Uterotonic effect of *Harpagophytum procumbens* DC (Pedaliaceae) secondary root aqueous extract on rat isolated uterine horns

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Received March 30, 2009; Accepted August 18, 2009

Abstract

Some traditional health practitioners of South Africa have claimed that *Harpagophytum procumbens* DC (family: Pedaliaceae) secondary root is a useful obstetric remedy for induction or acceleration of labour, as well as for expelling retained placentas in pregnant women. In the present study, therefore, we investigated the effect of *H. procumbens* secondary root aqueous extract (HPE) on longitudinal, tubular uterine horn muscle strips taken from non-pregnant and pregnant, young adult, female rats. HPE (10–800 $\mu$g/ml) induced concentration-related and significant ($P<0.05$) increases in the baseline tone, and caused powerful rhythmic, myogenic contractions of, oestrogen-dominated rat longitudinal uterine horn muscle strips taken from stilboestrol-pretreated, non-pregnant female rats. Relatively low to high concentrations of HPE (10–800 $\mu$g/ml) also provoked concentration-dependent and significant ($P<0.05$–0.001) increases in the baseline tone of, and contracted, longitudinal, tubular uterine horn muscle strips taken from female rats in the early, middle and late stages of pregnancy. Moderate to high concentrations of HPE (200–1,000 $\mu$g/ml) always provoked powerful contractions of isolated longitudinal, tubular uterine horn muscle preparations of non-pregnant and pregnant rats. The results of this *in vitro* study indicate that *H. procumbens* secondary root aqueous extract possesses spasmogenic, uterotonic action on mammalian uterine muscles. These findings lend pharmacological credence to the suggested folkloric obstetric uses of the plant’s secondary root for induction and/or acceleration of labour, as well as for expelling retained placentas in pregnant women.

Key words: *Harpagophytum procumbens*, secondary root, aqueous extract, uterotonic action

Introduction

*Harpagophytum procumbens* DC (family: Pedaliaceae) is a weedy, perennial plant with annual creeping stems spreading from a central thick, fleshy, tuberous tap root (Van Wyk *et al.*,
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). *H. 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 locally 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).

Dried secondary tap roots of *H. procumbens* are frequently used in South African traditional medicine in the form of infusions, decoctions, tinctures, powders and extracts, for a variety of human ailments. *H. procumbens* secondary root 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 (Watt and Breyer-Brandwijk, 1962; Van Wyk and Gericke, 2000; Van Wyk et al., 2002). *Devil’s claw* is also used *post-partum* as an analgesic, and to keep the uterus contracted (Watt and Breyer-Brandwijk, 1962; Van Wyk and Gericke, 2000; Van Wyk et al., 2002). The dry, powdered tuberous secondary 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 or 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* products (Van Wyk and Gericke, 2000).

Previous studies in our laboratories have indicated that *H. procumbens* secondary root aqueous extract stimulates and contracts the gastro-intestinal tract and vascular smooth muscles of certain experimental animals (Mohamed and Ojewole, 2004; Mahomed et al., 2005). Moreover, there are some anecdotal reports that *H. procumbens* secondary root extracts and/or preparations possess uterotonic actions in human subjects. In order to scientifically confirm or deny the possible oxytocin-like effect of *H. procumbens* secondary root extract on mammalian uterus, the present study was undertaken to investigate its plausible effect on longitudinal, tubular uterine muscle strips of non-pregnant and pregnant rats.

### Materials and Methods

**Ethical considerations**

Experimental protocols and procedures used in this study were approved by the Animal Ethics Committee of the University of Durban-Westville, Durban 4000, South Africa; and conform to 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 ‘Muthi’ Market in the Northern Cape Province of South Africa (between 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). A voucher specimen of the plant’s secondary root has been deposited in the University’s Botany Departmental Herbarium.

Preparation of *H. 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 milled into fine powder in a Waring commercial blender. The powdered root was macerated in distilled water and extracted twice, on each occasion with 2.5 l of distilled water at room temperature (26 ± 1°C) for 48 hours (with occasional shaking). The combined distilled water extractives were concentrated to dryness at 60 ± 1°C in a rotary evaporator. Freeze-drying and solvent elimination under reduced pressure finally gave 35.56 g (i.e., 3.56% yield) of a light-brown, powdery, crude aqueous root extract of *H. procumbens*. The crude aqueous extract thus obtained was used in our study without any further purification. Aliquot portions of the plant’s secondary root aqueous extract (HPE) residue were weighed and dissolved in distilled water for use on each day of our experiments.

Animals

Young adult, female Wistar rats (*Rattus norvegicus*) weighing 250–300 g (aged 4–6 months) were used. The animals were kept and maintained under conventional laboratory conditions of temperature, humidity, and light; and were allowed free access to food (standard pellet diet) and drinking tap water *ad libitum*. The animals were divided into two broad categories as follows:

Stilboesterol-treated, oestrogen-dominated, non-pregnant female rats

All the female rats in this group were pretreated with stilboesterol (0.1 mg/kg s.c.) for 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 they were in oestrus state. Oestrogen-dominated, female rats (in oestrus state) were used in this study.

Pregnant female 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.

Experimental procedure

Each of the pregnant and stilboesterol-treated, oestrogen-dominated, non-pregnant female rats was killed by applying a sharp blow to the back of its head and bled out. Following midline incision of the rat’s abdomen, the two uterine horns of the animal were exposed, cleaned free from
fatty and connective tissues, and trimmed. Longitudinal, tubular segments of approximately equal lengths (2–3 cm long) were removed from each uterine horn by cutting off both ends. The two isolated longitudinal, tubular uterine horn segments (2–3 cm long) were set-up under physiological conditions as described in detail earlier by Nyinawumuntu et al. (2008), and Chiwororo and Ojewole (2009). Each longitudinal, tubular uterine horn muscle strip was separately suspended in a 30-ml Ugo Basile Two-Chambered Organ Bath (model 4050) containing de Jalon’s physiological solution (DJS) of composition, in g/l: NaCl, 9.0; KCl, 0.42, CaCl₂·2H₂O, 0.06; MgCl₂, 0.005; NaHCO₃, 0.5; and glucose, 0.5; maintained at 32 ± 1 °C, and continuously aerated with carbogen (i.e., 5% CO₂ + 95% O₂ gas mixture). Two longitudinal, tubular uterine horn muscle preparations from the same animal (one used as ’control’ and the other one used as HPE- or reference drug-treated ‘test’ preparation) were always set-up to allow for changes in the uterine muscle sensitivity. Each longitudinal, tubular uterine horn muscle strip was subjected to an applied resting tension of 1.0 g, and allowed to equilibrate for 30–45 min (during which time the bathing de Jalon’s physiological solution was changed every 15 min) before it was challenged with HPE (or any of the standard drugs used). Concentrations of HPE (and other reference drugs used) were added to the bath-fluid either cumulatively or sequentially, and washed out 4–5 times after the maximum responses of the tissues were attained. Distilled water (i.e., the vehicle in which HPE and the standard drugs employed in this study were dissolved) was used as the ‘control’ fluid for HPE and reference drugs used. Concentrations of bath-applied HPE (and other drugs used) were repeated where appropriate and/or possible, at regular intervals of 5–20 min after the last washing. HPE- and other drug-induced responses of the longitudinal, tubular uterine horn muscle strips were recorded isometrically by means of Ugo Basile’s force-displacement transducers and Ugo Basile’s pen-writing, 2-Channel “Gemini” Recorders (model 7070).

Data analysis

Experimental data were pooled and presented as means (± SEM). Data obtained from distilled water-treated ‘control’ longitudinal, tubular uterine horn muscle strips were used as baseline values. In all cases, data obtained from the extract (HPE)- and reference drug-treated ‘test’ longitudinal, tubular uterine horn muscle strips were compared with those obtained from distilled water (vehicle)-treated ‘control’ uterine horn muscle strips. The differences between the data obtained from ‘test’ and vehicle-treated ‘control’ uterine horn muscle strips were subjected to one-way analysis of variance (ANOVA; 95% confidence interval), followed by Dunnett’s post-hoc test (Dunnett and Goldsmith, 1993). In all cases, statistical significance was established at values of \( P \leq 0.05 \).

Results

Stilboesterol-treated, oestrogen-dominated, non-pregnant female rat longitudinal, tubular uterine horn strips

Longitudinal, tubular uterine horn muscle strips taken from stilboesterol-pretreated, non-pregnant female rats were found to be quiescent and devoid of spontaneous, myogenic,
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rhythmic activity. However, relatively low to high concentrations of *H. procumbens* secondary root aqueous extract (HPE, 10–800 µg/ml) cumulatively-added to the bath-fluid, caused concentration-related and significant (*P*<0.05) increases in the baseline tone, and induced powerful rhythmic, myogenic contractions of the oestrogen-dominated rat longitudinal, tubular uterine horn muscle strips, like acetylcholine (ACh, 0.1–2.0 µg/ml) and oxytocin (OTC, 0.05–3.0 µU/ml). Figure 1 illustrates a typical trace obtained from an oestrogen-dominated, non-pregnant rat’s longitudinal, tubular uterine horn muscle preparation, while Fig. 2 summarizes the results obtained. Moderate to high concentrations of HPE (200–1,000 µg/ml) sequentially-added to the bath-fluid, always provoked powerful contractions of the longitudinal, tubular uterine horn muscle preparations (see Fig. 3). The spontaneous, rhythmic contractions of the quiescent, oestrogen-dominted rat longitudinal, tubular uterine horn muscle strips induced by relatively low to high concentrations of HPE (10–1,000 µg/ml) were inhibited or abolished in a concentration-dependent manner by bath-applied indomethacin (IDM, 0.1–5.0 µg/ml), atropine (ATR, 0.1–5.0 µg/ml) or nifedipine (NFD, 0.1–5.0 µg/ml). Relatively low to moderate concentrations of HPE (10–800 µg/ml) potentiated ACh- or OTC-induced contractions of the oestrogen-dominated rat longitudinal, tubular uterine horn muscle strips (see Fig. 4).

**Pregnant female rat uterine horn strips**

Longitudinal, tubular uterine horn muscle strips taken from pregnant female rats were found to be spontaneously active, producing rhythmic contractions on their own accord. The effects of HPE on longitudinal, tubular uterine horn muscle strips taken from pregnant female rats were found to be qualitatively and quantitatively similar to those produced by the plant’s
extract on longitudinal, tubular uterine horn muscle strips taken from stilboesterol-pretreated, oestrogen-dominated, non-pregnant female rats. Relatively low to high concentrations of HPE (10–1,000 µg/ml) provoked concentration-related and significant ($P<0.05$) increases in the baseline tone (basal tension), and contracted longitudinal, tubular uterine horn muscle strips taken from rats in early, middle or late stages of pregnancy (Fig. 5).

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Fig. 2. Concentration-response curve to sequentially-administered *Harpagophytum procumbens* secondary root aqueous extract (HPE, 1.0–800 µg/ml) on stilboesterol-treated, oestrogen-dominated, non-pregnant female rats’ longitudinal, tubular uterine horn muscle strips. Each point (●) represents the mean (± SEM) of 6–8 observations, while the vertical bars denote standard errors of the means.

Fig. 3. Effect of *H. procumbens* secondary root aqueous extract (HPE) on a longitudinal, tubular uterine horn muscle strip taken from a stilboesterol-treated, oestrogen-dominated female rat. HPE (400 µg/ml) was added to the bath-fluid at the left-hand-side upward-pointing, solid arrow, and washed out intermittently (by ‘overflow method’) 4–5 times, at the adjacent, right-hand-side downward-pointing, open arrow.
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Fig. 4. Effects of additions of separate and combined concentrations of *H. procumbens* secondary root aqueous extract (HPE) and oxytocin (OTC) on a longitudinal, tubular uterine horn muscle strip taken from a stilboesterol-treated, oestrogen-dominated female rat. Panels A, B and C represent additions of separate and combined concentrations of HPE (100 µg/ml), OTC (0.1 µg/ml) and combined HPE (100 µg/ml) plus OTC (0.1 µg/ml), respectively. In panels A, B and C, HPE (100 µg/ml), OTC (0.1 µg/ml) and combined HPE (100 µg/ml) plus OTC (0.1 µg/ml) were added to the bath-fluid at the left-hand-side, upward-pointing solid triangles (▲), and washed out intermittently (by ‘overflow method’) 4–5 times, at the adjacent, right-hand-side, downward-pointing open triangles (▼), respectively.

Fig. 5. Effect of *H. procumbens* secondary root aqueous extract (HPE) on a longitudinal, tubular uterine horn muscle strip taken from a female rat in its middle pregnancy. HPE (200 µg/ml) was added to the bath-fluid at the left-hand-side upward-pointing, solid arrow; and washed out intermittently (by ‘overflow method’) 4–5 times, at the adjacent, right-hand-side downward-pointing, open arrow.

effects of HPE on longitudinal, tubular uterine horn muscle strips taken from pregnant female rats were found to be qualitatively and quantitatively similar to those produced by the plant’s extract on longitudinal, tubular uterine horn muscle strips taken from stilboesterol-pretreated, oestrogen-dominated, non-pregnant female rats. Relatively low to high concentrations of HPE (10–1,000 µg/ml) provoked concentration-related and significant (P<0.05–0.001) increases in the
baseline tone (basal tension), and contracted longitudinal, tubular uterine horn muscle strips taken from rats in early, middle or late stages of pregnancy (Fig. 5).

Discussion

The results of the present longitudinal, tubular uterine horn muscle strips study are similar to those obtained on the chick parasympathetically-innervated oesophageal muscle preparations (Mahomed, 2004), and on some mammalian isolated gastro-intestinal tract smooth muscles (Mahomed et al., 2005). Relatively low to high concentrations of *H. procumbens* secondary root aqueous extract (HPE, 10–100 µg/ml) always induced concentration-related and significant, profound rhythmic, myogenic contractions of longitudinal, tubular uterine horn muscle strips taken from stilboesterol-pretreated, oestrogen-dominated, non-pregnant female rats. HPE produced similar contractile effects on longitudinal, tubular uterine horn muscle strips taken from pregnant rats. The precise mechanism of the contractile action of the plant’s extract on rat longitudinal, tubular uterine horn muscle strips is not fully understood at the moment. However, the ability of indomethacin, atropine or nifedipine to reduce or abolish HPE-induced contractions of the uterine muscle preparations would appear to suggest possible release of prostaglandins and/or other uterotonic substances or mediators, including acetylcholine, kinins, etc, by the plant’s extract. Furthermore, the ability of nifedipine, a calcium (Ca++)-channel blocker, to reduce or abolish HPE-induced contractions of the uterine muscle strips would appear to suggest that the contractile action of HPE on rat isolated longitudinal, tubular smooth muscles could be mediated through opening of the voltage-dependent L-type calcium-channels by the extract, thus facilitating and allowing influx of extracellular Ca++ into the muscle cells (thereby causing uterine muscle contraction). The observation that HPE possesses anticholinesterase activity (Mahomed, 2004) would also appear to buttress the possible involvement of cholinergic acetylcholine muscarinic (mACh) receptor stimulation in the uterotonic action of HPE.

*H. procumbens* secondary root has 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* secondary root that is/are responsible for the observed uterine contractile effect of the plant’s aqueous extract still remains speculative, the iridoid constituents of the plant, namely, harpagoside (a cinnamic acid ester), harpagide and procumbide, are speculated to account, at least to a large extent, for the uterotonic action of HPE. Taken together, experimental evidence obtained in the present *in vitro* study indicates that aqueous secondary root extract of *H. procumbens* possesses contractile, uterotonic action on mammalian uterus. This finding suggests that the use of *H. procumbens* secondary root should be contra-indicated in pregnancy. However, the findings of the present laboratory animal study lend pharmacological credence to the suggested folkloric, obstetric uses of the plant’s secondary root extract for induction or acceleration of labour, as well as for expelling retained placentas in pregnant women in some rural communities of southern Africa.
Acknowledgements

The authors are grateful to Mrs. Nirasha Nundkumar for her assistance in the extraction of *Harpagophytum procumbens* secondary roots, and to Miss Kogi Moodley for her technical assistance. Financial support from South African National Research Foundation (NRF) to one of us (IM Mahomed) is thankfully acknowledged.

References


