SOME PHARMACOLOGICAL PROPERTIES OF *HARPAGOPHYTUM PROCUMBENS* DC [PEDALIACEAE] SECONDARY ROOT EXTRACT

A THESIS SUBMITTED BY

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IN PARTIAL FULFILMENT FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE DEPARTMENT OF PHARMACOLOGY, SCHOOL OF PHARMACY & PHARMACOLOGY, FACULTY OF HEALTH SCIENCES, UNIVERSITY OF KWAZULU-NATAL

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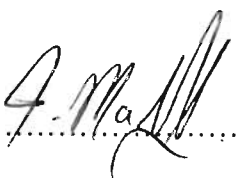
30 JULY, 2004

DECLARATION

I, Ismail Mall **Mahomed**, hereby declare that the thesis entitled:

"Some Pharmacological Properties of *Harpagophytum procumbens* DC [Pedaliaceae] Secondary Root Extract"

is the result of my original research investigation, that the work has not been submitted, in part or in full, for any degree in any other university, and that all the sources that I have used or quoted in the thesis have been indicated and acknowledged by complete references.



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Date

ABSTRACT

Harpagophytum procumbens DC [family: Pedaliaceae] is widely used in South African traditional medicine for the treatment, management and/or control of a variety of human ailments. In an attempt to scientifically appraise the '*healing powers*' and medicinal value of *Harpagophytum procumbens* DC secondary root aqueous extract (HPE), and throw some light on the efficacy and safety of the medicinal plant product, the pharmacological effects of the plant's root aqueous extract (HPE) have been investigated on a number experimental animal models *in vitro* and *in vivo*.

The results obtained in the studies reported in this thesis clearly indicate that *Harpagophytum procumbens* secondary root aqueous extract possesses a catalogue of pharmacological actions. The plant's aqueous extract (HPE, 10–1000 μg/ml) produced concentration-dependent, significant ($P<0.05$–0.001), atropine-sensitive contractions of mammalian and avian isolated gastro-intestinal smooth muscles. The plant's extract (HPE, 10–1000 μg/ml) also exhibited significant anticholinesterase activity. It is speculated that the contractile effects of *Harpagophytum procumbens* secondary root aqueous extract is mediated via cholinergic mechanism, possibly by stimulating cholinergic muscarinic receptors.

Harpagophytum procumbens secondary root aqueous extract (HPE, 10–1000 μg/ml) also provoked concentration-related, significant ($P<0.05$–0.001), contractions on the rat isolated uterine muscles. This observation appears to justify the use of the herb's

secondary root extracts for induction and acceleration of labour by traditional birth attendants. However, because *Harpagophytum procumbens* secondary root aqueous extract induced powerful contractions of uterine muscle strips taken from both pregnant and non-pregnant rats, the use of the plant's extract should be contra-indicated and avoided in pregnant women.

Harpagophytum procumbens secondary root aqueous extract produced a dose-related, slight but significant ($P<0.05–0.001$) hypotension in the arterial blood pressure of anaesthetized rats. The depressor effect of the plant's extract on the blood pressure of rats *in vivo* was resistant to atropine and mepyramine pretreatment. It was thought that the depressor effect could be due in part, to the vasorelaxant activity of the plant's extract, since *Harpagophytum procumbens* secondary root aqueous extract produced concentration-related, significant, secondary longer-lasting relaxations of the rat isolated portal vein.

The plant's extract produced biphasic effects on guinea-pig isolated cardiac muscles. The responses of guinea-pig isolated atrial muscles to the plant's extract consisted of concentration-dependent initial slight positive inotropic and chrotropic effects followed by long-lasting, significant negative inotropic and chronotropic effects. It was observed that relatively high concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 400–1000 μg/ml) always induced concentration-related, highly significant ($P<0.01–0.001$) negative inotropic and chronotropic responses of the guinea-pig isolated atrial muscles.

In the two analgesic test methods used, the plant's extract (HPE, 50–800 mg/kg i. p.) produced dose-related analgesic effects. Although to a smaller extent, the analgesic effects of the plant's extract were comparable with those of morphine, diclofenac or aspirin. It is, therefore, suggested that the analgesic effects of plant's extract are probably mediated centrally and peripherally.

Harpagophytum procumbens secondary root aqueous extract (HPE, 50–800 mg/kg i. p.) also produced dose-related, significant ($P<0.5–0.001$) anti-inflammatory activity against fresh egg albumin-induced inflammation in rats. Although qualitatively and quantitatively smaller, the anti-inflammatory effect of the plant's extract was comparable with that of diclofenac or aspirin. It is speculated that the plant's extract probably acts via a mechanism similar to those of the non-steroidal anti-inflammatory drugs (NSAIDs), i. e., by inhibiting cyclo-oxygenase and/or lipoxygenase, and thereby preventing the synthesis and release of inflammatory mediators such as prostaglandins.

In mice, *Harpagophytum procumbens* secondary root aqueous extract (HPE, 50–800 mg/kg i. p.) significantly delayed ($P<0.5–0.001$) the onset of seizures induced by pentylenetetrazole, picrotoxin and bicuculline. Since pentylenetetrazole- and picrotoxin-induced seizures are known to occur as a consequence of inhibition or attenuation of GABA neurotransmission and/or activity, it is speculated that the anticonvulsant activity of *Harpagophytum procumbens* secondary root aqueous extract might be due to potentiation or enhancement of GABA neurotransmission and/or activity by the plant's extract.

Harpagophytum procumbens secondary root aqueous extract (HPE, 50–800 mg/kg i. p.) produced dose-related, significant reductions in the blood glucose concentrations of streptozotocin-induced diabetic rats. The plant's extract also reduced the glucose levels of normal (normoglycaemic) rats. Although smaller, the hypoglycaemic and antidiabetic effects of the plant's extract are comparable to those of chlorpropamide in the experimental animal model used. It is speculated that *Harpagophytum procumbens* secondary root aqueous extract might be producing its hypoglycaemic and antidiabetic effects via mechanisms similar to those of sulphonylureas.

DEDICATION

This thesis is dedicated to:

ALL-MIGHTY ALLAH for sparing my life;

my parents, for their sympathetic approach to my problems;

my wife, for her love and care; and

my children, their understanding.

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my Promoter/Supervisor, Professor JAO Ojewole, for his unalloyed support, unparalleled encouragement and keen interest in my work. His kind and brotherly pieces of advice have made it possible for me to complete the work reported in this thesis on time.

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Table 5.

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Table 6.

Effects *of Harpagophytum procumbens* root aqueous extract (HPE), phenobarbitone (PBT) and diazepam (DZP) on picrotoxin (PCT)-induced seizures in mice.

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CHAPTER ONE

INTRODUCTION

BACKGROUND AND RATIONALE

Plants have been used for medicinal purposes in Africa for many centuries. Today, herbal products are being used worldwide in a variety of healthcare settings and as home remedies. Like other peoples in the world, Africans have long ago developed their own systems of healthcare delivery, including ways and means of classifying, diagnosing and treating diseases and illnesses. However, the history of '*Medicine and Healthcare*' in Africa has been shaped by complex socio-economic and political factors. In Africa, the concept of '*health*' and a '*healthy being*' has always been perceived in the light of the World Health Organization's (WHO's, 2002) document which defines "*health*" as: "*a state of complete physical, mental, social and psychological well being*".

There are millions of 'traditional heath practitioners' in Africa. For example, current estimates suggest that in South Africa, there is an approximate ratio of 1:500 of traditional healers (i. e., traditional health practitioners) to the people of an estimated population of 44 million, whereas the ratio of Western health practitioners (i. e., physicians) to the people in the country is 1:40 000. Current estimates also suggest that in many African countries, a large proportion of the population (approximately 75%) still rely heavily on traditional healers and medicinal plants for their daily healthcare needs.

Although modern, Western healthcare systems may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity among the people for historical, social, cultural and economic reasons. Moreover, the people, especially in the rural areas, are more likely to consult traditional healers first about their ailments because of the physical proximity. According to World Health Organization (WHO, 2002; 2003), *'Traditional Medicine'* may be defined as *'all knowledge and practices, understandable or not, that are used to diagnose, prevent, heal partially or completely, eliminate a physical, mental or social disorder (imbalance), based exclusively on the day-to-day experience of the practitioner and the observation/s transmitted orally or in writing, from generation to generation'.*

On the continent of Africa, only a few of the vast medicinal plants used as phytomedicines by traditional health practitioners have been subjected to scientific scrutiny and experimentation in laboratory animals and man, for their accredited medicinal values. Safety and efficacy data are not available for most of the commonly-used medicinal plants, their extractives and bioactive chemical constituents, and the preparations containing them. Although many of the plants have ethnobotanical reputation for traditional medicinal usage, there is very little or no scientific information on their active constituents, pharmacological actions, and clinical efficacy. Growing interests and concerns have, therefore, prompted greater in-depth studies on the properties and uses of medicinal plant materials and products, and raised concerns about the safety, efficacy and quality of medicinal plants and herbal products generally. Both the consumers and healthcare professionals need up-to-date, authoritative and accurate

information on the safety and efficacy of medicinal plant products and traditional medicines made from the plants. In the interest of millions of people who rely on traditional medicines for their daily healthcare needs, it is, therefore, thought imperative to scientifically probe the frequently-used medicinal plants of South Africa, with a view to obtaining convincing data on the pharmacological actions, safety and efficacy of extractives obtained from the plants.

The study reported in this thesis deals with some aspects of the pharmacology of *Harpagophytum procumbens* DC aqueous extract.

HARPAGOPHYTUM PROCUMBENS DC ("DEVIL'S CLAW")

Historical Background

The German explorers in Namibia in the early 19th century, learned about the multi-purpose and magnificent uses of '*harpago*' from the Bushmen. The plant was thus taken (from Namibia) and introduced to Europe via Germany at the beginning of the 20th century, where attention has focused on its use as an anti-inflammatory and anti-rheumatoid herb (Beresford, 2002).

Botanical Name: *Harpagophytum procumbens* DC

Family: Pedaliaceae

Vernacular (aka) Names: *Devil's claw, Grapple plant, Wood spider, Harpago*

Harpagophytum procumbens DC [family: Pedaliaceae] is a desert plant which is indigenous to southern Africa. It is found in abundance in the Kalahari desert and Namibian steppes where it has long been used as a folk remedy for the treatment of a variety of human ailments. The plant is also found in South Africa, Botswana, Zimbabwe, and to a very little extent, in some east African countries. *Harpagophytum procumbens* DC is a weedy, perennial plant with annual creeping stems spreading from a central, thick, fleshy, tuberous tap-root (Henderson and Anderson 1966; Van Wyk *et al.*, 2002; Van Wyk and Wink, 2004). 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) – see Figure 1. The plant is commonly referred to as '*Devil's claw*', a name derived from 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).

Devil's claw has been used for centuries by Africans as a folk remedy to treat a variety of human ailments, including skin cancer and lesions, fevers, gout, rheumatoid arthritis, diabetes, allergies, indigestion, and conditions affecting the gall bladder, pancreas,

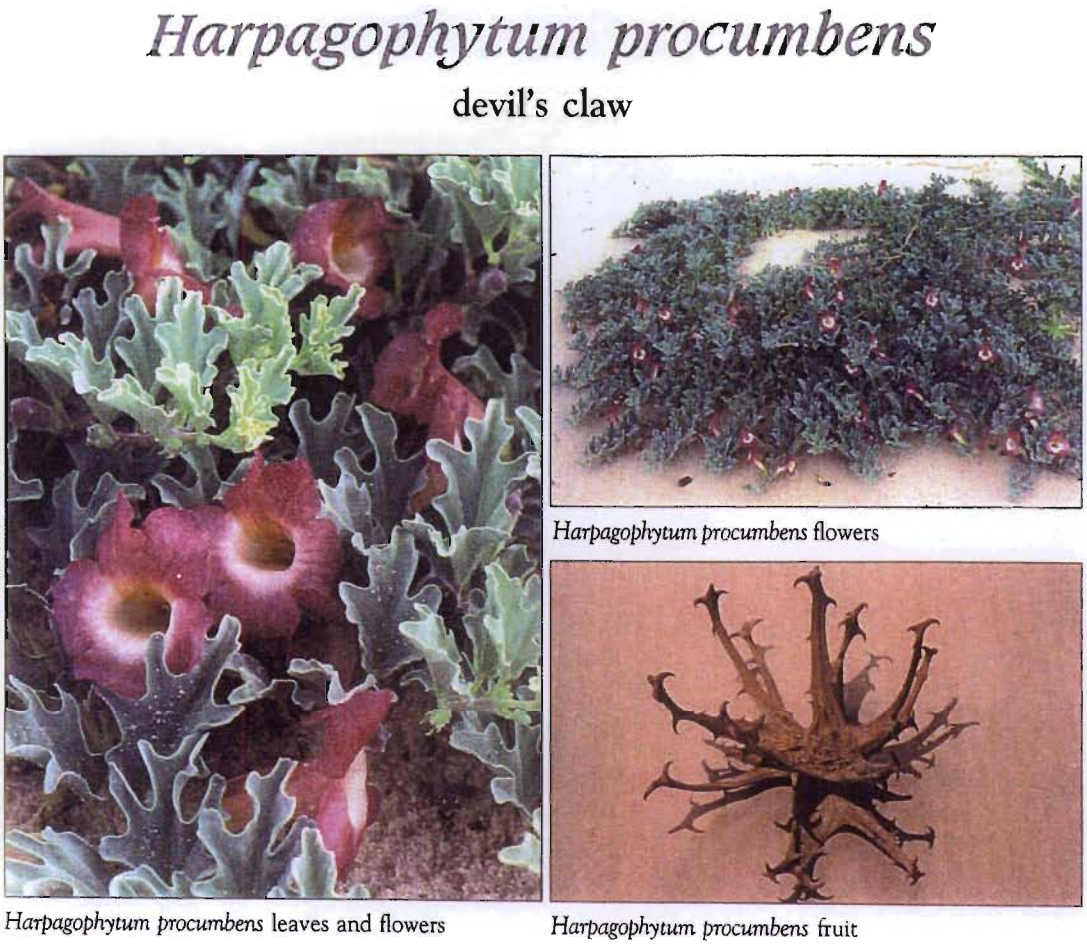

Figure 1.

Harpagophytum procumbens (Devil's claw). [Culled from Van Wyk and Wink, 2004].

stomach and kidneys (Beresford, 2002). In Europe, the root extract is recommended for arthritis, diabetes, allergies and senility, and is widely used as a digestive aid and appetite stimulant (Smith *et al.*, 2001). *Devil's claw* is widely used in European herbal tea formulations, and in recent years, many health food marketing centers carry formulations containing *harpago* extracts or root powders. The British and German Herbal Pharmacopoeias recognize *Devil's claw* as possessing analgesic, sedative and diuretic properties (Smith *et al.*, 2001). Also in Europe, a home remedy containing secondary roots of *Devil's claw* is used for lack of appetite, dyspeptic complaints and as a supportive therapy for degenerative disorders of the locomotor system (Smith *et al.*, 2001). Analgesic effects of the secondary roots of the plant have also been observed along with reductions in abnormally high cholesterol and uric acid levels. *Harpago* is reported to be useful in small joint pains. Current use of the herb in the Western world has focused on its application to painful conditions of the musculo-skeletal system and digestive problems (Smith *et al.*, 2001). It is frequently found in prescriptions for arthritis of all sorts, for rheumatic complaints and for low back pain, especially associated with spondylosis, lumbago, sciatica, fibrositis, neuralgia and polymyalgia (Smith *et al.*, 2001). 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. In South Africa, 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 (Van Wyk and Gericke, 2000). When taken on a regular daily basis, it has a subtle laxative effect. Small doses of the plant's secondary 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 ointments and creams of *H. procumbens* are applied topically for minor muscular aches and pains, and to painful joints (Watt and Breyer-Brandwijk, 1962; Van Wyk and Gericke, 2000; 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).

Active Constituents

The main active chemical constituents of *Harpagophytum procumbens* secondary root extract include:

Iridoid Glycosides – (0.5 – 3%) – Figure 2.

Harpagoside – Harpagoside was the first iridoid glycoside isolated from *Harpagophytum procumbens* secondary root in 1962, and is widely regarded as the main active constituent of the plant. Another glycoside discovered at the same time was named 'harpagide'.

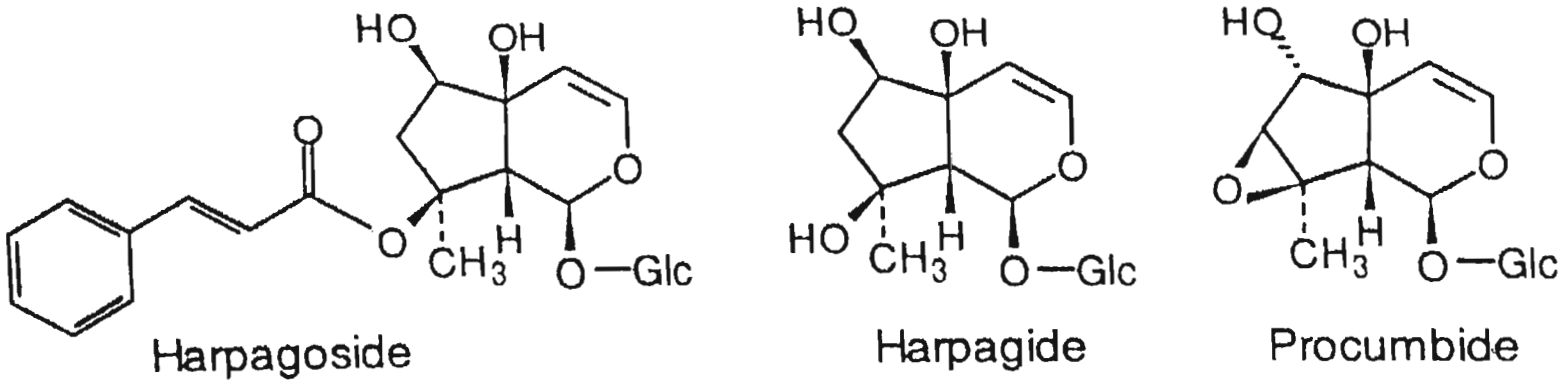

Figure 2.

Structural formulae of harpagoside, harpagide and procumbide. [Culled from Van Wyk *et al.*, 2002].

A third glycoside named 'procumbine' was also isolated in 1962 (Beresford, 2002). In 1983, three additional iridoid glycosides with similar names, but having different linkages were discovered (Beresford, 2002). Iridoids are synthesized through mevalonic acid pathway, and are technically known as 'cyclopentan-[c]-pyran monoterpenoids'. The aglycones of the iridoid glycosides have furane or pyrane structure (Beresford, 2002). By degradation of natural pentoses to furfuol, this gives rise, via furane-2-carbonic acid, to furane. The pyranes α-pyrane and gamma pyrane in their reactions resemble unsaturated aliphatic compounds, but the pyrylium salts derived from them are trebly unsaturated, and so are aromatic in character (Beresford, 2002). Some researchers believe that the therapeutic effects of the iridoid glycosides are due to furane and pyrane, the latter accepting hydrogen, and thus producing oxidation of the substrate, such as toxin (Beresford, 2002). Clinical studies have shown that the iridoid glycosides from *Harpagophytum procumbens* secondary root are hydrolysed into the pyridine monoterpene alkaloid 'aucubinine B' by human faecal bacteria (Beresford, 2002).

Flavonoids –

Mainly luteolin and kaempferol.

Phenolic Acids –

Chlorogenic acid, cinnamic acid and caffeic acid.

Quinones –

Mainly harpagoquinone

Phytosterols –

Stigmasterol and β-sitosterol

Sugars – (50%)

Consisting mainly of the tetrasaccharide stachyose (up to 46%), smaller amounts of raffinose, sucrose and monosaccharides (Beresford, 2002). With the absence of starch and high molecular weight polysaccharides, the sugars serve as reserve carbohydrate and lead to an unusually high water-soluble fraction of 50–70% (Beresford, 2002).

Triterpenes –

Oleanolic, ursolic and 3β-acetyloleanolic acid derivatives.

Other Compounds –

Acetosides, esters and minerals.

Pharmacology

Many studies have been conducted on laboratory animals and man over the last three-to-four decades, in an effort to scientifically validate the effectiveness of *Harpagophytum*

procumbens secondary root extract (and the iridoid glycoside, harpagoside) as an anti-inflammatory, anti-rheumatic and analgesic agent. The results of these studies have been conflicting (Beresford, 2002). As a result of these conflicting findings, several investigators have cited a lack of quality control (standardization) of the preparations used in the various studies, and the inactivation of *Harpagophytum procumbens* secondary root extracts in the stomach when the extracts are administered orally – due to deactivation of its active chemical constituents by gastric acid juices – as possible causes of the discrepancies. There is some evidence to support the latter view, with research indicating that the anti-inflammatory and analgesic effects of the plant's root extracts were abolished following an acid treatment in order to reproduce the physico-chemical conditions found in the stomach. The same acid treatment also abolished the analgesic effects of harpagoside, suggesting that it is also degraded by gastric acid (Lanhers *et al.*, 1992).

One area where researchers seem to be in agreement is that the iridoid glycoside harpagoside does not appear to be involved in the anti-inflammatory properties of *Harpagophytum procumbens* secondary root extracts (Lanhers *et al.*, 1992; Beresford, 2002). In addition, while harpagoside appears to exhibit some analgesic activity, this effect was about 42% less than that of the whole plant extract, suggesting that other compounds in the plant must also be involved in the analgesic activity (Lanhers *et al.*, 1992; Beresford, 2002).

The mechanism underlying the anti-inflammatory and anti-arthritic actions of *Harpagophytum procumbens* secondary root extracts is unclear. Research conducted in

the early 70s indicated that the plant's anti-arthritic action was due to the redox potential of the iridoid glycosides. However, more recent studies have indicated that the anti-inflammatory action of the plant's extracts does not appear to involve the arachidonic acid cascade and prostaglandins, as do conventional NSAIDs such as indomethacin (Lanhers *et al.*, 1992; Beresford, 2002). It is speculated that the analgesic action of *Harpagophytum procumbens* secondary root extracts may be due to a complex interaction between the various chemical constituents of the plant, thus suggesting that the compounds, especially harpagoside, interfere with the mechanisms which regulate calcium mobilization and sequestration in the cells.

Anti-oxidant Activity

Recent investigations have centred on the possibility that *Harpagophytum procumbens* secondary root extract possesses significant anti-oxidant action, and that this action may explain some of its other actions and effects, particularly the anti-inflammatory and anti-rheumatic actions (Beresford, 2002). There is substantial evidence that oxidative free radicals may be responsible for the induction of inflammation, and this may be an important aetiological factor in the genesis of inflammatory disease, such as rheumatoid arthritis (RA). Oxidative free radicals produced by polymorphonuclear leukocytes, and possibly other sources also, contribute to the complex pathogenesis of arthritic disease. Thus, they activate prostaglandin synthesis, and cause direct cellular damage or injury,

altering the biochemical, biophysical and structural properties of cellular proteins, including elastin, collagen and polysaccharides; degrading cartilage and reducing the viscosity of synovial fluid (Beresford, 2002). It has been shown that RA has many characteristics of a free-radical induced disease. The human synovial tissue has no anti-oxidant protection in the form of superoxide dismutase (SOD), catalase (CAT) or glutathione peroxidase (GPX), to protect against potential damage induced by oxygen free radicals. This would include lipid peroxidation and its consequences (Beresford, 2002). Experimental studies have shown that *Harpagophytum procumbens* secondary root extract possesses anti-oxidant activity similar to those of standard anti-oxidant agents. This anti-oxidant property may be responsible for the experimental and clinical anti-inflammatory activity of the herb (Beresford, 2002). This anti-oxidant activity of *Devil's claw* may also explain some of the other reported actions of the plant's secondary root extract, including its inhibitory effects on smooth, cardiac and skeletal muscles, and interfere with mechanisms that regulate the influx of calcium ions into cells. Perfusion injury following myocardial infarction is associated with an accumulation of oxidative free radicals (Beresford, 2002). Anti-oxidants have been shown to be effective in preventing or reducing such injury. Similarly, increased cellular calcium influx following lipid peroxidation of the cell membrane, may be involved in the mechanisms underlying cell damage or injury (Beresford, 2002).

Digestive Activity

Harpagophytum procumbens secondary root is reported to have a bitter action, equivalent to that of gentian root (Beresford, 2002). Much of this effect is thought to be due to its iridoid glycosides which cause reflex stimulation of the digestive processes. Recent studies have also shown that the plant product activates liver function, promoting detoxification of toxins such as urea (Beresford, 2002). Furthermore, the plant has been shown to be a useful remedy for reducing elevated cholesterol and neutral fat levels in patients with metabolic disorders (Beresford, 2002). Published research findings have shown beneficial effects of *Harpagophytum procumbens* secondary root in the treatment of a wide range of digestive disturbances, including dyspeptic conditions of the upper epigastrium, intestinal upsets, and liver and gall bladder complaints with or without pancreatic involvement (Beresford, 2002).

Immunomodulatory Activity

Harpagophytum procumbens secondary root has been shown to possess significant immunomodulatory activity, and according to traditional Indian medicine, the plant is a good stimulant for the lymphatic system (Beresford, 2002).

Toxicology

Harpagophytum procumbens secondary root is considered to be very safe in experimental animals and human beings (Beresford, 2002).

Contra-indications

Harpagophytum procumbens secondary root is said to have oxytocic properties and should be avoided in pregnancy. Furthermore, because of its reflex effect on digestive system, the use of the plant should be avoided in patients with gastric or duodenal ulcers (Beresford, 2002).

AIMS OF THIS STUDY

Although *Harpagophytum procumbens* DC secondary tap root is widely used in South African traditional medicine for the treatment, management and/or control of a variety of human ailments, very little or no scientific data exists in the literature on the efficacy, safety and quality of the plant product. Biomedical literature is also very sparse on the pharmacological actions of the herb in man or in laboratory animals. Since many people still use *Harpagophytum procumbens* DC secondary root daily for their health conditions, it has become imperative to scientifically scrutinize the '*healing powers*' of the plant, appraise and validate the ethnomedical claims of traditional healers on the herb, and throw more light on the efficacy and safety of the medicinal plant product. To meet the above objectives, the pharmacological effects of the plant's root aqueous extract (HPE) have been investigated on organ-systems and various tissues, including isolated gastro-intestinal muscle strips, cardiac muscle preparations and also on the cardiovascular system of some laboratory animals. It was also thought that such experimental, laboratory animal studies would probably provide rational explanations and pharmacological basis for some of the commonly-reported adverse effects of the plant product.

CHAPTER TWO

GASTRO-INTESTINAL SMOOTH MUSCLE PHARMACOLOGY OF *HARPAGOPHYTUM PROCUMBENS* DC

ABSTRACT

Harpagophytum procumbens DC is widely used in South African traditional medicine for the treatment, management and/or control of a variety of human ailments. In an attempt to scientifically appraise the '*healing powers*' and medicinal value of *Harpagophytum procumbens* DC secondary root aqueous extract (HPE), and throw some light on the efficacy and safety of the medicinal plant product, the pharmacological effects of the plant's root aqueous extract (HPE) on chick, guinea-pig and rabbit isolated gastro-intestinal smooth muscle preparations, and rat isolated uterine muscle strips have been examined. The results of this *in vitro* laboratory animal study indicate that relatively low to high doses of *Harpagophytum procumbens* root aqueous extract (HPE, 10–1000 μg/ml) provoked concentration-related, atropine-sensitive, significant ($P<0.05–0.001$) contractions of the chick isolated oesophagus, guinea-pig isolated ileum and rat uterine muscle preparations.

Relatively moderate to high concentrations of the plant's extract (HPE, 200–1000 μg/ml) always produced concentration-dependent, biphasic effects on rabbit isolated duodenum. Relatively low concentrations of the plant's extract (HPE, 10–100 μg/ml) usually caused

an initial slight, transient and non-significant (P>0.05) increase in the amplitude of the spontaneous, myogenic, rhythmic, pendular contractions of the rabbit duodenal muscle preparations. On the other hand, relatively moderate to high concentrations of the plant's extract (HPE, 200–1000 μg/ml) always produced initial slight, significant (P<0.05–0.01) reductions (inhibitions) of contractile amplitudes of the rabbit isolated duodenum. The initial slight depressions of the contractile amplitudes of the isolated rabbit duodenum caused by relatively moderate to high concentrations of the plant's extract (HPE, 200–1000 μg/ml) were always followed by dose-related, significant (P<0.05–0.001) elevated baseline tones (tensions) and augmentations of the contractile amplitudes; and then secondary, longer-lasting relaxations and reductions of the contractile amplitudes of the spontaneous, rhythmic, myogenic, pendular contractions of the rabbit isolated duodenal muscle preparations.

Some traditional birth attendants in South Africa have claimed that *Harpagophytum procumbens* DC secondary root is a useful obstetric remedy for the induction or acceleration of labour, as well as for expulsion of retained placentas in pregnant women. In the present study, therefore, the pharmacological effects of *Harpagophytum procumbens* secondary root aqueous extract (HPE) have been examined on isolated uterine muscle strips taken from pregnant and non-pregnant, young female rats. The plant's extract (HPE, 10–1000 μg/ml) induced concentration-related, significant (P<0.05–0.001) increases in the baseline tone (basal tension) and caused powerful spontaneous, rhythmic, myogenic contractions of the oestrogen-dominated uterine muscle strips taken from stilboesterol-pretreated, non-pregnant female rats. Relatively low to high

concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 10–1000 μg/ml) also provoked concentration-related, significant ($P<0.05–0.001$) increases in the baseline tone (basal tension) and contracted uterine muscle strips taken from rats in the early, middle and late stages of pregnancy. Moderate to high concentrations of the plant's extract (HPE, 200–800 μg/ml) always provoked powerful contractions of the uterine muscle preparations. The results of this *in vitro* study indicate that *Harpagophytum procumbens* secondary root aqueous extract (HPE) possesses significant contractile effect and/or uterotonic action on mammalian uterus. This observation suggests that the use of *Harpagophytum procumbens* secondary root should be contra-indicated and avoided in pregnancy.

In a separate set of experiments involving the use of chick isolated biventer-cervicis and oesophagus muscle homogenates for colorimetric anticholinesterase determination, *Harpagophytum procumbens* (HPE, 10–1000 μg/ml) was found to possess anticholinesterase activity. In this regard, however, the plant's extract was found to be less potent than physostigmine. The anticholinesterase action of the plant's extract is speculated to contribute, at least in part, to the contractile effects of the herb's extract on the isolated gastro-intestinal and uterine smooth muscle preparations used in this study.

INTRODUCTION

Reports on extra-vascular and vascular smooth muscle pharmacology of *Harpagophytum procumbens* DC secondary root extracts are very scanty in the literature. Occhiuto *et al.*, (1985) examined the effects of methanolic extract of *Harpagophytum procumbens* DC secondary roots and two of its active constituents, harpagoside and harpagide, on rabbit isolated duodenum and guinea-pig isolated ileum. The investigators observed that low to moderate concentrations of *Harpagophytum procumbens* secondary root extract (20–80 μg/ml) increased the basal tone of the gastro-intestinal smooth muscle preparations in a concentration-dependent manner. Occhiuto *et al.*, (1985) concluded that the action of *Harpagophytum procumbens* root extract on the non-vascular smooth muscle preparations is due to a complex interaction between the various active principles of the plant, and then suggested that the plant's active constituents, especially harpagoside, interfere with the mechanisms that regulate the influx of calcium in the cells. Besides this pioneering work of Occhiuto *et al.*, (1985), to the best of our knowledge, there is no other report in the literature, on the extra-vascular smooth muscle effect of *Harpagophytum procumbens* DC secondary root extract. Therefore, it was thought worthwhile investigating the effects of *Harpagophytum procumbens* DC secondary root aqueous extract on the chick isolated parasympathetically-innervated oesophageal smooth muscle preparation, guinea-pig isolated ileum, rabbit isolated duodenum and rat isolated uterine smooth muscles, with a view to meeting the following aims and objectives:

AIMS OF THIS STUDY

The studies reported in this section of the thesis were carried out in order to:

1. systematically study the effects of *Harpagophytum procumbens* DC secondary root aqueous extract (HPE) on a range of isolated smooth muscle preparations that have been, and that have not been, previously reported in the literature (for example, the chick oesophagus, and rat uterus);
2. elucidate the plausible mechanisms of action of *Harpagophytum procumbens* DC secondary root aqueous extract in the various isolated muscle preparations used;
3. examine the hypothesis that the uterotonic action of *Harpagophytum procumbens* DC secondary root aqueous extract may involve prostaglandin release;
4. examine if some of the commonly-reported adverse effects of the plant product (for example, gastro-intestinal disturbances and/or upsets) could be explained by an action on gastro-intestinal smooth muscles;
5. substantiate or deny the findings of Occhiuto *et al.*, (1985).

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 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°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, white albino rabbits of both sexes weighing 1.5–3.0 kg; male and female Dunkin-Hartley guinea-pigs weighing 300–450 g; young adult, female Wistar rats (*Rattus norvegicus*) weighing 250–300 g, and young chicks (aged between 3 and 10 days after hatching) weighing 50–75 g; were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and 12-hour light : 12-hour night cycle; and were allowed free access to food and water *ad libitum*.

A. Effects of *Harpagophytum procumbens* Root Aqueous Extract on Chick Isolated Parasympathetically-Innervated Oesophagus

Young chicks (aged between 3 and 10 days after hatching) were starved overnight (to empty their crops) and killed by deep petroleum ether inhalation. The upper oesophagus, as far as the crop, was carefully prepared together with as much as possible of the right parasympathetic nerve trunk which runs along the course of the jugular vein. Parasympathetically-innervated oesophageal muscle strips were removed from the chicks according to the method of Bowman and Everett (1964). In each case, the tubular

segment (3–4 cm long) of the entire upper oesophagus was removed, set-up, treated and chemically- or electrically-stimulated under physiological conditions as described in detail earlier by Ojewole (1976). Each isolated oesophageal muscle strip was 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°C and continuously aerated with carbogen (i. e., 5% carbon-dioxide + 95% oxygen gas mixture). Two oesophageal 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 oesophageal muscle sensitivity. Each preparation was subjected to a resting tension of 1.0 g, and allowed to equlibrate for 30–45 minutes before it was challenged with HPE (and other drugs used) or electrically stimulated. 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. In the electrically-stimulated preparations, each tubular muscle strip was indirectly- stimulated through the parasympathetic (vagus) nerve trunk by means of bipolar platinum ring electrodes. Maximal contractions of the isolated muscle preparations were provoked with trains of square wave pulses of 0.5–1.0 msec duration at a frequency of 10–30 Hz and supramaximal voltages of 50–100 volts delivered from SRI square wave stimulators. Electrical stimulation usually lasted for 10 seconds, and was repeated where necessary, at regular intervals of 5–10 minutes. In some cases, concentrations of HPE (and other drugs used) were added sequentially to the bath-fluid

in-between electrical stimulations. Concentrations of bath-applied 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 muscle prepations were recorded isometrically by means of Ugo Basile force-displacement transducers, 2-Channel "Gemini" Recorder, and pen-recording microdynamometers (model 7070).

B. Effects of *Harpagophytum procumbens* Root Aqueous Extract on Guinea-Pig Isolated Ileum

Tubular segments taken from distal ileum of guinea-pigs of either sex were prepared, isolated 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 deep petroleum ether inhalation and bled out. Tubular pieces (3–4 cm long) taken from distal ileum of each animal were suspended in 30-ml 'Ugo Basile Two-Chambered Organ Baths' (model 4050) containing Krebs-Henseleit physiological solution maintained at 36±1°C and continuously aerated with carbogen (i. e., 5% carbon-dioxide + 95% oxygen gas mixture). Two ileal 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 ileal muscle sensitivity. The tissues were subjected to a resting tension of 1.0 g, and allowed to equlibrate for 30–45

minutes 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, 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. HPE- (and other drugs-) induced contractions and responses of the isolated ileal muscle preparations were recorded isometrically by means of Ugo Basile force displacement transducers, 2-Channel "Gemini" Recorder, and pen-recording microdynamometers (model 7070).

C. Effects of *Harpagophytum procumbens* Root Aqueous Extract on Rabbit Isolated Duodenum

Tubular segments (3–4 cm long) taken from the duodenum of young adult rabbits of either sex (weighing 1.5–3.0 kg) were prepared, isolated and set-up under physiological conditions as described in detail earlier by Ojewole (1976). Each of the animals used was killed by deep petroleum ether inhalation and bled out. The abdomen of the animal was quickly opened by a midline incision, and tubular pieces (3–4 cm long) of the duodenum were carefully cleaned free of connective, extraneous and fatty tissues, and then removed. The tubular pieces (3–4 cm long) were separately suspended in 30-ml 'Ugo Basile Two-Chambered Organ Baths' (model 4050) containing Krebs-Henseleit physiological

solution maintained at 36±1°C and continuously aerated with carbogen (i. e., 5% carbon-dioxide + 95% oxygen gas mixture). Two isolated duodenal 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 duodenal muscle sensitivity. Each of the isolated duodenal muscle preparations was allowed to equilibrate for a period of 30–45 minutes under an applied resting tension of 1.5–2.0 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, 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, pendular, rhythmic contractions, as well as the HPE- (and other drug-) induced responses of the isolated muscle strips were recorded isometrically with the aid of Ugo Basile force-displacement transducers, 2-Channel "Gemini" Recorder, and pen-recording microdynamometers (model 7070).

D. Effects of *Harpagophytum procumbens* Root Aqueous Extract On Rat Isolated Uterine Muscles taken from Pregnant and Non-Pregnant Female Rats:

Young adult, female Wistar rats (*Rattus norvegicus*) weighing 250–300 g were used. The animals were kept and maintained under laboratory conditions of temperature, humidity,

and light; and were allowed free access to food (standard pellet diet) and water *ad libitum*. The animals were divided into two categories as follows:

(i) Oestrogen-dominated, non-pregnant rats

All the rats in this group were pretreated with stilboesterol (0.1 mg/kg s. c.) 20–24 hours before use (in order to induce oestrus state). Vaginal smears were taken immediately before the animals were sacrificed in order to ascertain that the animals were in oestrus state. Female rats in oestrus state were used in this set of experiments.

(ii) Pregnant Rats

Mated female rats were examined daily for the presence of cervical plug. The day on which cervical plug was first observed was taken as 'day one' of pregnancy of the female rat. Early pregnancy was regarded as day 1 to day 8, while late pregnancy was taken to be from day 16 to day 20 following cervical plug detection.

Each of the pregnant and stilboesterol-pretreated non-pregnant female rats was killed by deep petroleum ether inhalation and bled out. The two uterine horns of the animal were cleaned free from fatty and connective tissues and trimmed. Tubular segments of approximately equal lengths (2–3 cm) were removed from the uterine horns by cutting

off both ends. The two tubular uterine-horn segments (2–3 cm long) were set-up under physiological conditions as described in detail earlier by Ojewole (1976). Each isolated uterine muscle strip was separately suspended in 30-ml 'Ugo Basile Two-Chambered Organ Baths' (model 4050) containing de Jalon's physiological solution (of composition, in g/litre: NaCl, 9.0; KCl, 0.42; $CaCl_2$, 0.06; $MgCl_2$, 0.005; $NaHCO_3$, 0.5; and glucose, 0.5) maintained at 32±1°C and continuously aerated with carbogen (i. e., 5% carbon-dioxide + 95% oxygen gas mixture). Two uterine horn muscle preparations (one used as 'control' and the other one used as 'HPE- (or other drug-) treated test' preparation) were always set-up to allow for changes in the uterine muscle sensitivity. Each preparation was subjected to a resting tension of 1.0 g, and allowed to equilibrate for 30–45 minutes 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, and washed out three-to-five times after the maximum responses of the tissues were attained. Distilled water (the vehicle in which HPE and other drugs used in this study were dissolved) was used as the 'control' fluid for HPE and other drugs tested. Concentrations of bath-applied HPE (and other drugs used) were repeated where appropriate and/or possible, at regular intervals of 3–20 minutes after the last washing. HPE- (and other drug-) induced responses of the uterine muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers, Ugo Basile's 2-Channel "Gemini" Recorder, and pen-writing microdynamometers (model 7070).

E. Determination of Anticholinesterase Activity

Anticholinesterase activity of the plant's extract (HPE) was determined and compared with that of physostigmine by measuring the cholinesterase activity of the chick biventer-cervicis (and chick oesophagus) muscle homogenates, using the colorimetric assay method of Ellman *et al.*, (1961). Young chicks (aged between 3 and 10 days after hatching) were killed by deep petroleum ether inhalation. Their biventer-cervicis (and oesophageal) muscles were removed as described in detail by Ojewole (1976). The isolated muscles were separately homogenized with an Ultra Turrax homogenizer (type TP18/2) for one minute, using 20 mg of tissue per ml of 0.1M phosphate buffer (at pH 8.0). The homogenate was filtered through a fine gauze into a 25-ml glass flask immersed in ice. 0.5 ml of the homogenate was added to 5.1 ml of phosphate buffer (at pH 8.0) in the glass flask, and incubated at 37°C for 5 minutes with shaking (by means of a mechanical shaker). After 5 minutes, 0.2 ml of a graded concentration of HPE (or physostigmine) was added, and the solution was further incubated for another 15 minutes with shaking. Acetylthiocholine (0.2 ml; 1.0 mM) was added to serve as the substrate. Samples were taken at 15-minute intervals for the estimation of anticholinesterase activity. However, 5 minutes before sampling, 0.1 ml of 5,5-dithiobis-2-nitrobenzoic acid reagent was added to 2.9 ml of phosphate buffer (at pH 8.0) in a photocell, mixed gently, and then followed by addition of 0.2 ml of the incubated solution.

Absorbance of the sample was read against a blank at wavelength 412 mμ, using a Unicam SP600 spectrophotometer. The 'blank' solution was treated exactly in the same

way as the 'test' solution, except that the glass flask for the 'blank' solution contained 5.5 ml phosphate buffer (at pH 8.0), 0.5 ml homogenate, but without anticholinesterase agent (i. e., HPE or physostigmine) or acetylthiocholine. The anticholinesterase determination is based on the following reactions:

Acetylthiocholine —(cholinesterase enzyme)→ Thiocholine + acetate .....(i)

Thiocholine + dithiobisnitrobenzoate ⟶ Yellow colour .....(ii)

F. Analysis of Data

Data obtained from 'test' groups of isolated muscle strips (chick oesophagus, guinea-pig ileum, rabbit duodenum and rat uterus) treated with *Harpagophytum procumbens* root aqueous extract (HPE) and other drugs used, and those obtained from distilled water-treated 'control' 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. '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.

RESULTS

The results obtained in this set of experiments demonstrate that *Harpagophytum procumbens* root aqueous extract possesses an array of pharmacological actions on extra-vascular (i. e., non-vascular) smooth muscles, and confirm the findings of Occhiuto *et al.*, (1985).

Effects of *Harpagophytum procumbens* Root Aqueous Extract on Chick Isolated Parasympathetically-Innervated Oesophagus

Relatively low to high concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–1000 μg/ml) always raised the baseline tension (baseline tone) of, and contracted the chick isolated, parasympathetically-innervated oesophageal muscle preparations in a concentration-dependent manner. Furthermore, relatively small to high doses of the plant's extract (HPE, 50–1000 μg/ml) always produced dose-dependent, significant ($P<0.05–0.001$), atropine-sensitive contractions of the chick oesophageal muscle preparations. Figure 3 shows a typical trace, while Figure 4 summarizes the results obtained. Although qualitatively less, the chick oesophageal muscle contractions induced by moderate to high doses of the plant's extract (HPE, 50–1000 μg/ml) were comparable with those produced by acetylcholine (ACh, 0.1–5.0 μg/ml) and

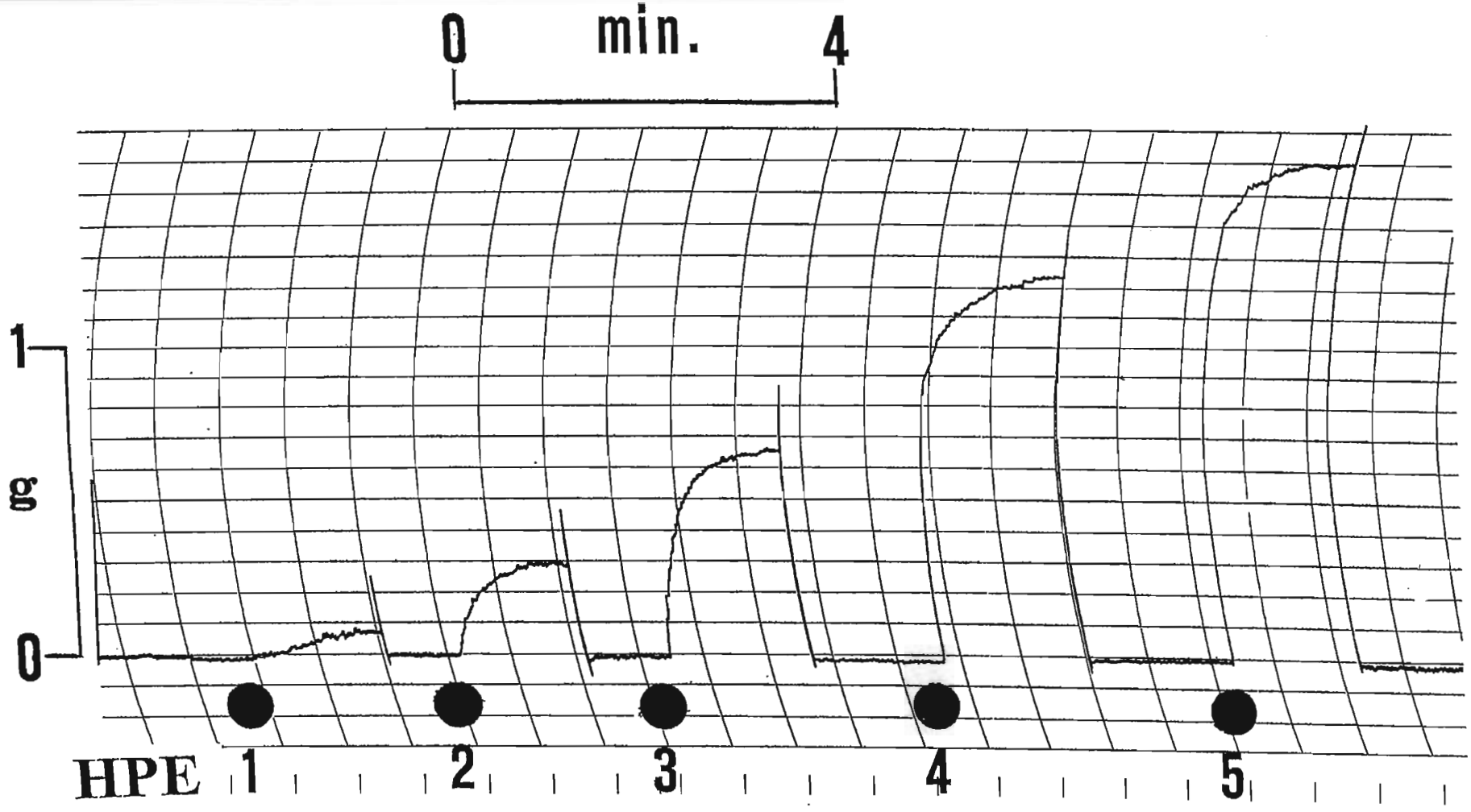

Figure 3.

Effects of graded concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE) on chick isolated oesophagus. HPE 1, 2, 3, 4 and 5 represent 50, 100, 200 400 and 800 μg/ml of *Harpagophytum procumbens* secondary root aqueous extract sequentially added to the bath fluid at the solid points (●) respectively.

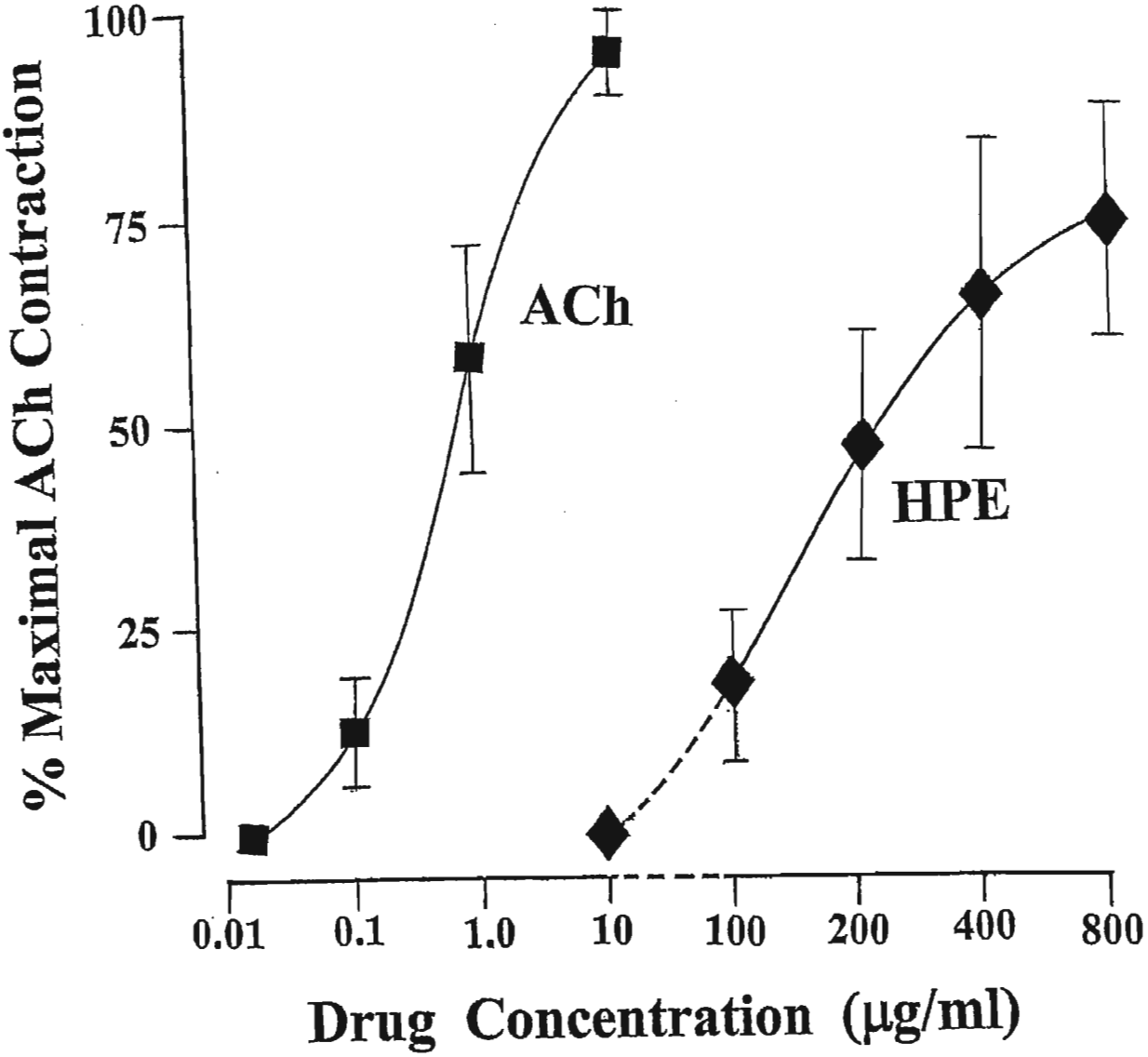

Figure 4.

Dose-response curves of graded concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 10–800 µg/ml) and acetylcholine (ACh, 0.01–10.0 µg/ml) on chick isolated oesophagus. Each point represents the mean (±SEM) of 6–8 observations, while the vertical bars denote standard errors of the means.

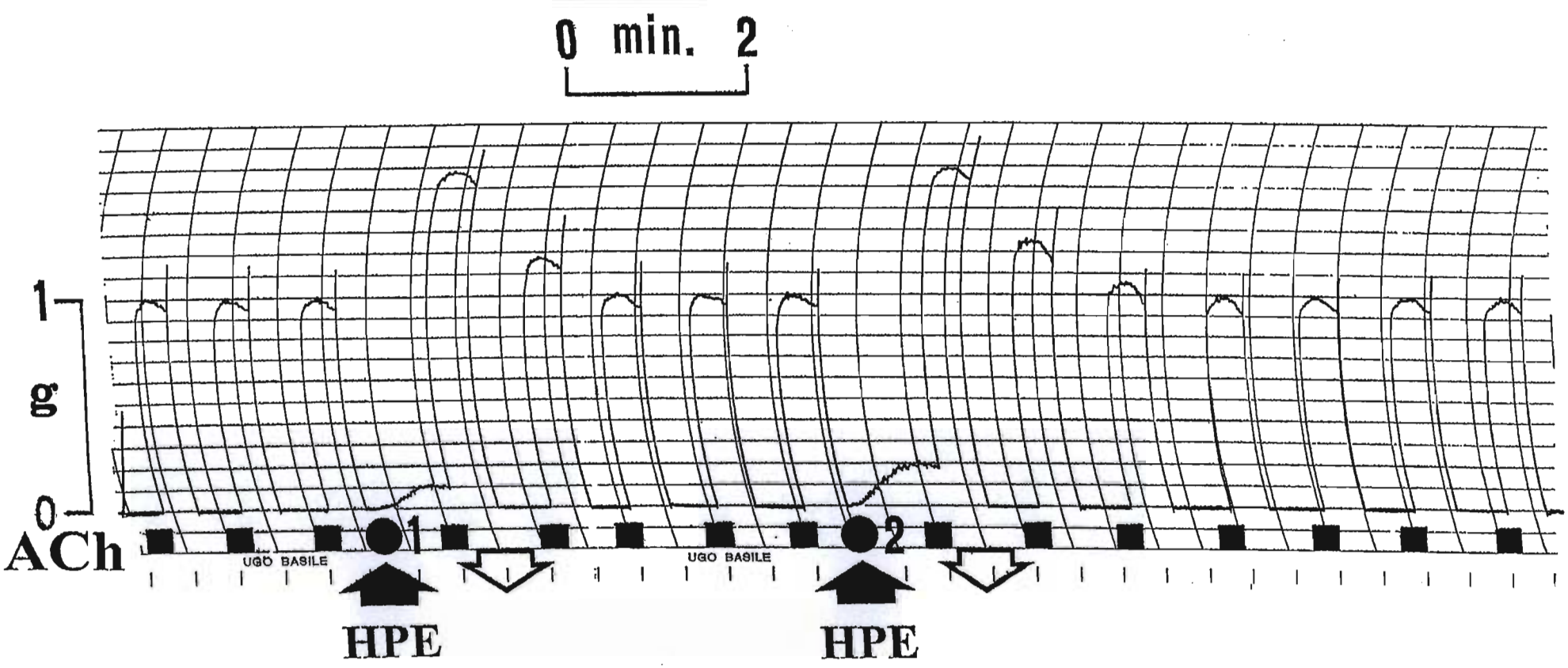

Figure 5.

Effects of *Harpagophytum procumbens* secondary root aqueous extract (HPE) on acetylcholine-(ACh-) induced contractions of the chick isolated oesophagus. Contractions of the muscle preparation were induced by acetylcholine (ACh, 0.5 μg/ml) sequentially added to the bath-fluid at the solid rectangles (■). HPE 1 and 2 represent 50 and 100 μg/ml of *Harpagophytum procumbens* secondary root aqueous extract sequentially added to the bath-fluid at the solid dots (●) and solid upright-pointing arrows respectively. The two different concentrations of the plant's extract sequentially added to the bath-fluid were washed out at the adjacent, open right-hand-side downward-pointing arrows respectively.

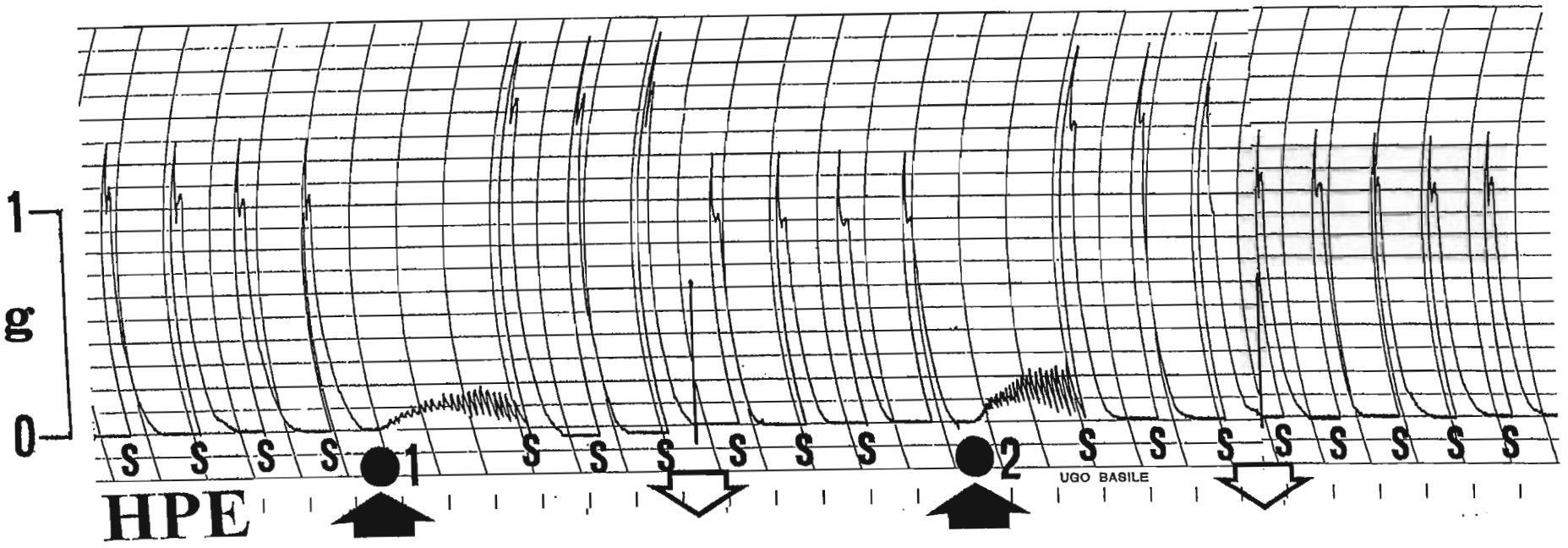

Figure 6.

Effects of *Harpagophytum procumbens* secondary root aqueous extract (HPE) on electrically-induced contractions of the chick isolated oesophagus. Contractions of the muscle preparation were induced by indirect, electrical stimulation instituted at the solid letter **S** (30 Hz, 70volts). HPE 1 and 2 represent 50 and 100 µg/ml of *Harpagophytum procumbens* secondary root aqueous extract sequentially added to the bath fluid at the solid dots (●) and solid upright-pointing arrows respectively. The two different concentrations of the plant's extract sequentially added to the bath-fluid were washed out at the adjacent, open right-hand-side downward-pointing arrows respectively.

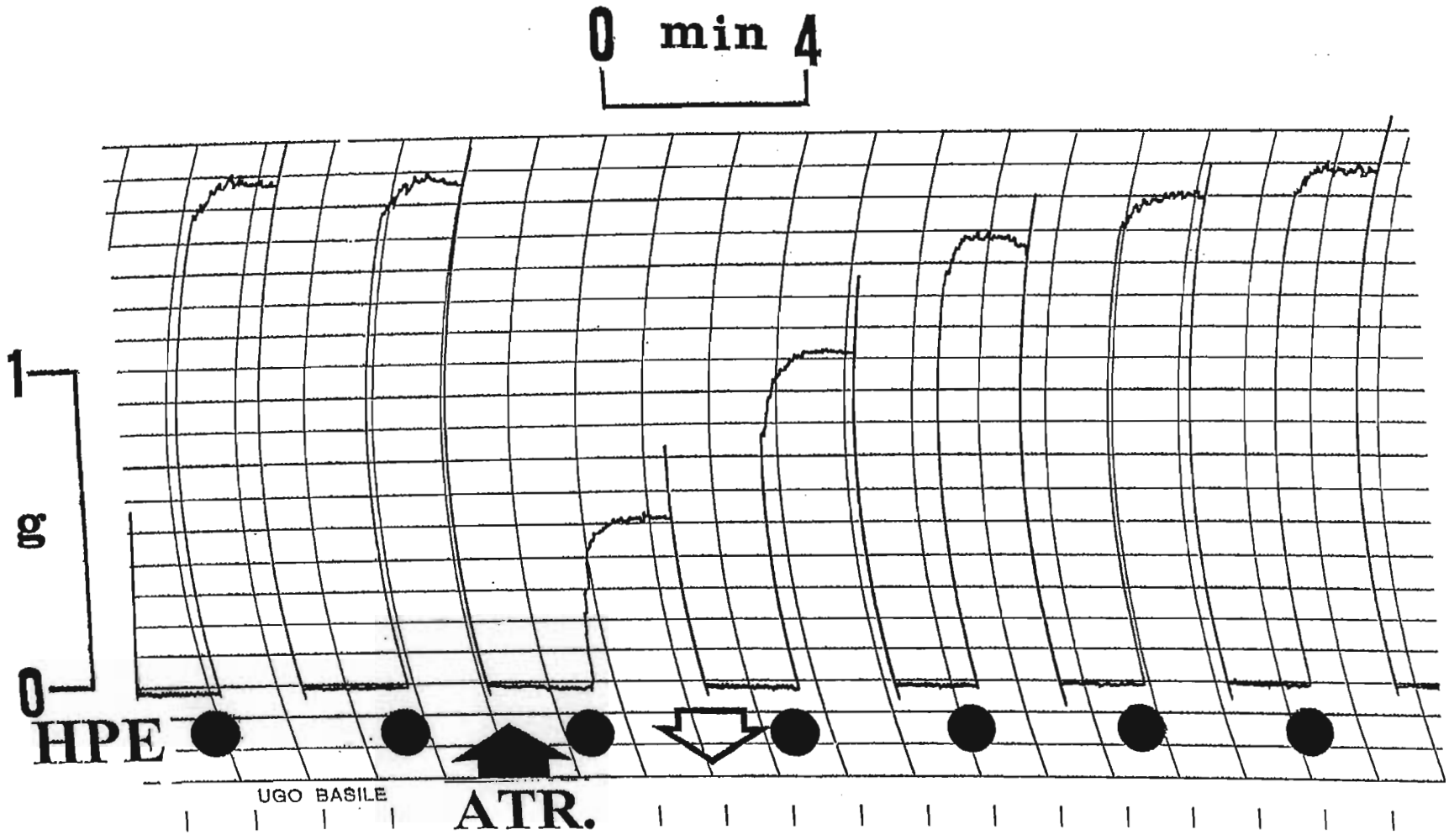

Figure 7.

Inhibitory effect of atropine (**ATR.**, 1.0 μg/ml) on *Harpagophytum procumbens* secondary root aqueous extract (HPE)-induced contractions of the chick isolated oesophagus. At each of the solid dots (●), HPE (800 μg/ml) was sequentially added to the bath fluid. Atropine (**ATR.**, 1.0 μg/ml) was added to the bath-fluid at the solid upright-pointing, left-hand-side arrow, and washed out at the adjacent, open right-hand-side downward-pointing arrow.

physosgmine (1.0–10.0 μg/ml). Relatively low to high concentrations of the plant's extract (HPE, 10–1000 μg/ml) also potentiated and/or enhanced acetylcholine- (ACh, 0.1–1.0 μg/ml), physostigmine- (PHY, 0.5–5.0 μg/ml) and electrical stimulation- induced contractions of the chick isolated oesophagus (Figures 5 and 6). Like acetylcholine- (ACh-) and physostigmine-provoked contractions of the chick oesophageal muscle preparations, HPE-induced contractions of the chick isolated oesophagus muscle preparations were also dose-dependently reduced, inhibited or abolished by bath-applied atropine (0.1–2.5 μg/ml) – see Figure 7.

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

In the guinea-pig isolated ileal muscle preparations, the pharmacological effects of relatively low to high concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–1000 μg/ml) were found to be similar to those produced by the plant's extract in the chick isolated oesophageal muscle preparations. However, HPE-induced responses of the guinea-pig isolated ileum were qualitatively and quantitatively smaller than those produced by the plant's extract in the chick isolated oesophagus. This observation appears to suggest that the chick oesophagus is more predominantly cholinergically-innervated than the guinea-pig ileum.

However, relatively low to high concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–1000 μg/ml) always raised the baseline tension (baseline tone) of, and contracted the guinea-pig isolated ileal muscle preparations in a concentration-dependent manner. Furthermore, relatively moderate to high concentrations of the plant's extract (HPE, 200–1000 μg/ml) always produced concentration-dependent, significant ($P<0.05–0.001$) and atropine-sensitive contractions of the guinea-pig ileal muscle preparations. Acetylcholine (ACh, 0.1–2.5 μg/ml), physostigmine (PHY., 1.0–10.0 μg/ml), histamine (HIST., 0,2–5.0 μg/ml), 5-hydroxytryptamine (5-HT, 0.5–5.0 μg/ml), nicotine (0.5–10 μg/ml) and potassium chloride (K^+, 5–40 mM) also contracted the guinea-pig isolated ileum in a concentration-related manner. Relatively low to high concentrations of the plant's extract (HPE, 10–1000 μg/ml) potentiated and enhanced acetylcholine- (ACh, 0.1–1.0 μg/ml)-induced contractions of the guinea-pig isolated ileum in a concentration-related manner. Like acetylcholine- (ACh-) and physostigmine-provoked contractions of the guinea-pig ileal muscle preparations, HPE-induced contractions of the guinea-pig isolated ileal muscle preparations were also dose-dependently reduced, inhibited or abolished by bath-applied atropine (0.1–2.5 μg/ml).

Effects of *Harpagophytum procumbens* Root Aqueous Extract on Rabbit Isolated Duodenum

Relatively low to high concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–1000 μg/ml) usually induced concentration-related complex, biphasic responses in rabbit isolated duodenum. Relatively moderate to high concentrations of the plant's extract (HPE, 200–1000 μg/ml) usually provoked dose-related, initial slight and transient, but significant ($P<0.05–0.01$) depressions of the amplitudes of the spontaneous, rhythmic, pendular contractions of the muscle preparations. These initial inhibitory effects of the plant's extract (HPE, 50–1000 μg/ml) were always immediately followed by sharp, significant ($P<0.05–0.001$) increases in the baseline tones (baseline tensions) of the muscle preparations. The increases in contractile amplitudes and baseline tones of the rabbit duodenal muscle preparations were always followed by gradual, dose-dependent, secondary, longer-lasting, significant ($P<0.05–0.001$) reductions in the amplitudes of the spontaneous, rhythmic, pendular contractions of the isolated muscle strips (Figure 8). The secondary, longer-lasting inhibitory effects of the plant's extract (HPE) were resistant to blockade by standard, receptor specific antagonists in all the rabbit isolated muscle preparations used.

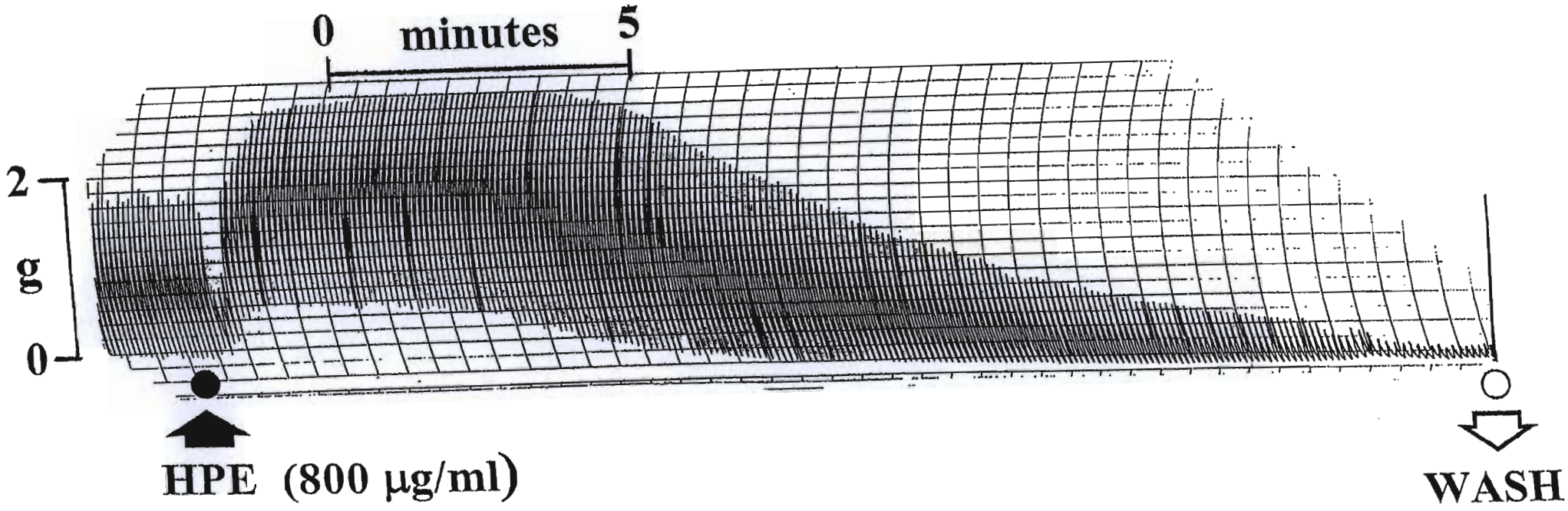

Figure 8.

Effects of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 800 µg/ml) on contractile amplitudes of the spontaneous, myogenic, pendular, rhythmic contractions of the rabbit isolated duodenum. *Harpagophytum procumbens* secondary root aqueous extract (HPE, 800 µg/ml) was sequentially added to the bath-fluid at the solid dot (●) and solid, left-hand-side upright-pointing arrow; and washed out at the adjacent, open dot (○) and open, right-hand-side downward-pointing arrow.

Effects of *Harpagophytum procumbens* Root Aqueous Extract on Rat Isolated Uterine Muscles:

(i) Oestrogen-dominated, non-pregnant uterine strips

Uterine muscle strips taken from stilboesterol-pretreated, non-pregnant female rats were found to be quiescent and devoid of spontaneous, myogenic, rhythmic activity. However, relatively low to high concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 10–1000 μg/ml) caused profound, concentration-related, and significant ($P<0.05–0.001$) increases in the baseline tone (basal tension) and induced powerful spontaneous, rhythmic, myogenic contractions of the oestrogen-dominated uterine muscle strips taken from stilboesterol-pretreated, non-pregnant female rats. Moderate to high concentrations of the plant's extract (HPE, 200–800 μg/ml) always provoked powerful contractions of the uterine muscle preparations. Figure 9 illustrates a typical trace obtained, while Figure 10 summarizes the results obtained. Acetylcholine (ACh, 0.1–2.0 μg/ml), physostigmine (PHY, 2.5–10.0 μg/ml), bradykinin (BKN, 1.0–10.0 ηg/ml) and oxytocin (OTC, 0.05–0.5 unit/ml) also induced powerful, concentration-dependent, significant ($P<0.05–0.001$) contractions of the oestrogen-dominated uterine muscle preparations taken from the oestrogen-dominated, non-pregnant rats. The spontaneous, rhythmic, myogenic contractions of the quiescent, oestrogen-dominated uterine muscle strips of the non-pregnant rats induced by relatively low to high

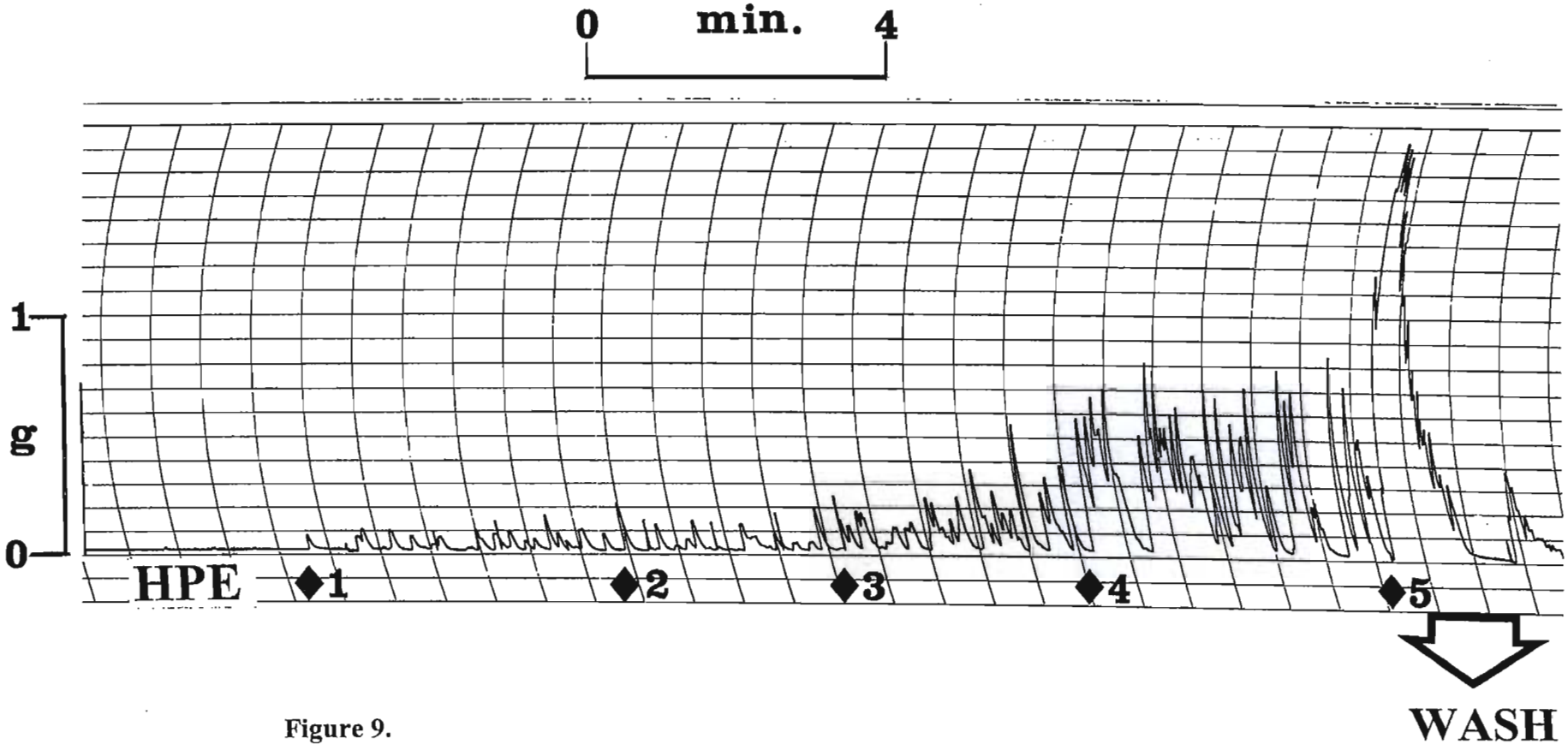

Figure 9.

Effects of graded concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE) on oestrogen-dominated rat isolated uterine muscle strip. HPE 1, 2, 3, 4 and 5 represent 25, 50, 100, 200 and 400 μg/ml of *Harpagophytum procumbens* secondary root aqueous extract cumulatively added to the bath fluid at the solid points (♦) respectively. HPE was washed out at the open, downward-pointing, right-hand-side arrow.

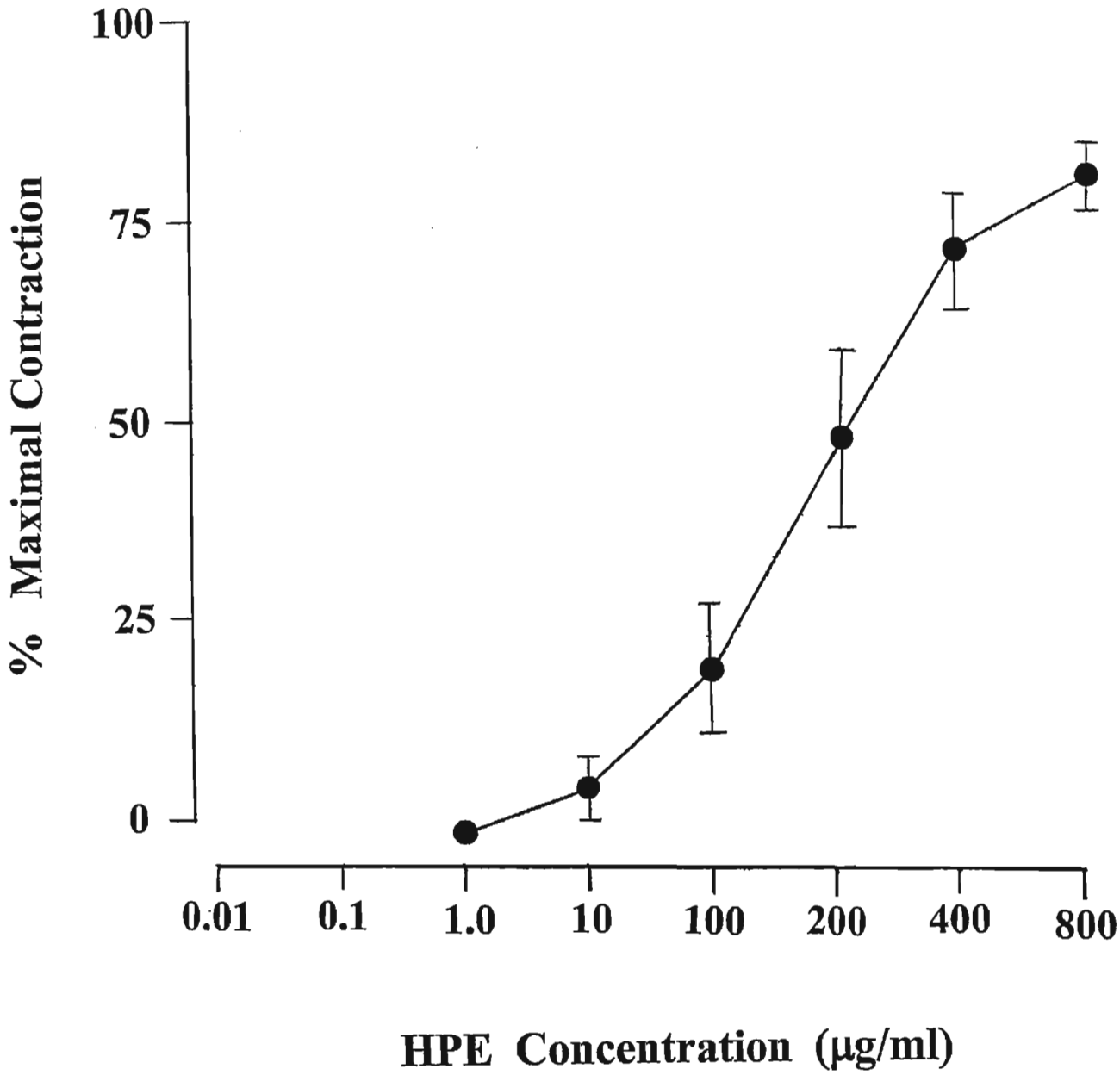

Figure 10.

Dose-response curve to sequentially-administered concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 10–800 µg/ml) on oestrogen-dominated rat isolated uterine muscle strip. Each point (●) represents the mean (±SEM) of 7–8 observations, while the vertical bars denote standard errors of the means.

concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 10–1000 μg/ml) were reduced in a concentration-dependent manner by bath-applied indomethacin (IDM, 0.1–5.0 μg/ml) or atropine (ATR, 0.1–5.0 μg/ml). However, the plant's extract (HPE)-induced spontaneous, myogenic contractions of the quiescent, oestrogen-dominated uterine muscle strips were resistant to the actions of all other receptor specific antagonists used. Relatively low to moderate concentrations of the plant's extract (HPE, 10–800 μg/ml) potentiated ACh-, PHY-, BKN- and OTC-induced contractions of the oestrogen-dominated isolated uterine muscle strips in a concentration-related manner.

(ii) Pregnant uterine strips:

Uterine muscle strips taken from pregnant rats were found to be spontaneously active, producing rhythmic contractions on their own accord. The pharmacological effects of HPE on isolated uterine muscle strips taken from pregnant rats were found to be qualitatively and quantitatively similar to those produced by the plant's extract on oestrogen-dominated isolated uterine muscle strips taken from stilboesterol-pretreated, non-pregnant female rats. Relatively low to high concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 10–1000 μg/ml) provoked concentration-related, significant ($P<0.05–0.001$) increases in the baseline tone (basal

tension) and contracted uterine muscle strips taken from rats in the early, middle or late stages of pregnancy.

The possibility that the HPE-induced responses of the chick isolated oesophagus muscle strips and guinea-pig isolated ileum muscle preparations used in this study might involve interaction with Ca^{2+} at the cell membrane was 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 increases in baseline tones of the chick oesophagus and guinea-pig isolated ileum muscle preparations induced by relatively low concentrations of HPE (10–200 μg/ml) were abolished or reduced in the presence of low calcium concentration [Ca^{2+} = 0.13 g/litre] in the bathing Krebs-Henseleit physiological solution. Similarly, the contractile responses of the isolated muscle strips induced by moderate to high concentrations of HPE (200–1000 μg/ml) decreased 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 (10–400 μg/ml) concentration-induced initial stimulant responses of the isolated muscle preparations. Similarly, the contractile responses of the isolated muscle strips induced by moderate to high concentrations of HPE (200–1000 μg/ml) were increased as the Ca^{2+} concentration of the external bathing fluid was increased. The secondary, longer-lasting inhibitory effects of moderate to high concentrations of the plant extract (HPE, 200–1000 μg/ml) on rabbit isolated duodenum increased with reduced calcium concentrations in the bathing fluid, and decreased when the external calcium concentration was increased.

In all cases, washing of the isolated 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.

Anticholinesterase Determination

The plant's extract (HPE, 10–1000 μg/ml) produced dose-related anticholinesterase activity in the colorimetric assay method used. However, the anticholinesterase activity of the plant's extract (HPE, 10–1000 μg/ml) was found to be far less than that of physostigmine (PHY, 0.01–10.0 μg/ml) – see Figure 11. In the colorimetric assay method used, the chick biventer-cervicis muscle homogenates gave better and more reproducible results than the chick oesophagus muscle homogenates.

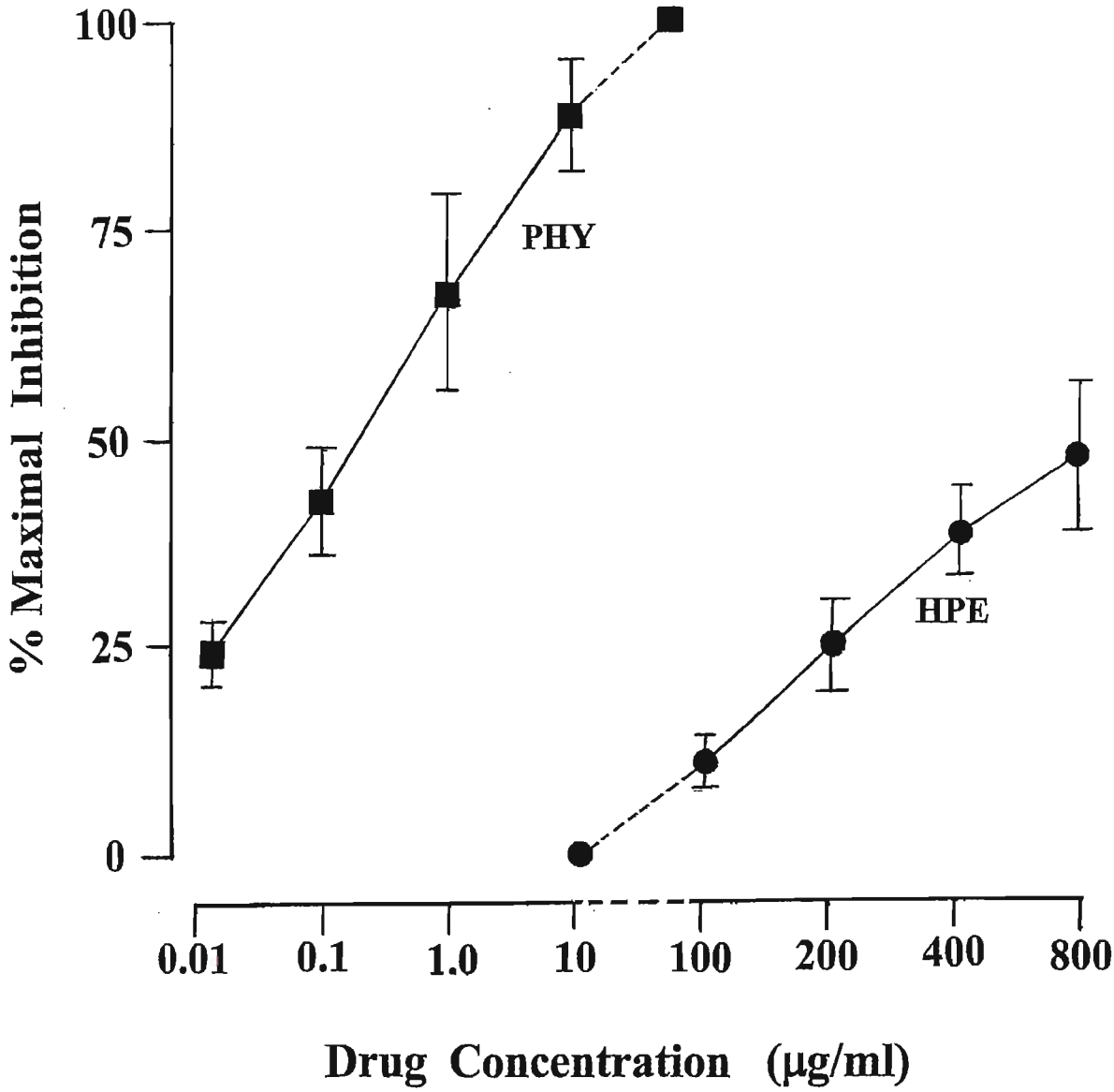

Figure 11.

Anticholinesterase activity of *Harpagophytum procumbens* secondary root aqueous extract (HPE, ●—●) compared with physostigmine (PHY, ■—■) on chick isolated biventer-cervicis muscle homogenates. HPE inhibited the chick cholinesterase enzyme in a concentration-related manner. In this regard, however, physostigmine (PHY) is far more potent than HPE. For both PHY and HPE, each point represents the mean (±SEM) of 6–9 determinations, while the vertical bars denote standard errors of the means.

DISCUSSION

Relatively low to high doses of *Harpagophytum procumbens* root aqueous extract (HPE) produced dose-related, significant ($P<0.05–0.001$) increases in the basal tone of, and contracted, the chick isolated oesophagus and guinea-pig isolated ileum in a concentration-dependent manner. This observation is in consonance with the findings of Occhiuto *et al.*, (1985). Bath-applied acetylcholine, physostigmine and indirect electrical stimulation of the chick oesophageal muscle preparations also induced contractions of the chick isolated muscle preparations in a concentration-dependent, and frequency-related manner respectively. Although the precise mechanism of the contractile action of the plant's extract on the muscle preparations used in the present study is obscure at present, it is speculated that (i) acetylcholine-like, direct stimulation and/or excitation of cholinergic muscarinic receptors, and (ii) inhibition of cholinesterase enzymes by the herb's extract, may have contributed significantly to the contractile effects of the plant's extract in the *in vitro* experimental animal models used in this study.

The precise mechanism of the secondary, inhibitory effects of the plant's extract on rabbit isolated duodenum muscle preparations is also unknown at the moment. However, because the secondary inhibitory 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 inhibitory and/or depressant effects of HPE on the rabbit duodenal 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 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 warranted to shed more light on this plausible mechanism of action of HPE.

However, the experimental evidence obtained in the present laboratory animal study indicates that the plant's extract contracts gastro-intestinal tract smooth muscles. This observation would appear to provide pharmacological basis for the frequently reported adverse effect of 'gastro-intestinal tract discomfort or upset' commonly associated with *Harpagophytum procumbens* root extract medication.

Relatively low to high concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE) always induced concentration-related, significant ($P<0.05–0.001$), persistent and profound spontaneous, rhythmic, myogenic contractions of, and contracted, uterine muscle strips taken from stilboesterol-pretreated female rats. The plant's extract produced similar contractile effects on uterine muscle strips taken from pregnant rats. The precise mechanism of this stimulant action of the plant's extract on uterine muscle strips is not fully understood at the moment. However, the ability of indomethacin and/or atropine to reduce HPE-induced contractions of the uterine muscle preparations used in this study may be taken to suggest possible release of prostaglandins and/or other uterotonic substances or mediators, including acetylcholine, by the plant's extract. The observation that *Harpagophytum procumbens* secondary root aqueous

extract (HPE) possesses anticholinesterase activity would appear to buttress the possible involvement of cholinergic muscarinic receptor stimulation in the uterine stimulant, uterotonic action of HPE.

Harpagophytum procumbens secondary 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 *Harpagophytum procumbens* secondary root extract have been reported in biomedical literature, the iridoids harpagoside (a cinnamic acid ester), harpagide and procumbide are speculated to have contributed significantly to the contractile effects of the plant's root extract on the isolated gastro-intestinal tract muscle preparations, and to the uterotonic action of the herb's extract on the uterine muscle strips. However, the experimental evidence obtained in the present *in vitro* study indicates that aqueous root extract of *H. procumbens* possesses contractile action on gastro-intestinal smooth muscles, and uterotonic action on mammalian uterus. These findings appear to provide pharmacological rational for the 'abdominal discomfort and/or upset' associated with *Harpagophytum procumbens* secondary root extract medication, and also suggest that the use of *Harpagophytum procumbens* root extract should be contra-indicated and avoided in pregnancy. The gastro-intestinal tract smooth muscle contractile effects and uterotonic action of the plant's extract (HPE) may be due, through the complex interactions that might occur among the various chemical constituents of the plant, to release of mediators and substances that are capable of contracting smooth muscles. However, the findings of this

study lend pharmacological credence to the suggested folkloric, obstetric uses of the plant's root extract for induction or acceleration of labour, as well as for expulsion of retained placentas in pregnant women in some communities of South Africa.

CHAPTER THREE

CARDIOVASCULAR PHARMACOLOGY OF *HARPAGOPHYTUM PROCUMBENS* DC

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 moderate to high doses of *H. procumbens* root aqueous extract (HPE, 10–500 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, 200–1000 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 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* root in the management and/or control of hypertension and certain cardiac disorders in some communities of South Africa.

INTRODUCTION

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*, 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 order to throw more light on the cardiovascular pharmacology of *Harpagophytum procumbens* DC secondary roots extracts, it was thought worthwhile examining the effects of the plant's aqueous extract on cardiovascular parameters that have not, to date,

been reported in the literature, using laboratory animal experimental models. In the experiments reported in this section of the thesis, the pharmacological effects of *Harpagophytum procumbens* DC secondary root aqueous extract have been investigated on:

(i) rat isolated portal vein;

(ii) guinea-pig isolated spontaneously-beating right atrial muscles;

(iii) guinea-pig isolated electrically-driven left atrial muscles;

(iv) systemic arterial blood pressure of rats.

MATERIALS AND METHODS

Plant Material

The plant material used, its preparation and extraction are the same as those described in detail under Chapter Two of this Thesis.

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 300–400 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.

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

(i) 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°C 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.

(ii) 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°C and continuously aerated with carbogen (i. e., 5% carbon-dioxide + 95% oxygen gas mixture). Each left atrial 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 equlibrate 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 200–350 g were used. Each of the animals used was killed by deep petroleum ether inhalation 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°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 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.

RESULTS

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 12 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-

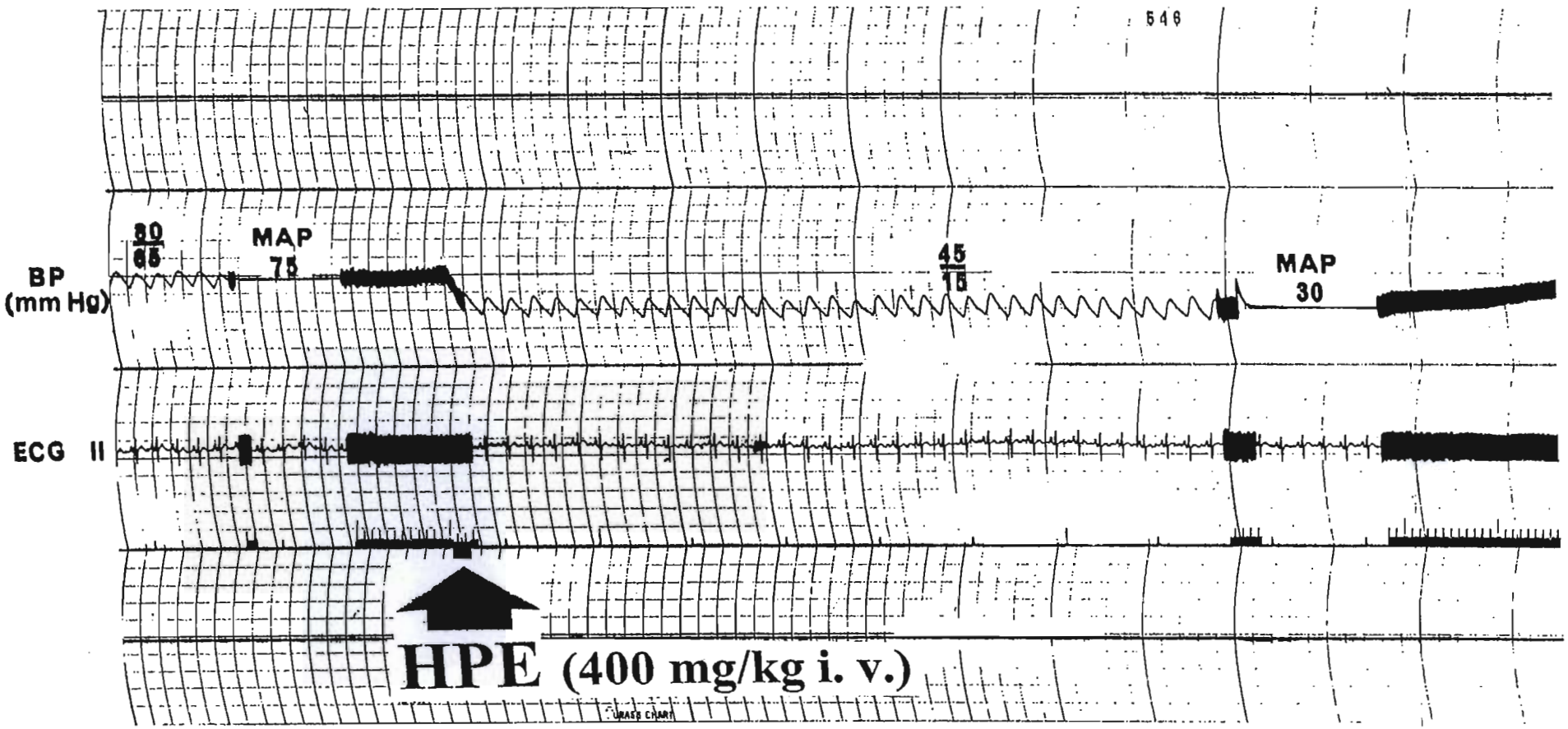

Figure 12.

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 arrow.

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

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 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 secondary gradual, longer-lasting, significant ($P<0.05$–0.001) negative chronotropic responses. Figure 13 illustrates a typical trace, while Figure 14 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.

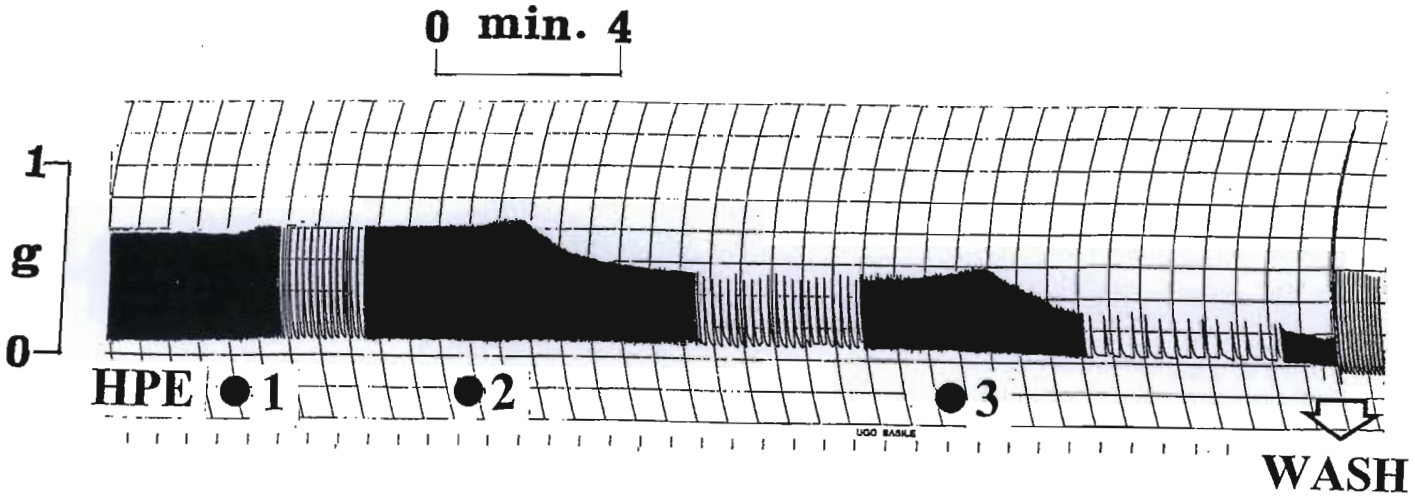

Figure 13.

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.

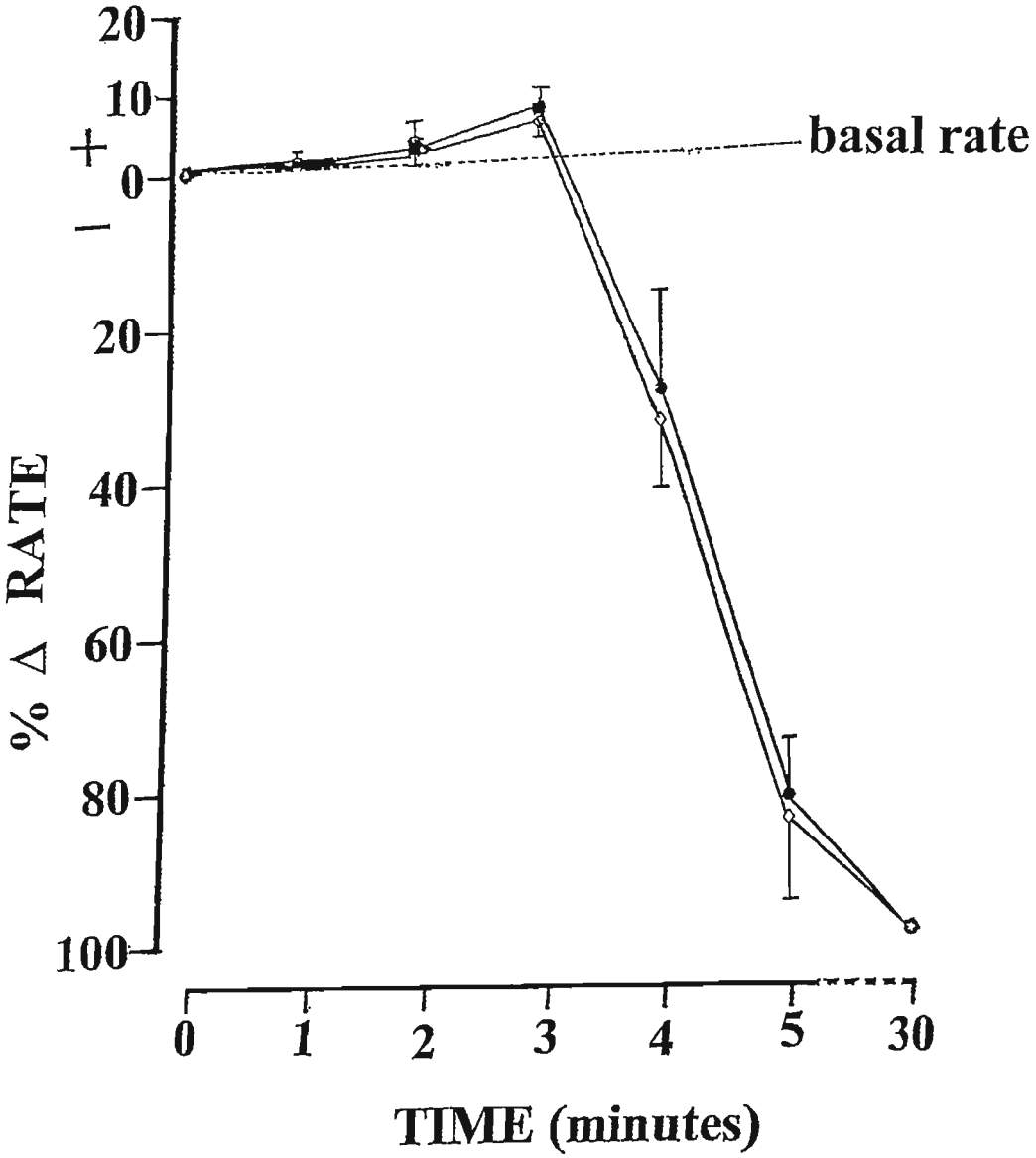

Figure 14.

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.

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 secondary gradual, longer-lasting, highly significant ($P<0.01–0.001$) negative inotropic responses. Figure 15 illustrates a typical trace, while Figure 16 summarizes the results obtained. 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.

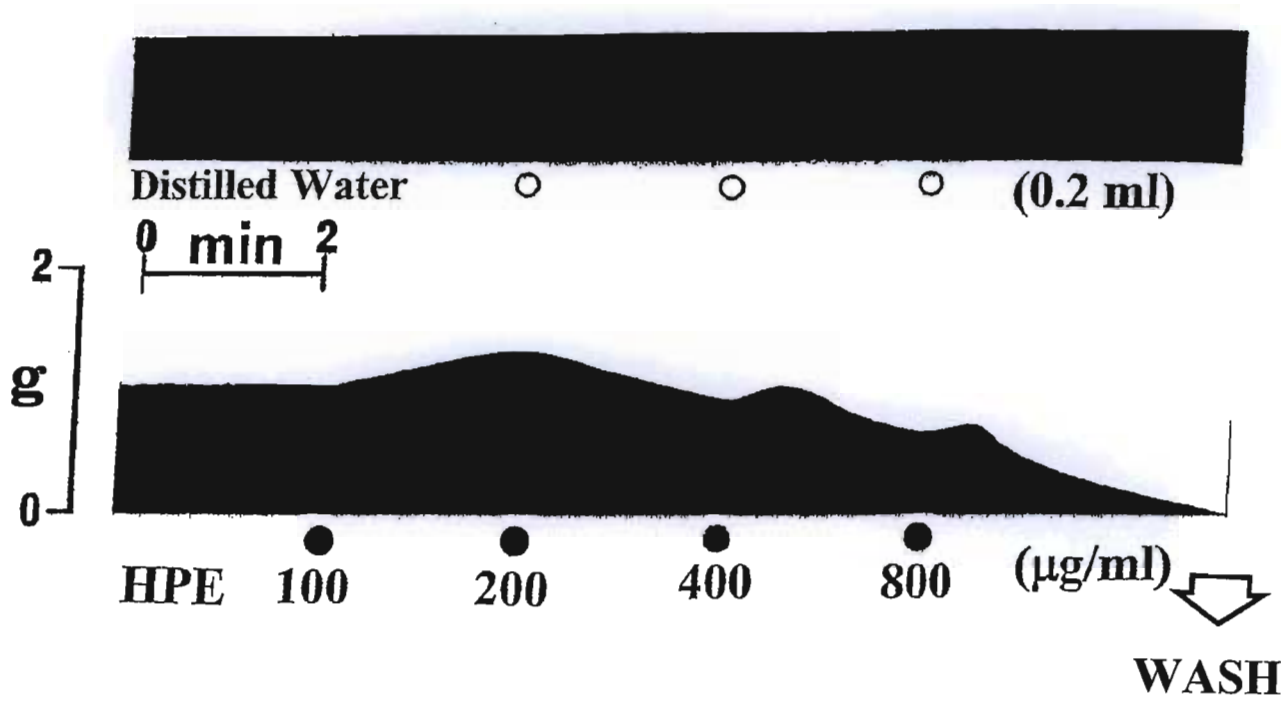

Figure 15.

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.

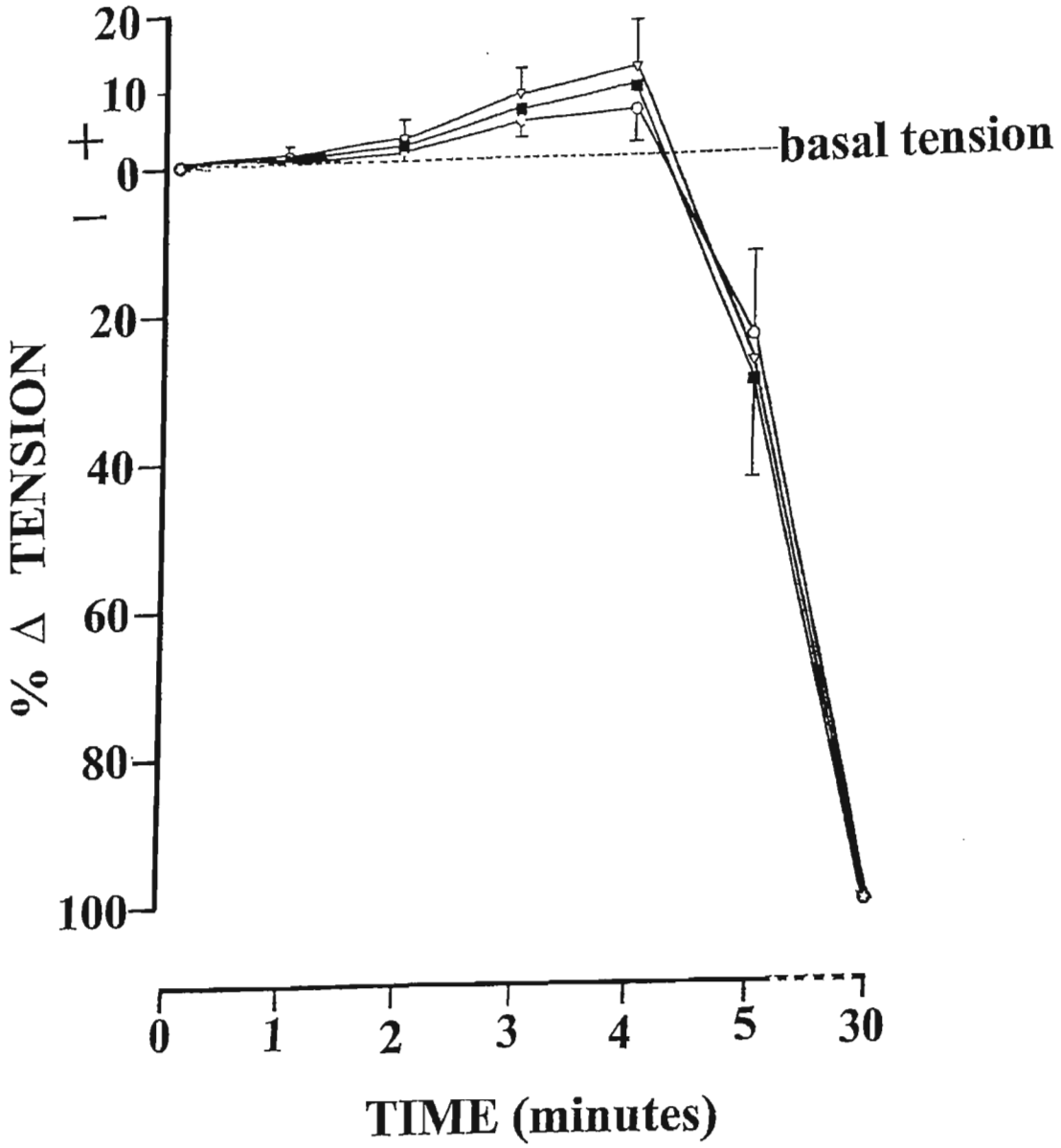

Figure 16.

Effects of relatively moderate to high concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, ○—○ 300, ■—■ 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.

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 17 shows a typical trace, while Figure 18 summarizes the results obtained.

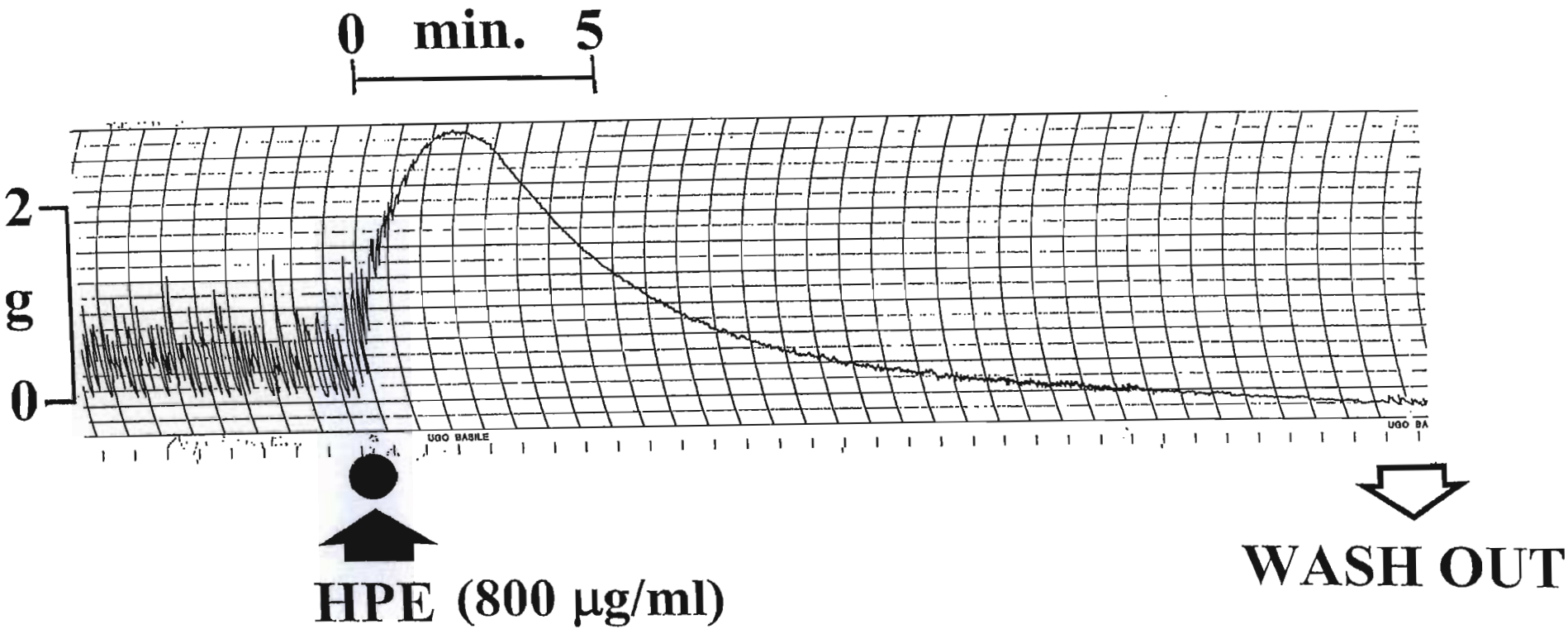

Figure 17.

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.

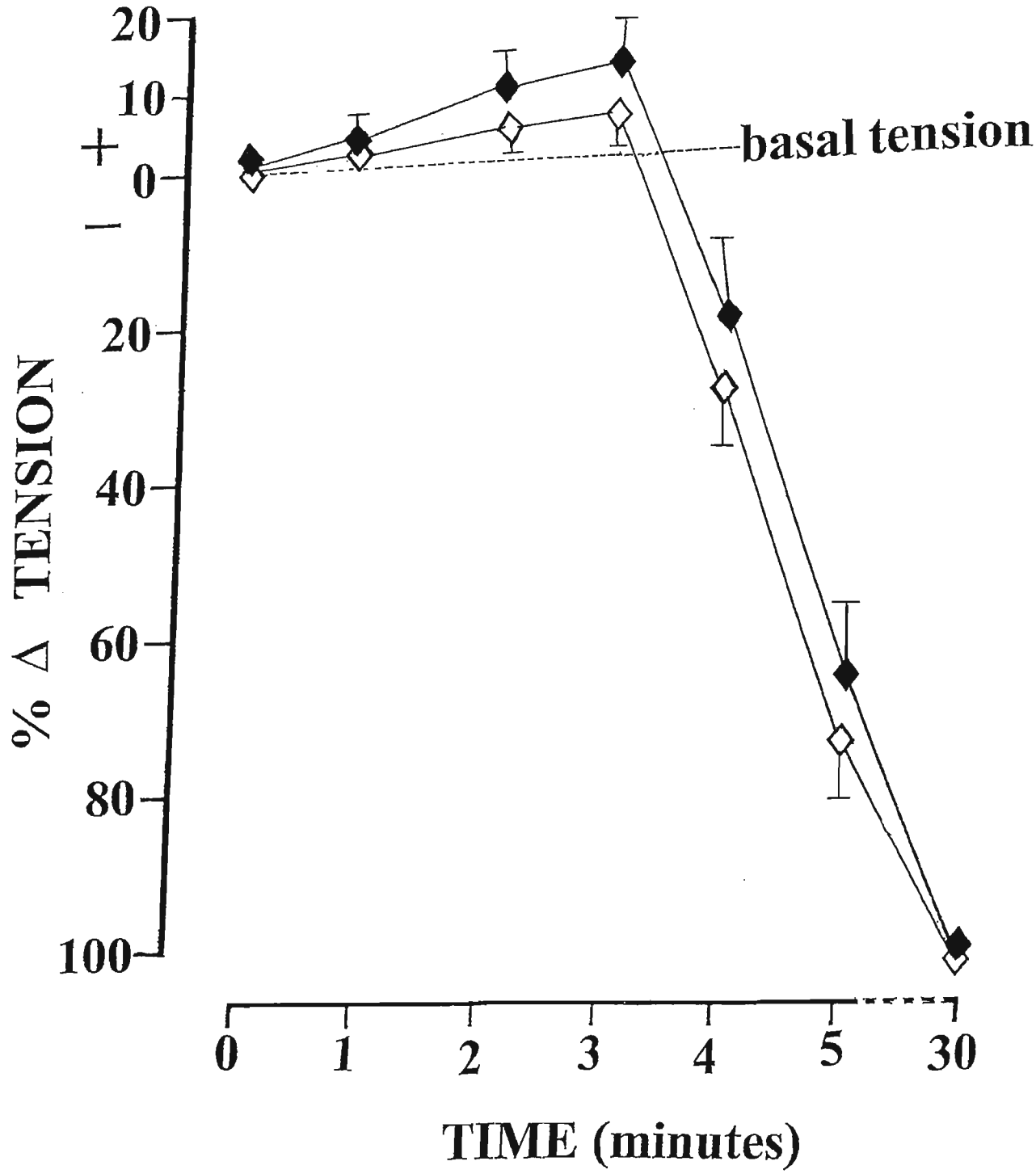

Figure 18.

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 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.

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 *Harpagophytum 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 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.

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.

CHAPTER FOUR

NEUROPHARMACOLOGY OF *HARPAGOPHYTUM PROCUMBENS* DC

A. EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES:

ABSTRACT

Harpagophytum procumbens DC is widely used in South African traditional medicine for the treatment, management and/or control of a variety of human ailments. In the present study, the analgesic effect of *H. procumbens* secondary root aqueous extract was evaluated in mice, using the 'hot-plate' and 'acetic acid' test methods; while the anti-inflammatory effects of the plant's root extract were investigated in rats. Fresh egg albumin-induced pedal oedema was used as experimental test model of inflammation. Morphine (MPN, 10 mg/kg i. p.) and diclofenac (DIC, 100 mg/kg i. p.) were used respectively as reference analgesic and anti-inflammatory agents for comparison. *Harpagophytum procumbens* secondary root aqueous extract (HPE, 50–800 mg/kg i. p.) produced dose-dependent, significant ($P<0.05$–0.001) analgesic effects against thermally-

and chemically-induced nociceptive pain stimuli in mice. It is speculated that that the analgesic effects of the plant's root extract (HPE, 50–800 mg/kg i. p.) are centrally- and peripherally- mediated. *Harpagophytum procumbens* secondary root aqueous extract (HPE, 50–800 mg/kg i. p.) also produced dose-related, significant reductions ($P<0.05$–0.001) in the fresh egg albumin-induced acute inflammation of the rat hind paw oedema. The results of this experimental animal study indicate that *Harpagophytum procumbens* secondary root aqueous extract possesses analgesic and anti-inflammatory properties. These observations lend pharmacological support to the suggested folkloric uses of *Harpagophytum procumbens* secondary root in the management and/or control of painful, arthritic and other inflammatory conditions in some communities of South Africa.

INTRODUCTION

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* is used for a variety of health conditions. In South Africa, the plant product 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 (Van Wyk and Gericke, 2000). Commercial ointments and creams of *H. procumbens* are applied topically for minor muscular aches and pains, and to painful joints (Watt and Breyer-Brandwijk, 1962; Van Wyk and Gericke, 2000; Van Wyk *et al.,* 2002; Van Wyk and Wink, 2004). In an effort to scientifically appraise the '*healing powers*' and medicinal value of *Harpagophytum procumbens*, the present study was designed to evaluate the analgesic and anti-inflammatory properties of the herb's secondary root aqueous extract in experimental models of pain and oedema.

Literature abounds with contradictory reports on the analgesic and anti-inflammatory actions of *Harpagophytum procumbens* secondary root extracts. Several contemporary pharmacological studies in laboratory animals and man have supported the efficacy of *Harpagophytum procumbens* secondary root extracts in the management and control of painful, arthritic and other inflammatory conditions (Lanhers *et al.*, 1992). The latter investigators concluded that the anti-inflammatory effects of the plant product are similar to, or greater than, those of potent non-steroidal anti-inflammatory agents (NSAIDs) such as phenylbutazone and indomethacin, and that its analgesic effects compared favourably to acetylsalicylic acid (aspirin). However, other pharmacological studies by some other researchers have indicated that *Harpagophytum procumbens* secondary root extracts have no or very little if any, anti-inflammatory, antirheumatic or analgesic activity (Grahame and Robinson, 1981; Whitehouse,1983; Moussard, 1992). The core aim of the present study was to substantiate or deny the efficacy of *Harpagophytum procumbens* secondary

root aqueous extract in experimental models of pain and inflammation, using mice and rats.

MATERIALS AND METHODS

Plant Material

The plant material used in this set of experiments, its preparation and extraction, are the same as those described in detail under Chapters Two and Three of this Thesis.

Animal Material

Balb C albino mice (*Mus domesticus*) weighing 20–25 g, and young adult Wistar rats (*Rattus norvegicus*) weighing 250–300 g were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light; and were allowed free access to food (standard pellet diet) and water *ad libitum*. The animals were divided into drug-treated 'test' and distilled water-treated 'control' groups of 8 animals per group. All the animals were fasted for 12 hours, but still allowed free access to water, before the commencement of our experiments. It has been shown that oral administration and subsequent passage of *Harpagophytum procumbens* root extract through the stomach (where the pH is acidic) leads to loss of pharmacological activity of the extract – due to

deactivation of its active constituents by gastric acid juices (Soulimani *et al.*, 1994). In the present study, therefore, *Harpagophytum procumbens* root aqueous extract has been administered intraperitoneally, a route that ensures adequate systemic bioavailability and pharmacological activity of the extract.

Evaluation of Analgesic Activity

Balb C albino mice (*Mus domesticus*) weighing 20–25 g were used for the analgesic evaluation experiments. The 'hot-plate' (thermal) and 'acetic acid' (chemical) test methods were used.

'Hot-Plate' Test Method:

The 'hot-plate' (thermal) test method employed in this study was modified from those described in detail by Eddy and Leimback (1953), Lanhers *et al.*, (1992) and Williamson *et al.*, (1996). A 600-ml glass beaker was placed on a 'Heidolph® MR 2002' hot-plate (with adjustable temperature). The temperature of the hot-plate was then regulated to 45±1°C. Each mouse was placed in the glass beaker (on the hot-plate) in order to obtain the animal's response to electrical heat-induced nociceptive pain stimulus (licking of the forepaws and eventually jumping out of the glass beaker). Jumping out of the beaker was taken as an indicator of the animal's response to heat-induced nociceptive pain stimulus.

The time taken for each mouse to jump out of the beaker (i. e., reaction time) was noted and recorded in seconds. Each mouse served as its own control. Thus, before treatment, its reaction time was determined thrice at 1-hour intervals. The mean of these three determinations constituted the 'initial reaction time' – that is, reaction time before treatment of the mouse. The mean reaction times for all the mice used were pooled to obtain the final, 'control' mean reaction time (Tb). Each of the test mice was thereafter treated with either distilled water, *Harpagophytum procumbens* root aqueous extract (HPE), or morphine (MPN). Twenty minutes after treatment with distilled water, the plant extract (HPE), or reference drug (MPN), the reaction time was again evaluated, but only once on this occasion. This value was pooled for the mice used in each treatment group, and the final 'test' mean value (Ta) for each treatment group was calculated. This final 'test' mean value represented 'after-treatment reaction time' (Ta) for each group of treated mice. This 'test' mean value (Ta) was subsequently used to determine percentage thermal pain stimulus relief or protection, by applying the formula:

$$\% \text{ protection against thermal pain stimulus} = \text{Test Mean} - \text{Control Mean} \times 100/\text{Control Mean}$$

$$= Ta - Tb \times 100/Tb$$

The plant extract (HPE) was tested at doses of 50, 100, 200, 400 and 800 mg/kg i. p. respectively. The reference drug, morphine (MPN), was used at a dose of 10 mg/kg i. p. only. Treated 'control' mice received distilled water (2 ml/kg i. p.) only.

'Acetic Acid' Test Method

The 'acetic acid' test method used in this study was adopted from those described earlier by Koster *et al.,*(1959); Williamson *et al.*, (1996); Zakaria *et al.,* (2001) and Silva *et al.,*(2003). The mice were divided into groups of 'test' and 'control' animals. There were two separate (A and B) groups of 'control' mice. Group A control mice were not pre-treated with anything at all, and the 8 mice in this group (A) served as the 'untreated control' animals for all the mice in all the other groups. Each of these 'untreated Group A' mice was, however, treated with intraperitoneally administered 0.2 ml of a 3% acetic acid solution (Koster *et al.,*1959). Each mouse in the control group (B) was pre-treated with distilled water (2 ml/kg i. p.) only. In this test, diclofenan (DIC, 100 mg/kg i. p.) was used as the reference analgesic drug for comparison. Each mouse in the treated 'control' group (B), and in the other 'test' groups, was treated with either distilled water (2 ml/kg i. p.), diclofenac (DIC, 100 mg/kg i. p.), or a graded dose of *Harpagophytum procumbens* root aqueous extract (HPE, 50, 100, 200, 400 and 800 mg/kg i. p.). Twenty minutes after pre-treatment with distilled water, reference drug (DIC), or a dose of the plant extract (HPE), 0.2 ml of a 3% acetic acid solution was injected intraperitoneally (i. p.) to each of the treated mice (Koster *et al.,*1959). The number of writhings (i. e., abdominal contractions and stretches) that occurred within the first 20 minutes following acetic acid administration were counted and recorded. The recorded numbers of acetic acid-induced writhings (abdominal contractions and stretches) that occurred in the HPE-,

DIC- and distilled water pre-treated mice were compared with those in the 'untreated Group A control' mice.

Evaluation of Anti-inflammatory Activity

Young adult Wistar rats (*Rattus norvegicus*) weighing 250–300 g were used. The animals were divided into three (A, B and C) groups of 8 rats per group. Group A rats were used as controls, and each of the animals in this group received distilled water (2 ml/kg i. p.) only. Group B 'test' rats received *Harpagophytum procumbens* root aqueous extract (HPE, 800 mg/kg i. p.). Group C 'test' rats received diclofenac (DIC, 100 mg/kg i. p.). The rat hind paw oedema was used as a model of acute inflammation. Rat hind paw oedema was induced by intra-plantar injection of fresh egg albumin (0.5 ml/kg), a cheap phlogistic agent (Ekpendu *et al*, 1994; Muko and Ohiri, 2000). Linear diameter of the injected paw was assessed for 3 hours at 30-minute intervals after the administration of the phlogistic agent. Increases in the linear diameter of the right hind paws were taken as an indication of paw oedema. Oedema was assessed in terms of the difference in the 'zero time' linear diameter of the injected right hind paw, and its linear diameter at 'time t' (i.e., 30, 60, 90, 120, 150 and 180 minutes) following fresh egg albumin administration. The increases in the right hind paw diameters (induced by injections of fresh egg albumin) were compared with those of the contra-lateral, non-injected left hind paw diameters (Hess and Milong, 1972; Oriowo, 1982; Ekpendu *et al*, 1994; Muko and Ohiri,

2000; Ojewole, 2003). *Harpagophytum procumbens* root aqueous extract (HPE) was separately administered at a dose of 800 mg/kg i. p. (a dose that was found to produce 76% - 89% of the maximal anti-inflammatory effect of the crude water extract in our pilot experiments) to each of the rats in the 'test' Group B, 20 minutes before inducing inflammation with the injection of fresh egg albumin. Rats in the reference, comparative 'test' Group C received diclofenac (DIC, 100 mg/kg p. o.); while rats in the 'control' Group A received distilled water (2ml/kg i. p.) only.

Percentage inflammation (oedema) was calculated from the formula: C_o/C_t X 100; while percent inhibition of the oedema was calculated from the formula: $C_o - C_t/C_o$ X 100 [where C_o is the average inflammation (hind paw oedema) of the 'control' Group A rats at a given time; and C_t is the average inflammation of the (Group B) plant extract- or (Group C) diclofenac-treated rats at the same time].

Data Analysis

Data obtained were pooled and presented as means (±SEM). Data from 'control' mice and rats were used as baseline values. The differences between the plant's extract- or diclofenac-treated 'test' mice, and distilled water-treated 'control' animals, were analysed statistically. The data were subjected to paired "Student's t-test", and the level of significance of the differences between the 'test' and 'control' group data was determined. In all cases, values of $P \leq 0.05$ were taken to imply statistical significance.

RESULTS

Analgesic Activity

Harpagophytum procumbens root aqueous extract (HPE, 50–800 mg/kg i. p.) produced dose-related, significant ($P<0.05–0.001$) analgesic effects against thermally- and chemically-induced nociceptive pain stimuli (see Tables 2 and 3). Pretreatment of the mice with the plant's extract (HPE, 50–800 mg/kg i. p.) caused significant ($P<0.05–0.001$) delays in the reaction times of the animals to thermally-induced pain. Similarly, morphine (MPN, 10 mg/kg i. p.) profoundly delayed the reaction times of the animals (Table 2). *Harpagophytum procumbens* root aqueous extract (HPE, 50–800 mg/kg i. p.) caused dose-dependent, significant ($P<0.05–0.001$) reductions in the acetic acid-induced writhes of the mice. Similarly, diclofenac (DIC, 100 mg/kg i. p.) profoundly inhibited acetic acid- induced writhes in the animals (Table 3).

Anti-inflammatory Activity

Subplantar injections of fresh egg albumin (0.5 ml/kg) produced profound and time-related increases in the rat hind paw oedema of the 'control' rats. Plantar swelling and/or oedema (which became evident approximately 5–8 minutes following fresh egg albumin administration) reached its peak approximately 90 minutes after the fresh egg albumin

Table 2.

Effects *Harpagophytum procumbens* root aqueous extract (**HPE**, 50–800 mg/kg i. p.) and morphine (**MPN**, 10 mg/kg i. p.) on electrical heat-induced pain stimulus. Each value represents the mean (±SEM) of 8 observations.

GROUP	**DOSE** (i. p.)	**Mean reaction time** (seconds)	**% Protection**
Control Group A (untreated)	0	10.10±1.44	0.00
Control Group B (distilled water treated)	2 ml/kg	10.15±1.45	0.50 NS
HPE	50 mg/kg	12.30±1.42*	21.78*
HPE	100 mg/kg	13.45±1.36*	33.17*
HPE	200 mg/kg	14.55±1.48**	44.06**
HPE	400 mg/kg	16.25±1.50**	60.89**
HPE	800 mg/kg	18.10±1.55***	79.21***
Morphine **(MPN)**	10 mg/kg	20.12±2.10***	99.21***

NS = P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001 vs Control

Table 3.

Effects of *Harpagophytum procumbens* root aqueous extract (**HPE**, 50–800 mg/kg i.p.) and diclofenac (**DIC**, 100 mg/kg i. p.) on acetic acid-induced pain stimulus. Each value represents the mean (±SEM) of 8 observations.

GROUP	**DOSE** (i. p.)	**Number of writhings (contractions) in 20 min.**	**% Inhibition**
Control Group A (untreated)	0	76.48±8.24	0.00
Control Group B (distilled water treated)	2 ml/kg	75.84±8.25	0.84 NS
HPE	50 mg/kg	58.48±7.72*	23.54*
HPE	100 mg/kg	42.40±7.56**	44.56**
HPE	200 mg/kg	36.44±6.80**	52.35**
HPE	400 mg/kg	29.96±6.64**	60.83**
HPE	800 mg/kg	22.72±6.60***	70.29***
Diclofenac **(DIC)**	100 mg/kg	8.42±6.34***	88.99***

NS = P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001 vs Control

Table 4.

Effects of *Harpagophytum procumbens* root aqueous extract (**HPE**, 400 and 800 mg/kg i. p.) and diclofenac (**DIC**, 100 mg/kg i. p.) on fresh egg albumin-induced rat paw oedema (inflammation). Each value represents the mean (±SEM) of 8 observations. Percentage inhibitions of the fresh egg albumin-induced inflammation (oedema) produced by **HPE** and **DIC** are indicated as (%).

Treatment	**Dose** (i. p.)	**Time (in minutes) diameter (in mm)**					
		30	**60**	**90**	**120**	**150**	**180**
Control Group A (untreated)	0	10.45±0.04	12.50±0.05	15.65±0.06	14.75±0.07	12.61±0.05	11.35±0.04
Control Group B (distilled water treated)	2 ml/kg	10.50±0.05	12.46±0.07	15.66±0.09	14.80±0.06	12.60±0.08	11.38±0.05
HPE	400 mg/kg	8.15±0.06* (22.01%)	7.56±0.04* (39.52%)	6.35±0.07** (59.42%)	5.40±0.09** (63.39%)	4.25±0.05*** (66.30%)	3.50±0.06*** (69.16%)
HPE	800 mg/kg	5.20±0.05** (50.24%)	4.70±0.05** (62.40%)	3.68±0.03*** (76.49%)	2.55±0.04*** (82.71%)	1.60±0.06*** (87.31%)	1.01±0.05*** (91.10%)
Diclofenac (**DIC**)	100 mg/kg	4.10±0.04** (60.77%)	3.61±0.06*** (71.12%)	2.75±0.05*** (82.43%)	1.32±0.06*** (91.05%)	0.40±0.03*** (96.83%)	0.00±00*** (100%)

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

administration. Like diclofenac (DIC, 100 mg/kg i. p.), *Harpagophytum procumbens* root aqueous extract (HPE, 50–800 mg/kg i. p.) produced dose-related, profound and significant reductions ($P<0.05–0.001$) in the fresh egg albumin-induced acute inflammation of the rat hind paw (Table 4). However, the anti-inflammatory effect of the plant's extract (HPE, 800 mg/kg i. p.) was found to be less than that of diclofenac (DIC, 100 mg/kg i. p.). Distilled water (2 ml/kg i. p.) alone did not significantly ($P>0.05$) modify the fresh egg albumin-induced rat paw oedema.

DISCUSSION

Over the past four decades, many investigators have conducted studies in experimental animals and man, to validate scientifically, the effectiveness of *Harpagophytum procumbens* root extract (and its iridoid glycoside, harpagoside), as an analgesic, anti-inflammatory and anti-rheumatic agent. Results of these studies have been conflicting, with some researchers reporting positive effects (Lanhers, *et al.*,1992), and other investigators reporting negative effects (Seeger, 1973; Grahame and Robinson, 1981; Whitehouse, 1983). However, the results of the present laboratory animal study indicate that aqueous root extract of *Harpagophytum procumbens* possesses analgesic and anti-inflammatory effects in the mammalian experimental animal models used.

It has been reported that oral administration and subsequent passage of *Harpagophytum procumbens* root extract through the stomach (where the pH is acidic) leads to loss of pharmacological activity of the plant's extract – due to

deactivation of its active constituents by gastric acid juices (Soulimani, *et al.*, 1994). In the present study, therefore, the root aqueous extract of *Harpagophytum procumbens* used has been administered intraperitoneally, a route that ensures high systemic bioavailability and adequate pharmacological activity of the extract.

Experimental evidence obtained in the present study show that *Harpagophytum procumbens* secondary root extract (HPE, 50–800 mg/kg i. p.) dose-dependently and significantly ($P<0.05–0.001$) delayed the reaction times of the mice used in the 'hot-plate' analgesic test method. In the same 'hot-plate' test method, morphine (MPN, 10 mg/kg i. p.) also profoundly delayed the reaction times of the animals. Moreover, the plant's extract (HPE, 50 – 800 mg/kg i. p.) significantly ($P<0.05–0.001$) inhibited acetic acid-induced writhes in mice. Similarly, diclofenac (DIC, 100 mg/kg i. p.) markedly reduced acetic acid-induced writhes in the mice. These observations suggest that *Harpagophytum procumbens* secondary root aqueous extract (HPE) probably possesses centrally- and peripherally-mediated analgesic properties. This hypothesis is in keeping with those of Eddy and Leimback (1953), Koster *et al.*, (1959) and Williamson *et al.*, (1996) who postulated that acetic acid-induced writhing and hot-plate test methods are useful and appropriate models for the evaluation of peripherally- and centrally- acting analgesic drugs respectively.

Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), is commonly employed in the treatment and/or management of rheumatoid arthritis, osteo-arthritis and ankylosing spondylitis (Siraux, 1977; Sigmeth and Sieberer, 1980; Brooks *et al.*, 1980) for its anti-inflammatory and analgesic effects (Small, 1989). Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis and/or production (Skoutakis *et al.*, 1988; Mahgoub, 2002). The drug also

affects polymorphonuclear leukocytes function *in vitro*, thereby reducing chemotaxis, superoxide toxic radical formation, oxygen-derived free radical generation, and neutral protease production (Freeman *et al.*, 1986; Mahgoub, 2002). Diclofenac has also been reported to suppress inflammation induced by various phlogistic agents in experimental animal models (Menasse *et al.*, 1978; Al-Tuwaijri and Mustafa, 1992; Mahgoub, 2002). The results of the present study tend to suggest that, like diclofenac, *Harpagophytum procumbens* root aqueous extract probably produces its analgesic and anti-inflammatory effects by inhibiting the release, synthesis and/or production of inflammatory mediators, including histamine, polypeptide kinins and prostaglandins.

Harpagophytum procumbens roots have been reported to contain sugars, flavonoids, iridoid glycosides, phenolic acids, quinones, phytosterols, triterpenoids, acetoside esters and minerals (Watt and Breyer-Brandwijk, 1962; Van Wyk and Gericke, 2000; Van Wyk *et al.*, 2002). Although the exact chemical constituent/s of *H. procumbens* roots that is/are responsible for the observed analgesic and anti-inflammatory effects of the plant's aqueous extract still remains speculative, a number of investigators have shown that a host of secondary plant metabolites with diverse chemical structures possess analgesic, anti-inflammatory and hypoglycaemic effects in various experimental animal models (Dongmo *et al.*, 2003; Taesotiku *et al.*, 2003; Ojewole, 2002; 2003; Adzu *et al.*, 2003; Akah and Okafor, 1992; Marles and Farnsworth, 1995). Since the secondary roots of *Harpagophytum procumbens* are known to contain triterpenoids, flavonoids and iridoids in addition to other chemical constituents, it is not unlikely that these chemical compounds might have contributed to the observed analgesic and anti-inflammatory effects of the plant's root extract. Several investigators have also attributed the anti-inflammatory (and hypoglycaemic)

properties of a number of plants to their triterpenoid, coumarin and flavonoid constituents (Gupta *et al.*, 1969; Singh *et al.*, 1992; Liu, 1995; Marles and Farnsworth, 1995; Ojewole, 2002; 2003).

Experimental evidence obtained in the present laboratory animal study indicates that aqueous root extract of *H. procumbens* possesses analgesic and anti-inflammatory properties. These pharmacological effects may be due to complex interactions among the various chemical constituents of the plant. However, the findings of the present study lend pharmacological support to the suggested folkloric uses of the plant's root in the management and/or control of painful, arthritic and other inflammatory conditions in some communities of South Africa.

B. EVALUATION OF ANTICONVULSANT ACTIVITY

ABSTRACT

Harpagophytum procumbens DC is widely used in South African traditional medicine for the treatment, management and/or control of a plethora of human ailments including febrile, childhood convulsions. In the present study, the anticonvulsant activity of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 50–800 mg/kg i. p.) has been examined against pentylenetetrazole (PTZ)-, picrotoxin (PCT)- and bicuculline (BCL)-induced seizures in mice. Phenobarbitone and diazepam were used as reference, standard anticonvulsant drugs for comparison. Like the standard anticonvulsant agents used, *Harpagophytum procumbens* secondary root aqueous extract (HPE, 100–800 mg/kg i. p.) significantly ($P<0.05$–0.001) delayed the onset of, and antagonized, pentylenetetrazole (PTZ)-induced seizures. The plant's extract (HPE, 100–800 mg/kg i. p.) profoundly antagonized picrotoxin (PCT)-induced seizures, but only partially and weakly antagonized bicuculline (BCL)-induced seizures. It is speculated that *Harpagophytum procumbens* secondary root aqueous extract (HPE) may be producing its anticonvulsant activity by enhancing GABAergic neurotransmission and/or facilitating GABAergic action in the brain. However, the results of this experimental animal study indicate that *H. procumbens* secondary root

aqueous extract possesses anticonvulsant activity, and thus lend pharmacological support to the suggested folkloric uses of the plant extract in the management and/or control of epilepsy and childhood convulsions in some communities of South Africa.

INTRODUCTION

Harpagophytum procumbens DC, 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 an array of human ailments, including febrile childhood convulsions. Some traditional health practitioners in KwaZulu-Natal province of South Africa have claimed that *Harpagophytum procumbens* secondary root extract is a useful remedy in the management and/or control of epilepsy and childhood convulsions. However, there is no record or report whatsoever in the literature on the anticonvulsant activity of *Harpagophytum procumbens* secondary root extract. In an effort to scientifically appraise the '*healing powers*' and medicinal value of *Harpagophytum procumbens* secondary root extract in epilepsy and/or convulsion, the present study was undertaken to examine the anticonvulsant activity of *H. procumbens* secondary root aqueous extract in mice.

MATERIALS AND METHODS

Plant Material

The plant material used in this study, its preparation and extraction, are the same as those described in detail under Chapters Two and Three of this Thesis.

Animal Material

Balb C male albino mice (*Mus domesticus*) weighing 20–25 g were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light; and were allowed free access to food (standard pellet diet) and water *ad libitum*. The animals were divided into drug-treated 'test' and distilled water-treated 'control' groups of 10 animals per group. All the animals were fasted for 12 hours, but still allowed free access to water, before the commencement of our experiments.

It has been shown that oral administration and subsequent passage of *Harpagophytum procumbens* root extract through the stomach (where the pH is acidic) leads to loss of pharmacological activity of the extract – due to deactivation of its active constituents by gastric acid juices (Soulimani *et al.*, 1994). In the present study, therefore, *H. procumbens* root extract has been administered intraperitoneally (i. p.), a route that ensures adequate systemic bioavailability and pharmacological activity of the extract.

Evaluation of Anticonvulsant Activity

Balb C male albino mice (*Mus domesticus*) weighing 20–25 g were used. The anticonvulsant testing method of Vellucci and Webster (1984) modified by Amabeoku and Chikuni (1993) was used to assess the anticonvulsant activity of the plant's extract (HPE) in the mice. Standard convulsant agents, pentylenetetrazole (PTZ, 90 mg/kg i. p.) picrotoxin (PCT, 10 mg/kg i. p) and bicuculline (BCL, 30 mg/kg i. p.) were used to induce convulsions in the mice. Phenobarbitone (PBT, 20 mg/kg i. p.) and diazepam (DZP, 0.5 mg/kg i. p.) were used as reference, standard anticonvulsant drugs for comparison. Following induction of convulsions in the 'test' mice (with intraperitoneal injections of the convulsant agents), the animals were observed for 30 minutes for signs of neurological deficits, especially, hind-limb tonic seizures or convulsions. Hind-limb tonic extensions of the mice were regarded as manifestations of seizures. The ability of the plant's extract (HPE, 50–800 mg/kg i. p.) to prevent the seizures or delay/prolong the latency or onset of the hind-limb tonic extensions was considered as an indication of anticonvulsant activity (Navarro-Ruiz *et al.*, 1995; Amabeoku *et al.*, 1998). The experimental procedures were repeated with other groups of 'test' mice pretreated for 20 minutes with graded doses of the plant's extract (HPE, 50–800 mg/kg i. p.) or with the standard anticonvulsant drugs used for comparison – [i. e., phenobarbitone (PBT, 20 mg/kg i. p.) and diazepam (DZP, 0.5 mg/kg i. p.)], before the administrations of the convulsant agents. Because the plant extract and other drugs used in this study were dissolved in distilled water each day at the beginning of our experiments, distilled water (2 ml/kg i. p.) pretreated mice were used as 'control' animals.

Data Analysis

Data obtained were pooled and presented as means (±SEM). Data from distilled-water (2 ml/kg i. p.) treated 'control' mice were used as baseline values. The differences between the plant extract-, and other drug-treated 'test' mice, and distilled water-treated 'control' animals,were analysed statistically using paired 'Student's t-test'. The level of significance of the differences between the 'test' and 'control' group data was determined. The proportion of mice convulsing was analysed by 'Chi-Square test' (Bienvenu *et* al., 2002). In all cases, values of $P \leq 0.05$ were taken to imply statistical significance.

RESULTS

Effect of *H*arpagophytum *procumbens* root aqueous extract (HPE) on pentylenetetrazole (PTZ)-induced seizures

Pentylenetetrazole (PTZ, 90 mg/kg i. p.) produced hind-limb tonic seizures in all the ten mice used. *Harpagophytum procumbens* root aqueous extract (HPE, 100–800 mg/kg i. p.) produced dose-related, significant ($P<0.05–0.001$) protection of the mice against PTZ-induced seizures (Table 5). The plant's extract (HPE, 100–800 mg/kg i. p.) significantly

Table 5.

Effects *of Harpagophytum procumbens* root aqueous extract (HPE), phenobarbitone (PBT) and diazepam (DZP) on pentylenetetrazole (PTZ)-induced seizures in mice.

Treatment Dose (mg/kg i. p.)				No. convulsed/ No. used	% Animals not convulsed (i.e., % animals protected)	Latency of tonic convulsion (min) [Mean (±SEM]
PTZ	HPE	Phenobarbitone	Diazepam			
90	-	-	-	10/10	0	7.30 ± 0.50
90	100	-	-	7/10	30	10.20* ± 1.45
90	200	-	-	6/10	40	12.40* ±2.30
90	400	-	-	5/10	50	15.50** ±2.25
90	800	-	-	3/10	70	19.36*** ± 2.30
90	-	20	-	0/10[c]	100	0.00***
90	-	-	0.5	0/10[c]	100	0.00***

*P<0.05; **P<0.01; ***P<0.001 vs pentylenetetrazole control (PTZ, 90 mg/kg i. p.); Paired Student's t-test.

[c]P<0.001 vs pentylenetetrazole control (PTZ, 90 mg/kg i. p.); Chi-Square test.

delayed (P<0.05–0.001) the onset of, and antagonized, PTZ-induced seizures. The standard anticonvulsant drugs used, phenobarbitone (PBT, 20 mg/kg i. p.) and diazepam (DZP, 0.5 mg/kg i. p.) also profoundly delayed the onset of, and significantly antagonized (P<0.001), PTZ-induced seizures (Table 5).

Effect of *H*arpagophytum *procumbens* root aqueous extract (HPE) on picrotoxin (PCT)-induced seizures

Picrotoxin (PCT, 10 mg/kg i. p.) produced hind-limb tonic seizures in all the ten mice used. *Harpagophytum procumbens* root aqueous extract (HPE, 100–800 mg/kg i. p.) produced dose-related, significant (P<0.05–0.001) protection of the mice against PCT-induced seizures (as in the PTZ-induced tonic seizures) – see Table 6. Furthermore, the plant's extract (HPE, 100–800 mg/kg i. p.) significantly delayed (P<0.05–0.001) the onset of PCT-induced seizures (as in the PTZ-induced tonic seizures). The standard anticonvulsant drugs used, phenobarbitone (PBT, 20 mg/kg i. p.) and diazepam (DZP, 0.5 mg/kg i. p.) profoundly delayed the onset of, and significantly antagonized (P<0.001), PCT-induced seizures (Table 6).

Table 6.

Effects *of Harpagophytum procumbens* root aqueous extract (HPE), phenobarbitone (PBT) and diazepam (DZP) on picrotoxin (PCT)-induced seizures in mice.

Treatment Dose (mg/kg i. p.)				No. convulsed/ No. used	% Animals not convulsed (i.e., % animals protected)	Latency of tonic convulsion (min) [Mean (±SEM]
PCT	HPE	Phenobarbitone	Diazepam			
10	-	-	-	10/10	0	10.20±0.58
10	100	-	-	7/10	30	12.45* ± 1.30
10	200	-	-	6/10	40	15.50* ±2.50
10	400	-	-	5/10	50	17.55** ±2.10
10	800	-	-	3/10	70	20.46*** ± 2.30
10	-	20	-	2/10[c]	80	27.54***
10	-	-	0.5	1/10[c]	90	28.10***

*P<0.05; **P<0.01; ***P<0.001 vs picrotoxin control (PCT, 10 mg/kg i. p.); Paired Student's t-test.
[c]P<0.001 vs picrotoxin control (PTZ, 10 mg/kg i. p.); Chi-Square test.

Effect of *H*arpagophytum *procumbens* root aqueous extract (HPE) on bicuculline (BCL)-induced seizures

Bicuculline (BCL, 30 mg/kg i. p.) produced hind-limb seizures in all the ten mice used. *Harpagophytum procumbens* root aqueous extract (HPE, 200–800 mg/kg i. p.) produced dose-related, significant ($P<0.05–0.01$) protection of the mice against BCL-induced seizures (Table 7). However, relatively low dose of the plant's extract (HPE, 50–100 mg/kg i. p.) did not significantly alter ($P>0.05$) the onset of BCL-induced seizures, whereas, relatively moderate to high doses of the plant's extract (HPE, 200–800 mg/kg i. p.) significantly delayed ($P<0.05–0.01$) the onset of BCL-induced seizures. The two standard anticonvulsant drugs used, phenobarbitone (PBT, 20 mg/kg i. p.) and diazepam (DZP, 0.5 mg/kg i. p.), profoundly antagonized, and significantly delayed ($P<0.001$) the onset of, BCL-induced seizures (Table 7).

Table 7.

Effects *of Harpagophytum procumbens* root aqueous extract (HPE), phenobarbitone (PBT) and diazepam (DZP) on bicuculline (BCL)-induced seizures in mice.

Treatment Dose (mg/kg i. p.)				No. convulsed/ No. used	% Animals not convulsed (i.e., % animals protected)	Latency of tonic convulsion (min) [Mean (±SEM]
BCL	HPE	Phenobarbitone	Diazepam			
30	-	-	-	10/10	0	6.40±0.56
30	100	-	-	8/10	20	8.10±1.40
30	200	-	-	7/10	30	9.50* ±2.20
30	400	-	-	6/10	40	11.55** ±2.45
30	800	-	-	5/10	50	13.46** ± 2.30
30	-	20	-	0/10[c]	100	0.00***
30	-	-	0.5	0/10[c]	100	0.00***

*P<0.05; **P<0.01; ***P<0.001 vs bicuculline control (BCL, 30 mg/kg i. p.); Paired Student's t-test.
[c]P<0.001 vs bicuculline control (BCL, 30 mg/kg i. p.); Chi-Square test.

DISCUSSION

Although *Harpagophytum procumbens* secondary root extract is widely used by the traditional health practitioners of southern Africa for a variety of human ailments, little or no scientific information exists in biomedical literature on the therapeutic efficacy of the plant product. It has been reported that oral administration and subsequent passage of *Harpagophytum procumbens* root extract through the stomach (where the pH is acidic) leads to loss of pharmacological activity of the plant's extract – due to deactivation of its active constituents by gastric acid juices (Soulimani, *et al.*, 1994). This is an interesting observation, especially because the plant product is always administered orally to patients! However, in the present study, *Harpagophytum procumbens* secondary root aqueous extract has been administered intraperitoneally, a route that ensures high systemic bioavailability and adequate pharmacological activity of the extract.

The results of the present laboratory animal study show that aqueous root extract of *Harpagophytum procumbens* (HPE) possesses anticonvulsant activity in the experimental animal model used. The data obtained in this study also indicate that relatively moderate to high doses of *Harpagophytum procumbens* secondary root aqueous extract (HPE) inhibited or attenuated pentylenetetrazole (PTZ)-induced seizures, while the standard anticonvulsant drugs used, phenobarbitone (PBT) and diazepam (DZP), completely abolished the seizures and protected all the animals against PTZ-induced seizures. Pentylenetetrazole (PTZ) has been reported to produce seizures by inhibiting gamma-aminobutyric acid (GABA) neurotransmission (Okada *et al.*, 1989; De Sarro *et al.*, 1999). GABA is the main inhibitory neurotransmitter

substance in the brain, and is widely implicated in epilepsy. Enhancement of GABAergic neurotransmission has been shown to inhibit or attenuate seizures, while inhibition of GABAergic neurotransmission or activity is known to promote and facilitate seizures (Meldrum, 1975; Olsen, 1981; Gale, 1992; Leonard, 2000). It would appear, therefore, that the complete protection of the mice by the standard anticonvulsant drugs used in this study against PTZ-induced seizures is in keeping with the above hypothesis, since the standard anticonvulsant drugs used are known to exert their anticonvulsant actions by enhancing GABAergic neurotransmission and activity (Olsen, 1981; Leonard, 2000, Rang *et al.*, 2000). The findings of the present study, therefore, tend to suggest that *Harpagophytum procumbens* secondary root aqueous extract (HPE) might have inhibited and/or attenuated PTZ-induced seizures of the mice by enhancing, or in some ways interfering with, GABAergic neurotransmission.

The standard anticonvulsant drugs used in the present study, phenobarbitone (PBT) and diazepam (DZP), as well as *Harpagophytum procumbens* secondary root aqueous extract (HPE), profoundly antagonized picrotoxin (PCT)-induced seizures. Postsynaptic $GABA_A$-receptors are functionally linked to benzodiazepine receptors, barbiturate receptors and chloride-ion channels to form GABA-chloride ionophore complex, which is intimately involved in the modulation of GABAergic neurotransmission (Olsen, 1981; Rang *et al.*, 2000; Bennett and Brown, 2003). According to Nicoll (2001), picrotoxin, a $GABA_A$-receptor antagonist, produces seizures by blocking the chloride-ion channels linked to $GABA_A$-receptors, thus preventing the entry of chloride ions into the brain. This process will, in turn, inhibit GABA neurotransmission and activity in the brain (Nicoll, 2001). Phenobarbitone and

diazepam are believed to enhance GABAergic neurotransmission by increasing chloride ion flux through the chloride-ion channels at $GABA_A$-receptor sites (Olsen, 1981; Rang *et al.*, 2000). This hypothesis may explain the observed protective effects, and/or antagonistic actions of, phenobarbitone and diazepam (a benzodiazepine) against picrotoxin (PCT)-induced seizures in the mice used. Since *Harpagophytum procumbens* secondary root aqueous extract (HPE) mimicked, to a large extent, the anticonvulsant actions of the standard anticonvulsant dugs used in this study, it is not unlikely that the plant's extract (HPE) antagonizes picrotoxin (PCT)-induced seizures by opening the chloride-ion channels associated with $GABA_A$-receptors. This proposal further buttresses the hypothesis that HPE may be interfering with GABAergic neurotransmission in one way or another.

Bicuculline (BCL), a potent, selective $GABA_A$-receptor antagonist, produces seizures by blocking the effect of GABA at central $GABA_A$-receptors, which have been associated with epilepsy (Gale, 1992; Rang *et al.*, 2000). Phenobarbitone and diazepam, which act by enhancing GABA neurotransmission, antagonized bicuculline (BCL)-induced seizures in the present study. However, *H. procumbens* secondary root aqueous extract (HPE) only partially and weakly antagonized BCL-induced seizures, and was much less effective in this regard compared with phenobarbitone and diazepam. This observation also tends to suggest that HPE interferes with GABAergic neurotransmission but probably not through $GABA_A$-receptor sites. *Harpagophytum* procumbens secondary roots have been reported to contain sugars, iridoid glycosides, flavonoids, phenolic acids, quinones, phytosterols, triterpenoids, acetoside esters and minerals (Watt & Breyer-Brandwijk, 1962; Van Wyk and Gericke, 2000; Van Wyk *et al.*, 2002). Although only a few pharmacological studies on *H. procumbens* have

been reported in the biomedical literature, the iridoids harpagoside (a cinnamic acid ester), harpagide and procumbide are speculated to account for the observed anticonvulsant activity of the plant's extract (HPE). However, the experimental evidence obtained in the present laboratory animal study indicates that aqueous root extract of *H. procumbens* (HPE) significantly delayed the onset of seizures induced by pentylenetetrazole (PTZ) and also significantly antagonized picrotoxin (PCT)-induced seizures. Since PTZ- and PCT-induced seizures have been shown to be due to inhibition and/or attenuation of GABAergic neurotransmission, it is reasonable to speculate that HPE probably produces its anticonvulsant activity by enhancing GABAergic neurotransmission. The observed anticonvulsant activity of the plant's extract may have been caused by complex interactions among the various chemical constituents of the herb. However, the findings of the present study lend pharmacological support to the suggested folkloric uses of the plant's root in the management and/or control of epilepsy and childhood convulsions in some communities of South Africa, and probably also account for the 'sedative' adverse effect that is often associated with *Harpagophytum procumbens* secondary root medication.

CHAPTER FIVE

METABOLIC PHARMACOLOGY OF *HARPAGOPHYTUM PROCUMBENS* DC

ABSTRACT

Harpagophytum procumbens DC is widely used in South African traditional medicine for the treatment, management and/or control of a variety of human ailments, including diabetes mellitus. In the present study, the hypoglycaemic and antidiabeticic effects of the plant's root extract have been investigated in rats using chlorpropamide (250 mg/kg p. o.) as reference hypoglycaemic agent for comparison. *Harpagophytum procumbens* secondary root aqueous extract (HPE, 50–800 mg/kg i. p.) produced dose-dependent, significant reductions ($P<0.05–0.001$) in the blood glucose concentrations of both fasted normal and fasted diabetic rats. The results of this experimental animal study indicate that *Harpagophytum procumbens* secondary root aqueous extract possesses hypoglycaemic/antidiabetic activity. These observations lend pharmacological support to the suggested folkloric uses of *Harpagophytum procumbens* secondary root in the management and/or control of adult-onset, type-2 diabetes mellitus in some communities of South Africa.

INTRODUCTION

In the form of infusions, decoctions, tinctures, powders and extracts, the dried thick, fleshy, tuberous secondary tap roots of *Harpagophytum procumbens* are usually used in South African traditional medicine for a variety of human ailments, including diabetes (Watt and Breyer-Brandwijk, 1962; Van Wyk and Gericke, 2000; Van Wyk *et al.,* 2002; Van Wyk and Wink, 2004). However, there is no record or report whatsoever in the literature on the hypoglycaemic/antidiabetic activity of *Harpagophytum procumbens* secondary root extract. In an effort to scientifically appraise the '*healing powers*' and medicinal value of *Harpagophytum procumbens* in diabetes, the present study was undertaken to investigate the antidiabetic property of the plant's secondary root aqueous extract in an experimental model of diabetes mellitus.

MATERIALS AND METHODS

Plant Material

The plant material used in this study, its preparation and extraction, are the same as those described in detail under Chapters Two, Three and Four of this Thesis.

Animal Material

Young adult Wistar rats (*Rattus norvegicus*) weighing 250–300 g were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light; and were allowed free access to food (standard pellet diet) and water *ad libitum*. The animals were divided into drug-treated 'test' and distilled water-treated 'control' groups of 8 animals per group. All the animals were fasted for 12 hours, but still allowed free access to water, before the commencement of our experiments. It has been shown that oral administration and subsequent passage of *Harpagophytum procumbens* root extract through the stomach (where the pH is acidic) leads to loss of pharmacological activity of the extract – due to deactivation of its active constituents by gastric acid juices (Soulimani *et al.*, 1994). In the present study, therefore, *Harpagophytum procumbens* root aqueous extract has been administered intraperitoneally, a route that ensures adequate systemic bioavailability and pharmacological activity of the extract.

Determination of Blood Glucose Levels

Young adult Wistar rats (*Rattus norvegicus*) weighing 250–300 g were used. The animals were randomly divided into two (A and B) groups of 'test' and 'control' rats. Diabetes mellitus was induced (in Group A diabetic 'test' rats) by intraperitoneal injections of streptozotocin (STZ, 90 mg/kg). Diabetes was allowed to develop and stabilize in these STZ-treated rats over a period 3 to 5 days. The 'control' (Group B)

normal (normoglycaemic) rats were treated with distilled water (2 ml/kg i. p.) only. All the animals were kept and maintained under laboratory conditions of temperature, humidity and light (12-hour day:12-hour night cycle); and were allowed free access to food (standard pellet diet) and water *ad libitum*. Before the commencement of our experiments, both the 'control' normal (normoglycaemic) and STZ-treated 'test' diabetic (hyperglycaemic) rats were fasted for 12 hours, but still allowed free access to water throughout. Fasted STZ-treated rats with blood glucose concentrations ≥ 500 mg/dL were considered to be diabetic, and used in this study. At the end of the 12-hour fasting period [taken as zero time (i. e., 0 hour)] blood glucose levels (initial glycaemia – G_o) of the fasted normal (normoglycaemic) and STZ-treated, diabetic rats were determined and recorded. For both the normoglycaemic and hyperglycaemic animals, chlorpropamide (250 mg/kg p. o.) was used as the reference hypoglycaemic agent for comparison. The test compounds [i. e., *Harpagophytum procumbens* root aqueous extract (HPE, 50–800 mg/kg i. p.) and chlorpropamide (250 mg/kg p. o.)] were administered to the groups of fasted normal and fasted diabetic 'test' rats. 1, 2, 4 and 8 hours following administration of the 'test' compounds to the animals, blood glucose concentrations (G_t) were determined. The rats were restrained in a cage, and blood samples (0.02 ml) were collected from the tail tip vein of each rat for blood glucose analysis. Blood samples were obtained by repeated puncture of the same tail tip vein. Blood glucose concentrations were determined by means of Bayer's Glucometer Elite® and compatible blood glucose test strips. Percentage glycaemic variation was calculated as a function of time (t) by applying the formula:

$$\% \text{ glycaemic change} = G_o - G_t \times 100 / G_o$$

where G_o and G_t represent (zero time or 0 hour) glycaemic values before, and glycaemic values at 1, 2, 4 and 8 hours after, intraperitoneal (or oral – in the case of chlorpropamide) administrations of the 'test' compounds respectively. At the same time, rats treated with distilled water (2 ml/kg i. p.) alone were used as 'controls'.

The 'test' and 'reference' compounds used in all experiments contained in this study [i. e., *Harpagophytum procumbens* root aqueous extract (HPE) and chlorpropamide] were dissolved in distilled water on each day of our experiment. In all cases, the vehicle (i. e., distilled water) was used as 'control' for the 'test' and 'reference' compounds used.

Data Analysis

Data obtained were pooled and presented as means (±SEM). Data from 'control' rats were used as baseline values. The differences between the plant extract- or chlorpropamide-treated 'test' rats, and distilled water-treated 'control' animals, were analysed statistically. The data were subjected to one-way analysis of variance (ANOVA), and Scheffe's multiple comparison was used to determine the level of significance of the differences between the 'test' and 'control' group data. Values of $P \leq 0.05$ were taken to imply statistical significance.

RESULTS

Antidiabetic Activity

In a separate set of experiments involving 12-hour fasted normal and STZ-treated diabetic rats, the mean blood glucose level of normal (normoglycaemic) rats was found to vary between 104±5.50 and 108±5.45 mg/dL. In the 'control' set of experiments, pre-treatment of the animals with distilled water (2 ml/kg i. p.) alone did not significantly modify ($P>0.05$) the blood glucose concentrations of either the fasted normal, or fasted diabetic rats. In these animals, pre-treatment with distilled water (2 ml/kg i. p.) for 1, 2, 4 and 8 hours either slightly but insignificantly ($P>0.05$) decreased, increased, or did not affect at all, the blood glucose concentrations of the fasted 'control' animals. The distilled water-induced changes in the blood glucose levels of the fasted rats varied by values ranging between 0.1% and 1.0% of the mean basal blood glucose concentrations. However, compared with the distilled water-treated 'control' rats, pre-treatment of the fasted animals with relatively moderate to high doses of *Harpagophytum procumbens* root aqueous extract (HPE, 50–800 mg/kg i. p.) produced significant reductions ($P<0.05$–0.001) in the blood glucose concentrations of both fasted normal and fasted diabetic rats (Tables 8 and 9). The maximal reductions in the blood glucose concentrations of the fasted 'test' rats occurred at the plant extract dose of 800 mg/kg (i. p.). Pre-treatment of fasted normal (normoglycaemic) and STZ-treated diabetic rats with chlorpropamide (250 mg/kg p. o.) or with *Harpagophytum procumbens* root aqueous extract (HPE, 800 mg/kg i. p.)

for 1, 2, 4 and 8 hours also produced highly significant reductions ($P < 0.01–0.001$) in the blood glucose concentrations of the animals (compared with distilled water-treated fasted 'control' rats – see Tables 8 and 9). The hypoglycaemic effect of the plant's root aqueous extract became significant ($P<0.05$) 1 hour following oral administration, reaching the peak of its hypoglycaemic effect 2–4 hours after administration. However, the hypoglycaemic effect of the plant's extract was still significant 8 hours after intraperitoneal administration of the extract (see Tables 8 and 9). Thereafter, the blood glucose concentrations of the animals gradually returned to normal, baseline values at the end of the 24^{th} hour.

Table 8.

Effects of *Harpagophytum procumbens* root aqueous extract (**HPE**, 800 mg/kg i. p.) and chlorpropamide (250 mg/kg p. o.) on blood glucose concentrations (mg/dL) of normal (normoglycaemic) rats. Values given represent the mean (±SEM) of 8 observations.

Treatment	**Before Treatment**	**After Treatment**				**Maximal Reduction**	**% Maximal Reduction**
	0 hr	**1 hr**	**2 hr**	**4 hr**	**8 hr**		
Control (2 ml/kg i. p. distilled water)	105.50±4.56	106.15±4.51	105.55±4.35	104.86±4.50	105.28±4.35	0.64	0.61
HPE (800 mg/kg i. p.)	107.35±4.50	101.45±4.45*	90.56±4.30**	79.75±4.35***	92.81±4.51**	27.60***	25.71***
Chlorpropamide (250 mg/kg p. o.)	106.40±4.45	96.45±4.48**	78.53±4.50***	69.50±4.38***	80.63±4.56***	36.90***	34.68***

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

Table 9.

Effects of *Harpagophytum procumbens* root aqueous extract (HPE, 800 mg/kg i. p.) and chlorpropamide (250 mg/kg p. o.) on blood glucose concentrations (mg/dL) of STZ-treated, diabetic rats. Values given represent the mean (±SEM) of 8 observations.

Treatment	**Before Treatment**	**After Treatment**				**Maximal Reduction**	**% Maximal Reduction**
	0 hr	**1 hr**	**2 hr**	**4 hr**	**8 hr**		
Control (2 ml/kg i. p. distilled water)	550.48±52.41	548.45±56.45	551.45±50.57	547.56±55.48	550.21±54.45	2.92	0.53
HPE (800 mg/kg i. p.)	560.52±55.46	470.53± 54.50*	387.65±53.55**	282.60±54.45***	364.59±50.41**	277.92***	49.58***
Chlorpropamide (250 mg/kg p. o.)	555.50±51.45	448.54±50.52*	347.50±51.56**	230.61±52.41***	290.51±53.40**	324.89***	58.49***

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

DISCUSSION

It has been reported that oral administration and subsequent passage of *Harpagophytum procumbens* root extract through the stomach (where the pH is acidic) leads to loss of pharmacological activity of the plant's extract – due to deactivation of its active constituents by gastric acid juices (Soulimani, *et al.*, 1994). In the present study, therefore, the root aqueous extract of *Harpagophytum procumbens* used has been administered intraperitoneally, a route that ensures high systemic bioavailability and adequate pharmacological activity of the extract.

Experimental evidence obtained in the present study shows that *Harpagophytum procumbens* secondary root extract (HPE, 50–800 mg/kg i. p.) dose-dependently and significantly ($P<0.05–0.001$) reduced the blood glucose concentrations of fasted normal and fasted STZ-treated diabetic rats. The main classes of synthetic oral hypoglycaemic agents currently available for the management and/or control of adult-onset, type-2, non-insulin-dependent (NIDDM) diabetes mellitus include the sulphonylureas, biguanides, thiazolidinediones, alpha-glucosidase inhibitors, and so on. Chlorpropamide, used as the reference hypoglycaemic agent in this study, is a member of the 'first-generation sulphonylureas'. As a class, sulphonylureas stimulate and increase the release of endogenous insulin from pancreatic β-cells. They also promote and facilitate peripheral tissue uptake and utilization of glucose. It has been proposed (Jackson and Bressler, 1981) that sulphonylureas produce their hypoglycaemic effects via three main mechanisms, viz: (i) *increased insulin release from pancreatic β-cells* – sulphonylureas

bind to pancreatic receptors associated with potassium (K^+) channels on the surface of the pancreatic β-cells. This binding inhibits K^+ efflux from the β-cells through the K^+-channels. Consequently, depolarization of pancreatic β-cells ensues. This depolarization opens the 'voltage-gated' calcium (Ca^{2+}) channels. This process allows and/or facilitates Ca^{2+} influx into the pancreatic β-cells, subsequently resulting in the release of preformed insulin from pancreatic β-cells; (ii) *potentiation of insulin's action on target tissues and increased glucose removal from the blood* – sulphonylureas increase the binding of insulin to peripheral tissue insulin receptors, thereby enhancing glucose uptake and utilization by peripheral tissues; (iii) *reduction of blood glucagon levels* – administration of sulphonylureas may lead to reduced blood glucagon concentrations. This effect can be attributed, at least in part, to the hypoglycaemic action of sulphonylureas, since the primary actions of glucagon are to enhance the metabolism of stored glycogen, and to facilitate and/or increase gluconeogenesis and ketogenesis. Enhanced release of insulin (and somatostatin) inhibits glucagon secretion via inhibition of pancreatic α-cell secretion. Thus, any plant product or chemical constituent that is capable of affecting the pancreatic β- or α-cell secretion in any of the three ways illustrated above will be a good mimicker of sulphonylureas, and will produce hypoglycaemic effects in mammals via mechanisms similar to those of sulphonylureas.

Since streptozotocin is known to destroy insulin-producing pancreatic β-cells, the STZ-treated rat model would appear to represent a good laboratory NIDDM experimental diabetic state, with residual or remnant insulin production by the pancreatic β-cells. The diabetic state of STZ-treated diabetic rats is, therefore, not the same as that obtained by

total pancreatectomy, as daily administration of insulin is not required for survival in STZ-treated diabetic animals. Acute treatment of the 'control' rats with distilled water alone did not produce any significant change in the blood glucose concentrations of either the fasted normal or the fasted STZ-treated, diabetic rats. However, the aqueous plant's extract, like chlorpropamide – a sulphonylurea antidiabetic agent – produced significant reductions in the blood glucose levels of the fasted normal and fasted STZ-treated diabetic rats. The hypoglycaemic and antidiabetic effects of *Harpagophytum procumbens* root aqueous extract would, therefore, appear to be most probably exerted via a mechanism that is similar to that of chlorpropamide.

Harpagophytum procumbens roots have been reported to contain sugars, flavonoids, iridoid glycosides, phenolic acids, quinones, phytosterols, triterpenoids, acetoside esters and minerals (Watt and Breyer-Brandwijk, 1962; Van Wyk and Gericke, 2000; Van Wyk *et al.,* 2002). Although the exact chemical constituent/s of *H. procumbens* roots that is/are responsible for the observed hypoglycaemic effects of the plant's aqueous extract still remains speculative, several investigators have attributed the anti-inflammatory and hypoglycaemic properties of a number of plants to their triterpenoid, coumarin and flavonoid constituents (Gupta *et al.*, 1969; Singh *et al.*, 1992; Akah and Okafor, 1992; Liu, 1995; Marles and Farnsworth, 1995; Ojewole, 2002; 2003; Adzu *et al.*, 2003).

The experimental evidence obtained in the present laboratory animal study indicates that aqueous root extract of *H. procumbens* possesses hypoglycaemic/antidiabetic property. This pharmacological action may be due to complex interactions among the various chemical constituents of the plant. However, the findings of this study lend

pharmacological support to the suggested folkloric uses of the plant's root in the management and/or control of adult-onset, type-2 diabetes mellitus in some communities of South Africa.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER STUDY

CONCLUSIONS

The studies reported in this thesis clearly indicate that *Harpagophytum procumbens* secondary root aqueous extract possesses a catalogue of pharmacological actions.

The plant's extract produced concentration-dependent, significant ($P<0.05–0.001$), atropine-sensitive contractions of mammalian and avian isolated gastro-intestinal smooth muscles. The plant's extract also exhibited significant anticholinesterase activity. It is speculated that the contractile effects of *Harpagophytum procumbens* secondary root aqueous extract is mediated via cholinergic mechanism, possibly by stimulating cholinergic muscarinic receptors. The contractile effects of the herb's extract on gastro-intestinal smooth muscles would appear to provide a pharmacological basis for the troublesome 'gastro-intestinal disturbance and/or upset' commonly associated with *Harpagophytum procumbens* secondary root aqueous extract medication in some communities of South Africa.

Harpagophytum procumbens secondary root aqueous extract also provoked concentration-related, significant ($P<0.05–0.001$) contractions of the rat isolated uterine muscles. This observation appears to justify the use of the herb's secondary root extracts

for induction and acceleration of labour by traditional birth attendants. The findings also provide pharmacological justification for the use of the plant's extract in expelling retained placentas during child birth. The uterotonic action of *Harpagophytum procumbens* secondary root aqueous extract is thought to be due to the release of uterotonic mediators, such as prostaglandins, polypeptide kinins, acetylcholine, and so forth. However, because *Harpagophytum procumbens* secondary root aqueous extract induced powerful contractions of uterine muscle strips taken from both pregnant and non-pregnant rats, the use of the plant's extract should be contra-indicated and avoided in pregnant women.

Harpagophytum procumbens secondary root aqueous extract produced a dose-related, slight but significant ($P<0.05–0.001$) hypotension in the arterial blood pressure of anaesthetized rats. The depressor effect of the plant's extract on the blood pressure of rats *in vivo* was resistant to atropine and mepyramine pretreatment. It is thought that the depressor effect could be due in part, to the vasorelaxant activity of the plant's extract, since *Harpagophytum procumbens* secondary root aqueous extract produced concentration-related, significant, secondary longer-lasting relaxations of the rat isolated portal vein.

The plant's extract produced biphasic effects on guinea-pig isolated cardiac muscles. The responses of guinea-pig isolated atrial muscles to the plant's extract consisted of concentration-dependent, initial slight positive inotropic and chrotropic effects followed by long-lasting, significant negative inotropic and chronotropic effects. It was observed that relatively high concentrations of *Harpagophytum procumbens* secondary root

aqueous extract (HPE, 400–1000 μg/ml) always induced concentration-related, highly significant (P<0.01–0.001) negative inotropic and chronotropic responses of the guinea-pig isolated atrial muscles.

Harpagophytum procumbens secondary root aqueous extract dose-dependently delayed the reaction time in the 'hot-plate' analgesic test model, and also significantly (P<0.5–0.001) reduced or inhibited the writhes produced by 0.2 ml of 3% acetic acid in mice. In the two analgesic test methods used, the plant's extract produced dose-related analgesic effects. Although to a smaller extent, the analgesic effects of the plant's extract were comparable with those of morphine, diclofenac or aspirin. It is, therefore, suggested that the analgesic effects of plant's extract are probably mediated centrally and peripherally.

Harpagophytum procumbens secondary root aqueous extract also produced dose-related, significant (P<0.5–0.001) anti-inflammatory activity against fresh egg albumin-induced inflammation in rats. Although qualitatively and quantitatively less, the anti-inflammatory effect of the plant's extract was comparable with that of diclofenac or aspirin. It is speculated that the plant's extract probably acts via a mechanism similar to those of the non-steroidal anti-inflammatory drugs (NSAIDs), i. e., by inhibiting cyclo-oxygenase and/or lipoxygenase, and thereby preventing the synthesis and release of inflammatory mediators such as prostaglandins.

In mice, *Harpagophytum procumbens* secondary root aqueous extract significantly delayed (P<0.5–0.001) the onset of seizures induced by pentylenetetrazole, picrotoxin and bicuculline. Since pentylenetetrazole- and picrotoxin-induced seizures are known to occur as a consequence of inhibition or attenuation of GABA neurotransmission and/or

activity, it is speculated that the anticonvulsant activity of *Harpagophytum procumbens* secondary root aqueous extract might be due to potentiation or enhancement of GABA neurotransmission and/or activity by the plant's extract.

Harpagophytum procumbens secondary root aqueous extract produced dose-related, significant reductions in the blood glucose concentrations of streptozotocin-induced diabetic rats. The plant's extract also reduced the glucose levels of normal (normoglycaemic) rats. Although smaller, the hypoglycaemic and antidiabetic effects of the plant's extract are comparable to those of chlorpropamide in the experimental animal model used. It is speculated that *Harpagophytum procumbens* secondary root aqueous extract might be producing its hypoglycaemic and antidiabetic effects via mechanisms similar to those of sulphonylureas.

SUGGESTIONS FOR FURTHER STUDY

Although evidence from the present study indicates that *Harpagophytum procumbens* DC secondary root aqueous extract possesses a battery of pharmacological actions, the mechanisms via which the pharmacological effects are produced still remain largely speculative. For example, the plant's extract has been shown to produce contractile effects on isolated gastro-intestinal and uterine smooth muscles of mammalian experimental animals. The plant's extract has also been shown to possesses both cardio-

tonic and cardio-depressant activities. The mechanisms of action of the plant's extract on the various smooth and cardiac muscle preparations used need to be probed further and established.

The pharmacological actions of the plant's extract on skeletal muscles were not investigated in the studies reported in this thesis. It will be interesting to know what *Harpagophytum procumbens* DC secondary root aqueous extract does to mammalian skeletal muscles *in vitro* and *in vivo*.

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APPENDICES

APENDIX I

ETHICAL CLEARANCE AND ETHICAL ISSUES

The experimental protocols used in this thesis was approved by the Ethics Committee of the University of KwaZulu-Natal, Durban 4000, South Africa [please, see the attached "Ethical Clearance" Certificate]; and conforms with the "*Guide to the care and use of animals in research and teaching*" [published by the Univerity of KwaZulu-Natal, Durban 4000, South Africa]. The animals used for isolated muscle experiments were subjected to deep petroleum ether inhalation before they were bled out.

UNIVERSITY OF
KWAZULU-NATAL

RESEARCH OFFICE (FRANCIS STOCK BUILDING)
HOWARD COLLEGE
TELEPHONE NO.: 031 – 2603587

23 JULY 2004

MR. I. MALL
PHARMACOLOGY

Dear Mr. Mall

ETHICAL CLEARANCE

I wish to confirm that ethical clearance has been granted for the following project:

"Studies on some pharmacological properties of *Harpagophytum procumbens* [Devils claw] extracts"

Yours faithfully

MS. PHUMELELE XIMBA
(FOR) MANAGER: RESEARCH AFFICE

PS: The following general condition is applicable to all projects that have been granted ethical clearance:

THE RELEVANT AUTHORITIES SHOULD BE CONTACTED IN ORDER TO OBTAIN THE NECESSARY APPROVAL SHOULD THE RESEARCH INVOLVE UTILIZATION OF SPACE AND/OR FACILITIES AT OTHER INSTITUTIONS/ORGANISATIONS. WHERE QUESTIONNAIRES ARE USED IN THE PROJECT, THE RESEARCHER SHOULD ENSURE THAT THE QUESTIONNAIRE INCLUDES A SECTION AT THE END WHICH SHOULD BE COMPLETED BY THE PARTICIPANT (PRIOR TO THE COMPLETION OF THE QUESTIONNAIRE) INDICATING THAT HE/SHE WAS INFORMED OF THE NATURE AND PURPOSE OF THE PROJECT AND THAT THE INFORMATION GIVEN WILL BE KEPT CONFIDENTIAL.

cc. Director of School
cc. Supervisor

APENDIX II

PLANT MATERIAL USED

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 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°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 root 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.

APENDIX III

DRUGS USED

The following compounds, chemicals, reagents and drugs were used:

Harpagophytum procumbens secondary root extract (HPE); pentobarbitone sodium (Abbot Laboratories); phenobarbitone sodium (Sigma); acetylthiocholine; (-)-acetylcholine chloride (Sigma); pentylenetetrazole, picrotoxin, bicuculline (Sigma); morphine (Bodene); diazepam (Roche); aspirin (sny. acetylsalicylic acid), diclofenac (Sigma); acetic acid (Merk); (-)-noradrenaline hydrochloride, chlorpropamide (Sigma); atropine sulphate, reserpine, 5,5-dithiobis-2-nitrobenzoic acid, histamine dihydrochloride, physostigmine sulphate, tyramine hydrochloride, nicotine hydrogen tartrate, stilboesterol, potassium chloride (British Drug Houses); phentolamine mesylate (Ciba); 5-hydroxytryptamine (Koch-Light); heparin (Evan Medical); hexamethonium bromide, mepyramine maleate, (May and Baker); oxytocin (Park-Davis); indomethacin (Merck, Sharp and Dohme); petroleum ether (Saarchem) and streptozotocin (Fluka).

Except where otherwise indicated, HPE and all drugs used were dissolved in distilled water. Distilled water was used as the 'control' vehicle (solution) in all experiments. Drug concentrations quoted in the *in vitro* studies refer to the final organ-bath concentrations.

In the *in vivo* experiments, pilot (preliminary) studies were carried out to establish the final doses of HPE and other drugs used, and also to determine pretreatment times. Solutions of HPE and other drugs used were prepared on each day of the experiment.

APENDIX IV

Acronyms/Abbreviations of Standard Terms and Units

Kg	kilogram
g	gram
mg	milligram
µg	microgram
ηg	nanogram
M	molar
l	litre
ml	milliliter
hr	hour
min	minute
s	second
msec	millisec
Hz	Herts
^{o}C	degrees Celcius