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**PHARMACOLOGICAL EFFECTS OF *HARPAGOPHYTUM PROCUMBENS* DC
[PEDALIACEAE] SECONDARY ROOT AQUEOUS EXTRACT ON ISOLATED
GASTRO-INTESTINAL TRACT MUSCLES OF THE CHICK, GUINEA-PIG
AND RABBIT**

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Abstract

In an attempt to scientifically appraise the ‘*healing powers*’ and medicinal value of *Harpagophytum procumbens* DC [family: Pedaliaceae], and throw some light on the efficacy and safety of the medicinal plant product, we have examined the pharmacological effects of the plant’s secondary root aqueous extract (HPE) on the chick, guinea-pig and rabbit isolated gastro-intestinal smooth muscle preparations. The results of this laboratory animal study indicate that relatively low to high doses of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 10–1000 µg/ml) provoked concentration-related, significant ($P < 0.05$ – 0.001), atropine-sensitive contractions of the chick isolated oesophagus and guinea-pig isolated ileum. Relatively moderate to high concentrations of the plant’s extract (HPE, 200–1000 µg/ml) usually induced concentration-dependent, biphasic responses in the rabbit isolated duodenum. Relatively low concentrations of the plant’s extract (HPE, 10–100 µg/ml) usually produced 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

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concentration-related, significant ($P < 0.05$ – 0.001) elevated baseline tones (tensions) and augmentations of the contractile amplitudes; followed by secondary, longer-lasting relaxations and reductions of the contractile amplitudes of the spontaneous, rhythmic, myogenic, pendular contractions of the rabbit isolated duodenal muscle preparations. 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 $\mu\text{g/ml}$) 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 smooth muscle preparations used. The results of this laboratory animal study indicate that *H. procumbens* secondary root aqueous extract (HPE) induced concentration-related contractions of gastro-intestinal tract smooth muscles. These findings may account for the commonly reported adverse effect of 'gastro-intestinal discomfort or upset' usually associated with *Harpagophytum procumbens* secondary root extract medication.

Key Words: *Harpagophytum procumbens*, Secondary Root, Aqueous Extract, Gastro-intestinal Tract Muscles.

Introduction

Harpagophytum procumbens DC [family: Pedaliaceae] is a medicinal plant with a remarkable therapeutic reputation in southern African folklore medicine. *Harpagophytum procumbens*, locally known as "Devil's claw, Grapple plant, Wood spider, or Harpago", is widely used in South African traditional medicine for the treatment, management and/or control of a variety of human ailments.

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). 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). *Harpagophytum procumbens* is virtually restricted to the southern part of Africa, occurring mainly in South Africa, Namibia, Botswana and Zimbabwe. The plant is commonly referred to as 'Devil's claw', 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). 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 root is used for a variety of human health conditions. It has an ethnomedical reputation for efficacy in anorexia, indigestion, diabetes mellitus, hypertension, gout, fevers, skin cancer,

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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). Serum cholesterol and uric acid levels are also reduced by *H. procumbens* preparations (Van Wyk and Gericke, 2000).

Reports on vascular and extra-vascular smooth muscle pharmacology of *Harpagophytum procumbens* DC secondary root extracts are 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 used is due to a complex interaction between the various active principles of the plant, and suggested that the plant's active constituents, especially harpagoside, interfere with the mechanisms that regulate the influx of calcium in 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. It was, therefore, thought worthwhile investigating the effects of *Harpagophytum procumbens* DC secondary root aqueous extract on chick isolated oesophagus, guinea-pig isolated ileum and rabbit isolated duodenum because 'gastro-intestinal discomfort or upset' is one of the few commonly reported adverse effects associated with *Harpagophytum procumbens* secondary root extract medication.

Materials and Methods

The experimental protocol used in this study was approved by the Ethics Committee of the University of KwaZulu-Natal, Durban 4000, South Africa; and conforms with the "Guide to the care and use of animals in research and teaching" [published by the University of Durban-Westville, Durban 4000, South Africa].

Plant Material

Fresh pieces of *Harpagophytum procumbens* DC secondary roots were purchased from Upington 'Muti' Market in the Northern Cape Province of South Africa (between

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November, 2002 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 secondary 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 $30\pm 1^\circ\text{C}$. 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.

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; and young chicks (aged between 3 and 10 days after hatching) weighing 50–100 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 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, 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 $32\pm 1^\circ\text{C}$ 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

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oesophageal 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) or electrically stimulated. Doses of HPE (and other drugs used) were added to the bath-fluid either cumulatively or non-cumulatively (sequentially), 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 preparations were recorded isometrically by means of Ugo Basile force-displacement transducers, 2-Channel “Gemini” Recorder, and pen-writing microdynamometers (model 7070).

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 applying a sharp blow to the back of its head 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 $36\pm 1^{\circ}\text{C}$ 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 equilibrate 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-writing microdynamometers (model 7070).

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 applying a sharp blow to the back of its head and bled out. The abdomen of each animal was quickly opened by a midline incision, and 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 $36\pm 1^{\circ}\text{C}$, 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 ‘test’ 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.

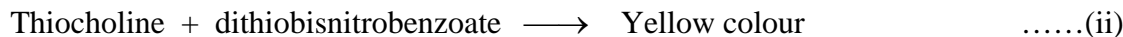
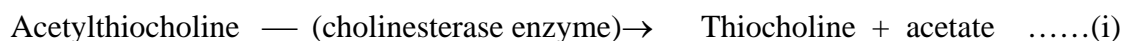
Determination of HPE’s Anticholinesterase Activity

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.

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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 m μ , 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:



Drugs used

The following compounds and/or drugs were used: *Harpagophytum procumbens* dried secondary root aqueous extract (HPE); acetylcholine chloride; physostigmine sulphate; atropine sulphate; mepyramine maleate; histamine dihydrochloride; (–)-noradrenaline hydrochloride; phentolamine mesylate; acetylthiocholine; 5,5-dithiobis-2-nitrobenzoic acid; and petroleum ether. HPE and all other drugs used in this study were dissolved in distilled water each day at the beginning of our experiments. Drug concentrations quoted in the text refer to the salts (except HPE), and denote final organ-bath concentrations.

Data Analysis

Data obtained from ‘test’ groups of isolated muscle strips (chick oesophagus, guinea-pig ileum and rabbit duodenum) 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 (\pm 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 Cochran, 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

Relatively low to high concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–1000 $\mu\text{g/ml}$) 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 moderate to high doses of the plant's extract (HPE, 50–1000 $\mu\text{g/ml}$) always produced dose-dependent, significant ($P < 0.05$ – 0.001), atropine-sensitive contractions of the chick oesophageal muscle preparations. Figure 1 shows a typical trace, while Figure 2 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 $\mu\text{g/ml}$) were comparable with those produced by acetylcholine (ACh, 0.1–3.0 $\mu\text{g/ml}$) and physostigmine

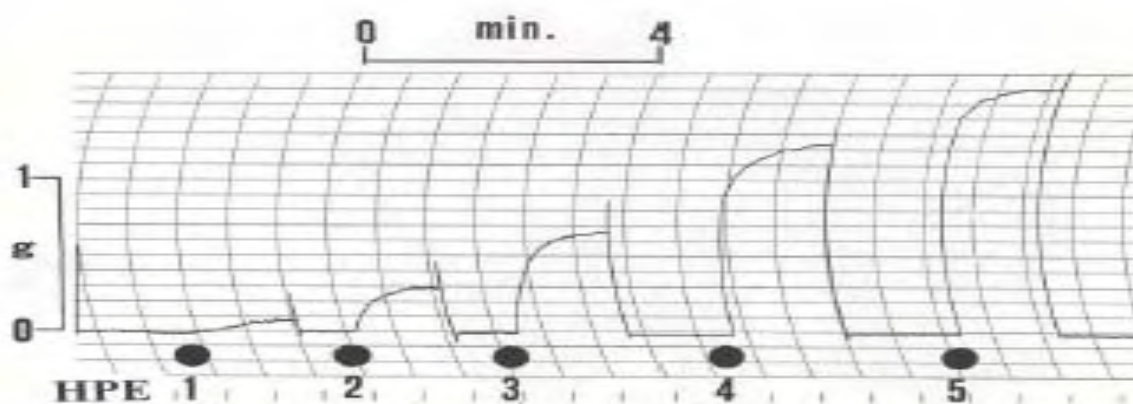


Figure 1. Effects of graded concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE) on chick isolated oesophagus. HPE 1, 2, 3, 4 and 5 represent 50, 100, 200, 400 and 800 $\mu\text{g/ml}$ of *Harpagophytum procumbens* secondary root aqueous extract sequentially added to the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

(1.0–10.0 $\mu\text{g/ml}$) – (data not shown). Relatively low to high concentrations of the plant's extract (HPE, 10–1000 $\mu\text{g/ml}$) also potentiated and/or enhanced acetylcholine- (ACh, 0.1–1.0 $\mu\text{g/ml}$), physostigmine- (PHY, 0.5–5.0 $\mu\text{g/ml}$) and electrical stimulation-induced contractions of the chick isolated oesophagus (Figure 3a & b). 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 $\mu\text{g/ml}$) – see Figure 4.

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In the guinea-pig ileal muscle preparations, the pharmacological effects of relatively low to high concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–1000 $\mu\text{g/ml}$) 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.

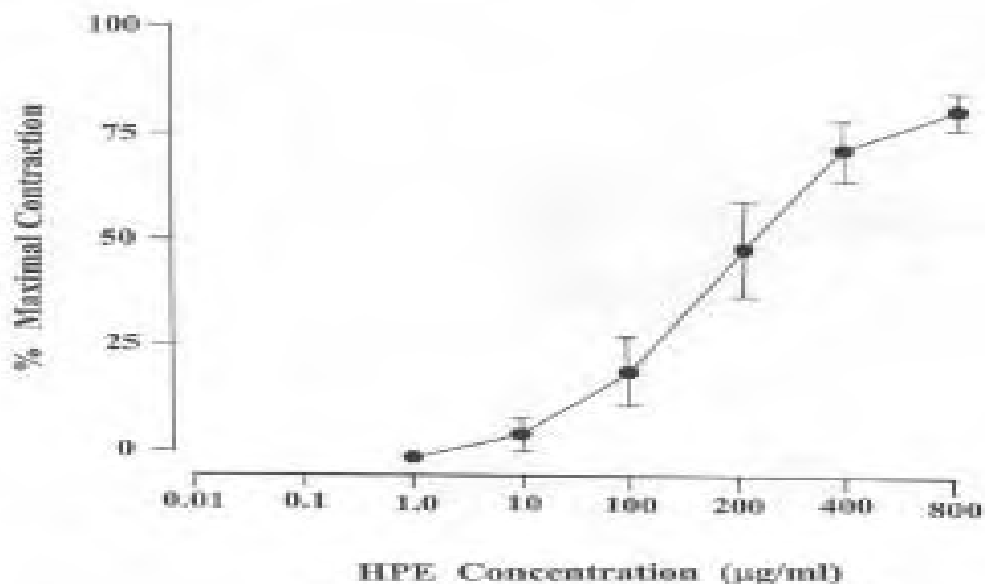


Figure 2. Dose-response curve of graded concentrations of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 10–800 $\mu\text{g/ml}$) on chick isolated oesophagus. Each point represents the mean ($\pm\text{SEM}$) of 6–8 observations, while the vertical bars denote standard errors of the mean.

Relatively low to high concentrations of *Harpagophytum procumbens* root aqueous extract (HPE, 10–1000 $\mu\text{g/ml}$) usually induced concentration-related complex, biphasic responses in rabbit isolated duodenum. Relatively moderate to high concentrations of the plant's extract (HPE, 50–1000 $\mu\text{g/ml}$) 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 $\mu\text{g/ml}$) 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 5). The secondary, longer-lasting inhibitory effects of the plant's extract (HPE) were resistant to blockade by standard, receptor specific antagonists in all the isolated muscle preparations used (data not shown).

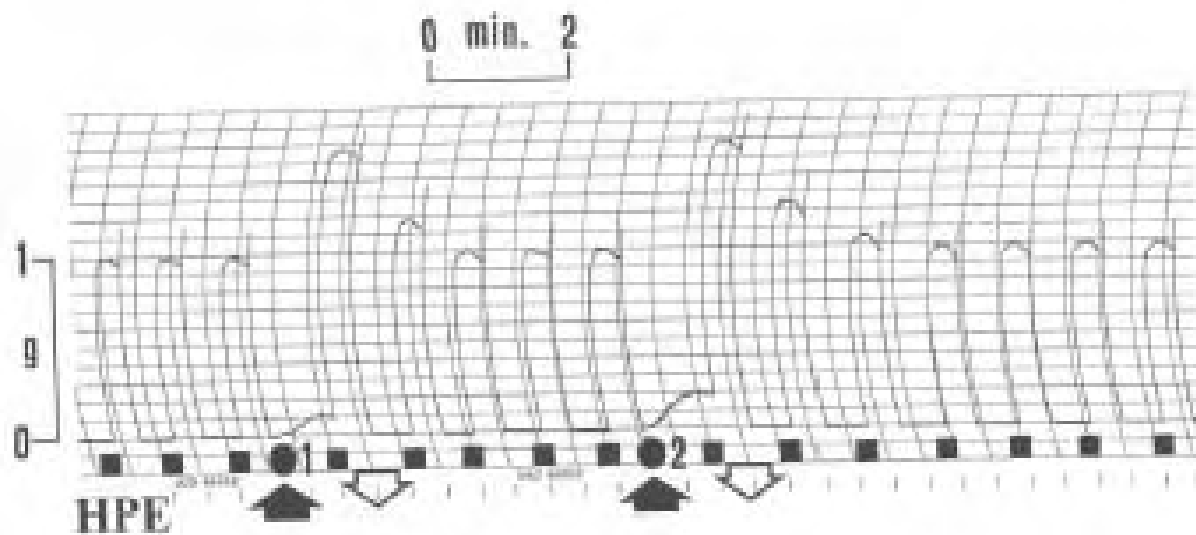


Figure 3a. Effects of *Harpagophytum procumbens* secondary root aqueous extract (HPE) on acetylcholine-(ACh-) induced contractions of the chick isolated oesophagus. Contractions of the muscle preparation were induced by acetylcholine (ACh, 0.5 µg/ml) sequentially added to the bath-fluid at the solid 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.

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 less than that of physostigmine (PHY, 0.01 – 10.0 µg/ml) – see Figure 6. In the colorimetric assay method used, the chick biventer-cervicis muscle homogenates gave better and more reproducible results than the chick oesophagus muscle homogenates.

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 physiological solution was reduced from 0.26 g/litre to 0.13

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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 $\mu\text{g/ml}$) were abolished or reduced in the presence of low calcium concentration [$\text{Ca}^{2+} = 0.13$ g/litre] in the bathing physiological solution. Similarly, the contractile responses of the isolated muscle strips induced by moderate to high concentrations of HPE (50–1000 $\mu\text{g/ml}$) 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 (10–40 $\mu\text{g/ml}$) 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 $\mu\text{g/ml}$) were increased as the Ca^{2+} concentration of the external bathing fluid was increased (data not shown). The secondary, longer-lasting inhibitory effects of moderate to high concentrations of the plant extract (HPE, 200–1000 $\mu\text{g/ml}$) on rabbit isolated duodenum increased with reduced calcium concentrations in the bathing fluid, and decreased when the external calcium concentration was increased.

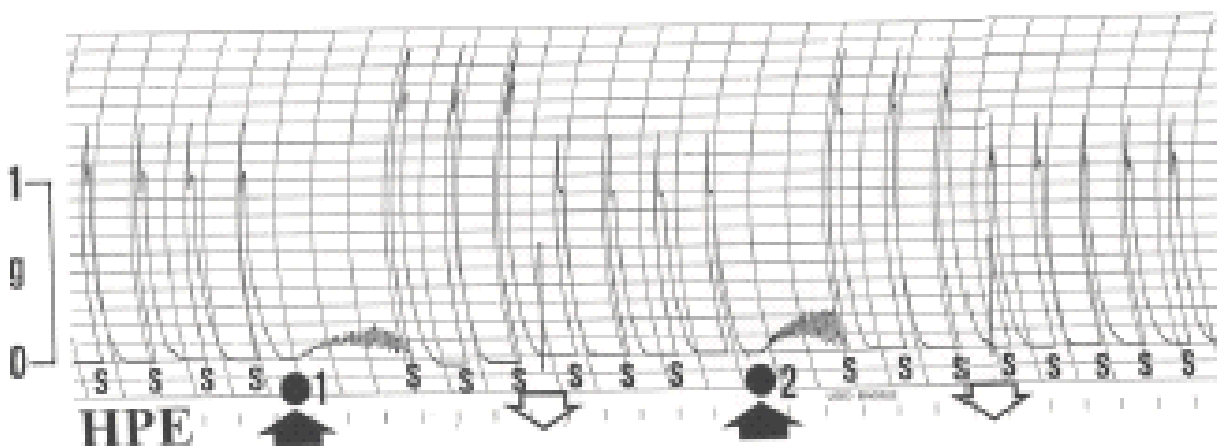


Figure 3b. Effects of *Harpagophytum procumbens* secondary root aqueous extract (HPE) on electrically-induced contractions of the chick isolated oesophagus. Contractions of the muscle preparation were induced by indirect, electrical stimulation instituted at the solid letter S (30 Hz, 70 volts). HPE 1 and 2 represent 50 and 100 $\mu\text{g/ml}$ 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.

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.

Discussion

Relatively low to high doses of *Harpagophytum procumbens* root aqueous extract (HPE) produced concentration-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. Bath-applied acetylcholine, physostigmine and indirect electrical stimulation of the chick muscle preparations also induced contractions of the chick oesophagus 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, we speculate 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 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 used (data not shown), we speculate that the secondary, longer-lasting inhibitory and/or depressant effects of HPE on the rabbit duodenal muscle preparations may be non-specific in nature. Since the plant's extract-induced contractions of the isolated muscle preparations were sensitive to atropine, it is not unlikely that the contractile effect of the plant's extract

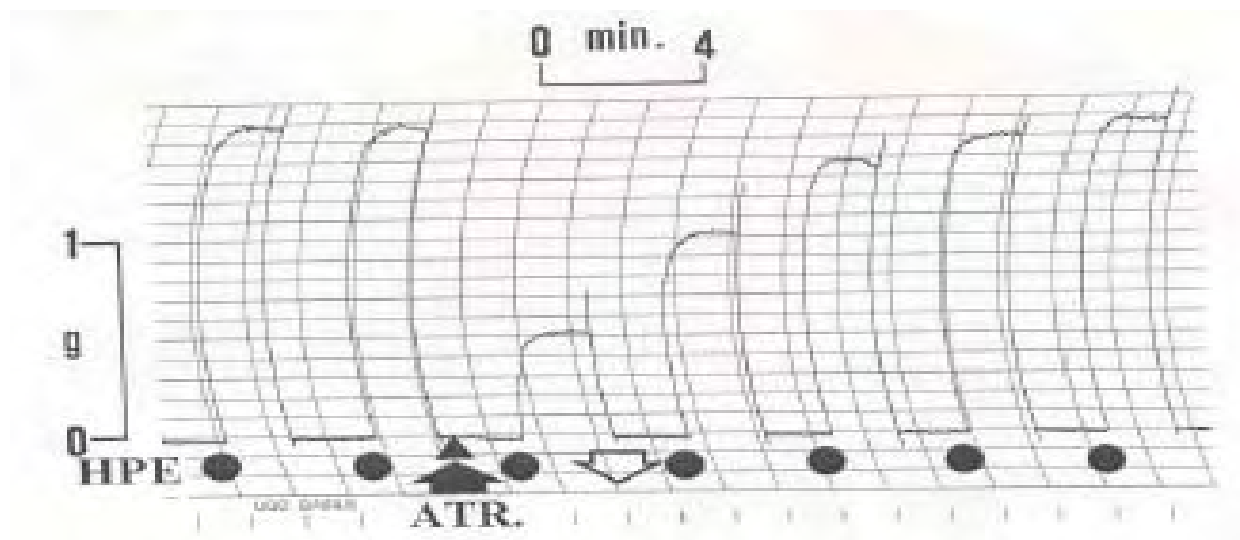


Figure 4. Inhibitory effect of atropine (ATR., 0.5 µg/ml) on *Harpagophytum procumbens* secondary root aqueous extract (HPE)-induced contractions of the chick isolated oesophagus. At each of the solid dots (●), HPE (800 µg/ml) was sequentially added to the bath fluid. Atropine (ATR., 0.5 µg/ml) was added to the bath-fluid at the solid triangle (▲) and solid upright-pointing, left-hand-side arrow, and washed out at the adjacent, open right-hand-side downward-pointing arrow.

(HPE) on guinea-pig isolated ileum may also involve cholinergic muscaric receptor stimulation. The finding that changes (decrease or increase) in the calcium ion concentrations of the bathing physiological solution altered 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, judging from the action of HPE on the isolated GIT smooth muscles used, and the proposed mechanism of action of the plant's extract, it would appear that HPE may be useful in the management or treatment of GIT disorders such as oesophageal reflux. The plant product may also be useful in treating and/or managing urinary bladder disorders such as common urinary retention and/or post-partum urinary retention. The proposed anti-acetylcholinesterase (Anti-AChE) action of HPE can be gainfully utilized as a miotic, and to treat or manage glaucoma. The plant's extract can also be utilized in the treatment of poisoning due to atropine, imipramine and tricyclic antidepressants, all of which possess strong anticholinergic activities. Furthermore, the plant product may be useful in the management of *myasthenia gravis*, and in tachy-arrhythmias. It would also appear that HPE may be useful in Alzheimer's disease caused by degeneration of central cholinergic neurons.

Harpagophytum procumbens secondary roots have been reported to be rich in sugars, phytosterols, triterpenoids, coumarins, flavonoids and iridoids (Watt and Breyer-Brandwijk

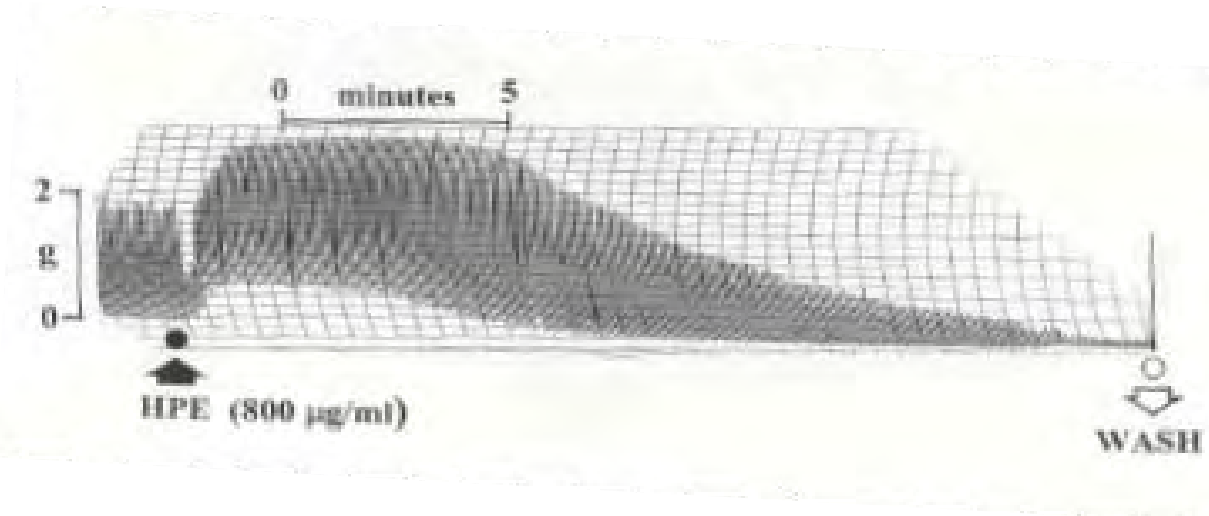


Figure 5. Effects of *Harpagophytum procumbens* secondary root aqueous extract (HPE, 800 µg/ml) on contractile amplitudes of the spontaneous, myogenic, pendular, rhythmic contractions of the rabbit isolated duodenum. *Harpagophytum procumbens* secondary root aqueous extract (HPE, 800 µg/ml) was sequentially 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.

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1962; Van Wyk and Gericke, 2000; Van Wyk *et al.*, 2002). Although only a few pharmacological studies on *Harpagophytum procumbens* secondary root extract have been reported in the 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 strips used in this study. 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 the pharmacological basis for the frequently reported adverse effect of 'gastro-intestinal tract discomfort or upset' commonly associated with *Harpagophytum procumbens* root extract medication.

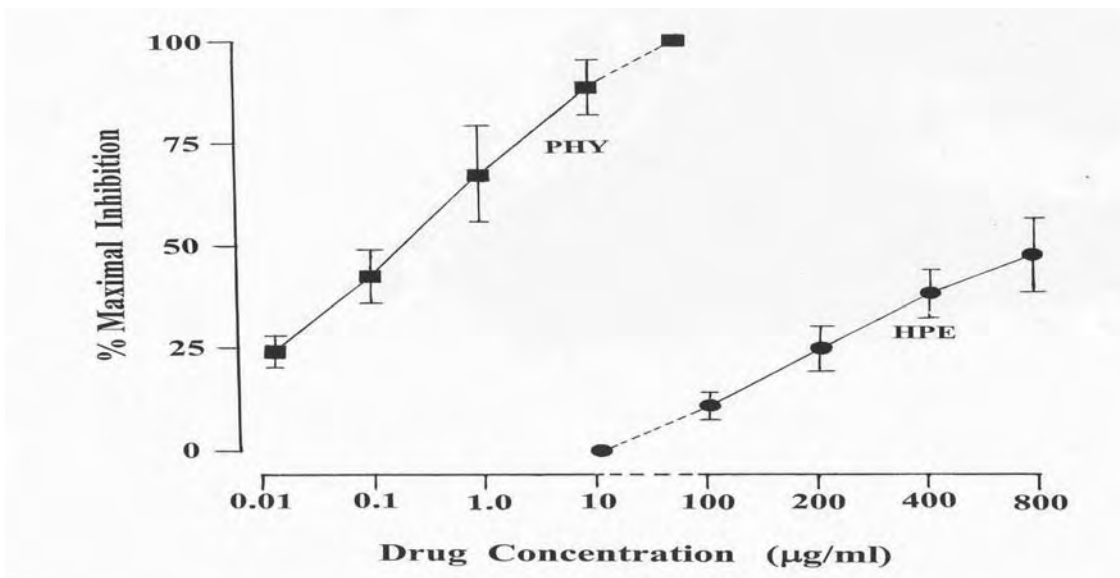


Figure 6. Anticholinesterase activity of *Harpagophytum procumbens* secondary root aqueous extract (HPE, ●—●) compared with physostigmine (PHY, ■—■) on chick isolated biventer-cervicis muscle homogenates. HPE inhibited the chick cholinesterase enzyme in a concentration-related manner. In this regard,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

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