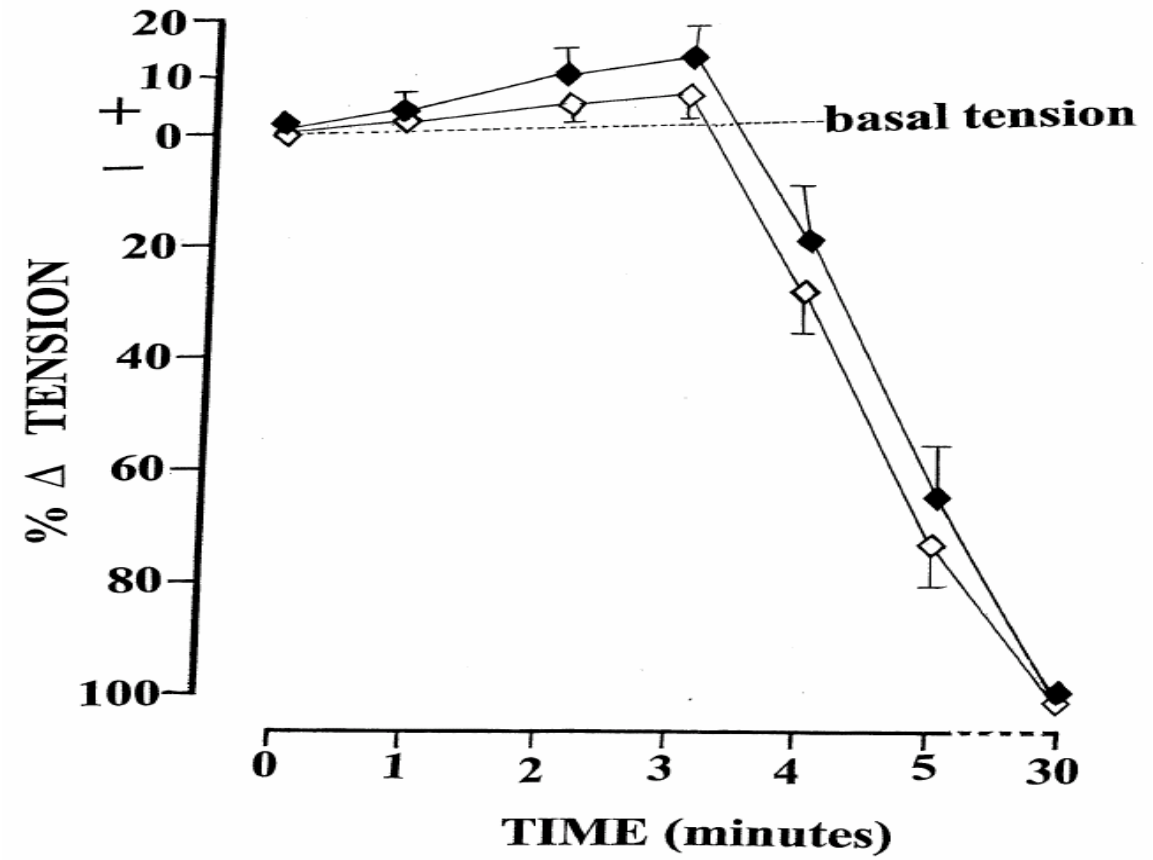

Figure 7. Effects of relatively high concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, ◇—◇ 400 and ◆——◆ 600 µg/ml) on the amplitude (force) of contractions of rat isolated portal veins. Each point represents the mean (±SEM) of 6–8 observations, while the vertical bars denote standard errors of the means.

The exact mechanisms of the hypotensive and cardio-depressant actions of *H. procumbens* root aqueous extract are obscure at the moment. Similarly, the exact chemical compound/s responsible for the hypotensive and cardio-depressant effects of the plant's root aqueous extract in the experimental animal models used in this study still remain/s speculative. However, the experimental evidence obtained in the present laboratory animal study lends pharmacological support to the suggested folkloric uses of the plant's root in the management and/or control of hypertension and certain cardiovascular disorders in some communities of South Africa.

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References

Bunag, R. D. (1984). Experimental and Genetic Models of Hypertension. In: 'Handbook of Hypertension', W. H. Birkenhager and J. L. Reid eds., Volume 4, Elsevier, Amsterdam, New York & Oxford; pp. 1–12.

Circosta, C., F. Occhiuto, S. Ragusa, A. Trovato, G. Tumino, F. Briguglio and C. De Pasquale (1984). A drug used in traditional medicine: *Harpagophytum procumbens* DC. II. Cardiovascular activity. J. Ethnopharmacol. **11:** 259–274.

Costa De Pasquale, R., G. Busa, C. Circosta, L. Iauk, S. Ragusa, P. Ficarra and F. Occhiuto (1985). A drug used in traditional medicine: III. Effects on hyperkinetic ventriclar arrhythmias by reperfusion. J. Ethnopharmacol. **13:** 193–199.

Henderson, M. and J. G. Anderson (1966). Common weeds in South Africa. Memoirs of the Botanical Survey of South Africa 37.

Ojewole, J. A. O. (1976). Studies on the Pharmacology of Some Antimalarial Drugs. PhD Thesis, University of Strathclyde, Glasgow, UK.

Snedecor, G. W. and W. G. Cochrane (1967). Statistical Methods. 6th Edition; Ames, Iowa: The Iowa State University Press; USA.

Van Wyk, B-E. and N. Gericke (2000). People's Plants. A Guide to Useful Plants of Southern Africa. 1st Edition, Briza Publications, Pretoria; p.146.

Van Wyk, B-E and M. Wink (2004). Medicinal Plants of The World. 1st Edition, Briza Publications, Pretoria, p. 165.

Van Wyk, B-E., B. Van Oudtshoorn and N. Gericke (2002). Medicinal Plants of South Africa, 2nd Edition, Briza Publications, Pretoria; p. 144.

Watt, J. M. and M. G. Breyer-Brandwijk (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd Edition, Livingstone, London; p. 830.