Bronchorelaxant property of ‘African potato’ (*Hypoxis hemerocallidea* corm) aqueous extract *in vitro*

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**Abstract**

This study was undertaken to investigate the bronchorelaxant effect of *Hypoxis hemerocallidea* corm (‘African potato’) aqueous extract (APE) on spasmogen-provoked contractions of guinea-pig isolated tracheal smooth muscle preparations. APE (25–400 mg/ml) relaxed spasmogen (histamine-, carbachol- and potassium-) induced contractions of the isolated tracheal muscle preparations in a concentration-dependent manner. The relaxant effects of APE on spasmogen-evoked contractions of the tracheal muscle preparations were not altered by bath-applied propranolol (0.1–5.0 µg/ml), which markedly inhibited or completely abolished the relaxant effects of isoprenaline (0.1–5.0 µg/ml). Although the precise mechanism of the bronchorelaxant effect of APE could not be established in the present study, it is unlikely that the herb’s aqueous extract stimulates the β2-adrenoceptors present on the bronchial smooth muscles to produce its bronchodilatation. The finding that APE significantly relaxed (*P*<0.05) histamine-, carbachol- and high potassium ion concentration (K+, 80 mM)-induced contractions of guinea-pig isolated bronchial muscle preparations appears to suggest that the bronchospasmolytic effect of the plant’s extract is probably not mediated through a specific receptor, but rather, probably mediated via a non-specific bronchospasmolytic mechanism.

Key words: African potato (*Hypoxis hemerocallidea* corm), aqueous extract, guinea-pig, tracheal smooth muscle, bronchorelaxant activity

**Introduction**

Available biomedical evidence suggests that approximately 70% of South Africans rely on traditional health practitioners and medicinal plants for their daily healthcare needs. Unfortunately, however, very little data exist in the literature on the quality, safety and efficacy
of the various plant medicines used in South African traditional medicines (Johnson et al., 2007). Recently, we have investigated some of the commonly-used South African medicinal plants for their chemical constituents and pharmacological properties (Drewes et al., 1984; Ojewole, 2001; 2002; 2003; 2004; 2005; 2006; 2008; Musabayane et al., 2005a; 2005b), in an effort to establish a scientific basis for their folkloric, ethnomedical uses. One such frequently used medicinal plant in South Africa is Hypoxis hemerocallidea (Fisch. & C.A. Mey.; family, Hypoxidaceae). This ‘cure-all’ medicinal plant of southern Africa is a tuberous, perennial herb with long, strap-shaped leaves and yellow, star-shaped flowers. The broad and slightly hairy leaves of H. hemerocallidea are arranged one above the other to form three distinct groups of leaves spreading outwards from the centre of the plant; while the bright yellow, star-shaped flowers are borne on long, slender stalks (Van Wyk et al., 2002). The tuberous rootstock (i.e., the ‘corm’) of the herb, popularly known locally as ‘African potato’ in southern Africa, is widely used in South African traditional medicine as a remedy for an array of human ailments. The traditional healers of South Africa have employed the corm of the plant as a “muthi” (isiZulu word for “medicine”) for centuries, and now, ‘African potato’ has been claimed to be a miracle plant medicine in the fight against various modern and 21st century diseases of mankind (Owira and Ojewole, 2009). This South African wonder plant medicine has been claimed to be an effective remedy against HIV/AIDS-related diseases, arthritis, yuppie flu, hypertension, diabetes mellitus, cancer, psoriasis, gastric and duodenal ulcers, tuberculosis, urinary tract infections, asthma, and some central nervous system (CNS) disorders, especially epilepsy and childhood convulsions (Watt and Breyer-Brandwijk, 1962; Hutchings, 1989; Pujol, 1990; Hutchings et al., 1996; Albrecht, 1995; 1996; Van Wyk et al., 2002).

Previous studies in our laboratories and elsewhere have indicated that Hypoxis hemerocallidea corm possesses anti-inflammatory, hypoglycaemic, analgesic, anticonvulsant, antibacterial, antioxidant and other pharmacological properties (Ojewole, 2002; 2006; 2008; Mahomed and Ojewole, 2003; Steenkamp et al., 2006; Musabayane et al., 2005b). The various ethnomedical and pharmacological effects of the herb’s corm have been attributed to the phytosterols and sterolins present in the corm. The three best known and established constituents of the plant’s corm are: (i) hypoxoside, (ii) rooperol and (iii) β-sitosterol. In addition to these three well-established chemical constituents, the plant’s corm is also known to contain stigmastanol and other sterolins.

The present study was prompted by the claim of some traditional health practitioners in KwaZulu-Natal Province of South Africa, that decoctions and infusions of ‘African potato’ are effective remedies for the management, control and/or treatment of bronchial asthma and other respiratory disorders. Consequently, the present study was undertaken to evaluate the bronchospasmolytic effect of ‘African potato’ aqueous extract (APE) on carbachol-, histamine- and potassium-induced contractions of guinea-pig isolated tracheal smooth muscles in vitro.

Materials and Methods

Ethical considerations

Experimental protocols and procedures used in this study were approved by the Animal
Ethics Committee of the University of KwaZulu-Natal, Durban 4000, South Africa; and conform to the “Guide to the Care and Use of Animals in Research and Teaching”, published by the Animal Ethics Committee of the University of KwaZulu-Natal, Durban 4000, South Africa.

**Plant material**

Fresh corms of *Hypoxis hemerocallidea* were purchased from a fruit kiosk along West Street in Durban, KwaZulu-Natal Province of South Africa, between February and July, 2007. The corms were identified by Prof. H. Baijnath (the former Chief Taxonomist/Curator of the University of Durban-Westville’s Department of Botany) as the corms of *Hypoxis hemerocallidea* Fisch. & C. A. Mey. (family, Hypoxidaceae). A voucher specimen of the plant has been deposited in the Botany Departmental Herbarium of the University.

**Preparation of Hypoxis hemerocallidea corm aqueous extract**

One kilogramme (1 kg) of *H. hemerocallidea* fresh corms were washed with distilled water, cleaned, cut into smaller pieces and milled in a Waring commercial blender. The milled corm 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 solubles obtained were concentrated to dryness under reduced pressure in a rotary evaporator at 60 ± 1°C. The resulting crude aqueous extract was freeze-dried, finally giving 78 g (i.e., 7.8% yield) of a dark-brown, powdery aqueous extract (APE). Without any further purification, aliquot portions of APE thus obtained were weighed and dissolved in distilled water (at room temperature) for use on each day of our experiments.

**Animals**

Healthy, male and female Dunkin-Hartley guinea-pigs (*Cavia porcellus*) weighing 300–350 g 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*. All the animals were fasted for 16 hours, but still allowed free access to drinking tap water, before the commencement of our experiments.

**Guinea-pig isolated tracheal chain smooth muscle preparations**

The method used for these preparations was adopted from those described earlier by Foster (1960) and Ojewole (1977). The guinea-pigs used were starved overnight (but given free access to drinking tap water) and subsequently euthanized with intraperitoneal injection of sodium pentobarbital (70 mg/kg). The chest of each animal was opened, and the entire trachea of the animal was quickly removed and transferred to a Petri-dish containing warm Krebs-Henseleit (K-H) physiological solution (composition in mM: NaCl, 118.4; KCl, 4.7; NaH₂PO₄, 1.2; NaHCO₃, 25.0; MgSO₄·7H₂O, 1.2; CaCl₂·2H₂O, 2.5; glucose, 11.1, and the pH of the solution adjusted to 7.4), and continuously bubbled with carbogen (*i.e.*, 5% CO₂ + 95% O₂ gas mixture). After removal of the excess connective tissues and fat, the trachea was cut into 6–8 approximately equal small rings of about 2 mm in length. Each ring was subsequently cut open through the cartilage, and 3–4 of such open rings were tied together to form a chain. Tracheal chain muscle preparations
thus obtained were separately suspended in 30-ml Ugo Basile Two-Chambered Organ Baths (model 4050) containing Krebs-Henseleit solution continuously aerated with carbogen gas and maintained at 35 ± 1°C. Each muscle preparation was subjected to an applied resting tension of 1 g, and allowed to equilibrate for 45–60 min, during which time the bathing physiological solution was changed every 15 min, before it was challenged with graded concentrations of histamine, carbachol or potassium. In order to make allowance for changes in muscle sensitivity, two muscle preparations from the same animal, one used as drug-treated (test), and the other one used as distilled water-treated (control) preparation, were always set-up at a time. Sub-maximal contractions (i.e., 70–80% of the maximal contractions) of the drug-treated test preparations were elicited by sequential exogenous additions of either histamine (1.2 µg/ml), carbachol (1.5 µg/ml), or potassium (K+, 80 mM) to the bath-fluid. The sub-maximal muscle tensions developed by the spasmogens used were similar, and approximately equal to 1.5 g. APE- and other drug-induced decreases in the spasmogen-provoked muscle tensions were considered as relaxant effects of the compounds. The relaxant effects of APE on the sub-maximal muscle tensions developed by each of the spasmogens used were investigated by cumulative additions of stepwise, graded concentrations of APE (25–400 mg/ml) to the bath-fluid when the peak contractile effects of the spasmogens had been obtained. In all cases, after the maximal relaxation to each of the graded concentrations of APE (or other relaxant drugs used) had been achieved, the muscle preparation was washed out 4–5 times with fresh K-H physiological solution, and then left to recover (for 20–30 min) and return to pre-spasmogen treatment baseline level before it was contracted again with any of the standard spasmogens. The control muscle preparations were always treated with distilled water (0.1–0.5 ml) only. In these in vitro experiments, isoprenaline (0.1–5.0 µg/ml) and aminophylline (0.1–5.0 µg/ml) were used as standard tracheal muscle relaxants for comparison.

Changes in tension developed by the muscle preparations (contractions or relaxations) were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing, Gemini recorders (model 7070).

Drugs
The following drugs were used: carbachol chloride, histamine dihydrochloride, (±)-isoprenaline hydrochloride, aminophylline hydrate, (±)-propranolol hydrochloride, atropine sulphate and mepyramine maleate (Sigma Chemical, Co.). All drugs were dissolved or diluted in distilled water on each day of our experiments. Drug concentrations quoted in the text refer to final organ-bath concentrations.

Data analysis
Data obtained were expressed as means (± SEM) of at least six experiments (n≥6). Values obtained with distilled water-treated control preparations were used as baseline values for comparison. In each set of experiments, the differences between the control and test values were statistically determined by one-way analysis of variance (ANOVA, 95% confidence interval), followed by Dunnett’s t-test (Montgomery, 1991). P values less than 0.05 were considered to be statistically significant.
Bronchorelaxant effect of 'African potato'

Results

The sub-maximal muscle tensions developed by each of the spasmogens used were similar, and approximately equal to 1.5 g. APE (25–400 mg/ml), like the standard tracheal smooth muscle relaxants used, significantly (P<0.05) relaxed histamine (1.2 µg/ml)-, carbachol (1.5 µg/ml)-, or potassium (K+, 80 mM)-induced contractions of the guinea-pig isolated tracheal muscle preparations in a concentration-dependent manner. Figure 1 shows a typical trace obtained with stepwise, graded concentrations of APE on histamine-induced contraction of a guinea-pig isolated tracheal chain muscle preparation. Isoprenaline (0.1–5.0 µg/ml), mepyramine (0.1–5.0 µg/ml), atropine (0.1–5.0 µg/ml) and aminophylline (0.1–5.0 µg/ml) also provoked concentration-related relaxations of the isolated tracheal muscle preparations. The concentrations of isoprenaline, mepyramine, atropine, aminophylline and APE that produced 50% relaxations of the spasmogen-evoked contractions (IC<sub>50</sub>) are presented in Table 1. The maximal relaxant effects of the highest concentration of APE (400 mg/ml) used in this study were found, respectively, to be approximately 86% on histamine-induced contractions, 71% on carbachol-provoked contractions, and 63% on potassium-evoked contractions of the tracheal muscle preparations.

The tracheal chain muscle relaxant effect of APE (25–400 mg/ml) on histamine-, carbachol- and potassium-induced tracheal muscle contractions were not altered (P>0.05) by prior administration to the bath-fluid of the β-adrenoceptor antagonist, propranolol (3.0 µg/ml), a concentration that markedly inhibited or completely abolished the bronchorelaxant effect of

Fig. 1. Relaxant effects of stepwise, graded concentrations of APE on histamine-induced contraction of a guinea-pig isolated tracheal muscle preparation. Histamine (1.0 µg/ml) was added to the bath-fluid at the left-hand-side, upward-pointing, solid arrow and dot (HIST. ●). 1–5 right-hand-side, downward-pointing, solid arrows represent APE (25, 50, 100, 200 and 400 mg/ml), respectively, cumulatively added to the bath-fluid. Both histamine and APE were washed out (4–5 times) at the right-hand-side, downward-pointing, open arrow and dot (○).
isoprenaline (0.1–5.0 µg/ml) on histamine- or carbachol-evoked contractions of the tracheal muscle preparations. Concurrent administration to the bath-fluid of each of the tracheal muscle relaxants employed in this study with APE (25–400 mg/ml) also significantly reduced the IC₅₀ concentrations of the standard tracheal muscle relaxant drugs used.

### Discussion

Extracts, decoctions and infusions of *Hypoxis hemerocallidea* corm (‘African potato’) have been shown to be relatively safe in experimental animals (Ojewole, 2006), and in human subjects (Johnson et al., 2007). The results of the present study indicate that APE, like isoprenaline (a β₂-adrenoceptor stimulant), mepyramine (a classical histamine H₁-receptor antagonist), atropine (a cholinergic, muscarinic-receptor antagonist), and aminophylline (a phosphodiesterase inhibitor), relaxed spasmogen-evoked contractions of the guinea-pig isolated tracheal muscle preparations in a concentration-related manner, suggesting that APE possesses bronchodilator property. To the best of our knowledge, this is the first scientific report on the bronchorelaxant effect of APE in biomedical literature.

Although the exact mechanism of the bronchodilatory action of APE is still obscure, it is unlikely that it stimulates the β₂-adrenoceptors present on bronchial smooth muscles to produce its bronchodilatation. This hypothesis is strengthened by the observations that (i) mepyramine, atropine and aminophylline produced pharmacological effects that are similar to that of APE on the tracheal muscle preparations used, and (ii) concentrations of propranolol which markedly inhibited or completely abolished the bronchospasmolytic effect of isoprenaline did not affect the bronchospasmolytic action of APE. The finding that APE also inhibited the spasmogenic action of solution containing high concentration of potassium ions (high-K solution; K⁺ = 80

### Table 1

| Spasmogens (Agonists) | Inhibitory Agents (Antagonists) | Mean IC₅₀ of the Inhibitory Agents (Antagonists) | Numbers of Observations |
|-----------------------|---------------------------------|-----------------------------------------------|------------------------|
| Histamine (1.2 µg/ml) | Isoprenaline                    | 1.15 ± 0.12 (µg/ml)                          | n=8                    |
|                       | Mepyramine                      | 0.93 ± 0.37 (µg/ml)                          | n=6                    |
|                       | Atropine                        | 1.14 ± 0.29 (µg/ml)                          | n=7                    |
|                       | Aminophylline                   | 1.11 ± 0.32 (µg/ml)                          | n=8                    |
|                       | APE                             | 131.56 ± 12.23 (mg/ml)                        | n=7                    |
| Carbachol (1.5 µg/ml) | Isoprenaline                    | 1.18 ± 0.19 (µg/ml)                          | n=8                    |
|                       | Mepyramine                      | 0.96 ± 0.15 (µg/ml)                          | n=7                    |
|                       | Atropine                        | 0.83 ± 0.26 (µg/ml)                          | n=6                    |
|                       | Aminophylline                   | 1.34 ± 0.31 (µg/ml)                          | n=8                    |
|                       | APE                             | 142.43 ± 13.12 (mg/ml)                        | n=7                    |
| High-K (K⁺, 80 mM)    | Isoprenaline                    | 2.54 ± 0.52 (µg/ml)                          | n=7                    |
|                       | Mepyramine                      | 2.53 ± 0.49 (µg/ml)                          | n=6                    |
|                       | Atropine                        | 2.56 ± 0.51 (µg/ml)                          | n=8                    |
|                       | Aminophylline                   | 2.73 ± 0.36 (µg/ml)                          | n=7                    |
|                       | APE                             | 187.36 ± 16.27 (mg/ml)                        | n=8                    |
mM) on the tracheal muscle preparations seems to suggest that it is unlikely that the plant’s extract produces its bronchodilatory effects through a specific adrenergic, cholinergic or histaminergic receptor, but rather via a non-specific bronchospasmolytic mechanism. However, the ability of APE to relax (or inhibit) contractions produced by high-K solution suggests that, at least in part, APE probably inhibits influx of extracellular Ca++ into muscle cells by some yet unknown mechanisms, like a classical calcium-channel blocker, or alternatively APE may reduce Ca++ sensitivity of smooth muscle cells in the guinea-pig trachea. The possibility also exists that the bronchorelaxant effects of APE may be attributed, at least partially, to its ability to antagonize the post-junctional, receptor-mediated, spasmodic actions of carbachol and histamine. Since APE is known to inhibit several enzyme systems, such as cyclo-oxygenase (COX) and lipoxygenase enzymes, angiotensin-converting enzyme (ACE) and many other enzyme systems (Owira and Ojewole, 2009), the bronchospasmolytic action of APE could also be partly mediated via inhibition of phosphodiesterase enzyme (PDE), like aminophylline. However, further studies are required to clarify these speculations and determine the precise mechanism by which APE produces relaxation of tracheal smooth muscles.

In conclusion, the bronchorelaxant effect of APE unveiled in the present study lends pharmacological support to the folkloric, ethnomedical uses of african potato as a natural supplementary remedy in the management, control and/or treatment of bronchial asthma in some rural communities of southern Africa.

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**References**

Albrecht, C.F. (1995). Hypoxoside: A putative prodrug for the treatment of malignancies, HIV infections, and inflammatory conditions. *S. Afr. Med. J.* 85: 302–307.

Albrecht, C.F. (1996). Hypoxoside: A putative, non-toxic prodrug for the possible treatment of certain malignancies, HIV infections, and inflammatory conditions. In: Chemistry, Biological and Pharmacological Properties of African Medicinal Plants. Proceedings of the First International IOCD Symposium; Victoria Falls, Zimbabwe, ed. by K. Hostettmann, F. Chinyanganya, M. Maillard and J.L. Wolfender, University of Zimbabwe Press, Harare, pp. 303–307.

Drewes, S.E., Hall, A.J., Learmonth, R.A. and Uphold, U.J. (1984). Isolation of hypoxoside from *Hypoxis rooperi* and synthesis of [E]-1, 5-bis [3', 4'-dimethoxyphenyl 1] pent-4-en-1-yne. *Phytochemist.* 23: 1313–1316.

Foster, R.W. (1960). The paired tracheal chain preparation. *J. Pharm. Pharmacol.* 12: 189–191.

Hutchings, A. (1989). A survey and analysis of traditional medicinal plants as used by the Zulu, Xhosa and Sotho. *Bothalia.* 19: 111–123.

Hutchings, A., Scott, A.H., Lewis, G. and Cunningham, A.B. (1996). Zulu Medicinal Plants—An Inventory, University of Natal Press, Pietermaritzburg, pp. 55–56.

Johnson, Q., Syce, J., Nell, H., Rudeen, K. and Folk, W.R. (2007). A randomized, double-blind, placebo-controlled trial of *Lessertia frutescens* in healthy adults. *PLos Clin. Trials* 2: e16 (1–7).
Mahomed, I.M. and Ojewole, J.A.O. (2003). Hypoglycaemic effect of Hypoxis hemerocallidea corm (‘African Potato’) aqueous extract in rats. Methods Find. Exp. Clin. Pharmacol. 25: 617–623.

Mongomery, D. (1991). Diseño y Análisis de Experimentos. ed. by Grupo Editorial Ibero-americana. SA. de CV, Mexico, pp. 45–81.

Musabayane, C.T., Mahlalela, N., Shode, F.O. and Ojewole, J.A.O. (2005a). Effects of Syzygium cordatum (Hochst.) [Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetic rats. J. Ethnopharmacol. 97: 485–490.

Musabayane, C.T., Xozwa, K. and Ojewole, J.A.O. (2005b). Effects of Hypoxis hemerocallidea (Fisch. & C.A. Mey.) [Hypoxidaceae] corm (‘African potato’) aqueous extract on renal electrolyte and fluid handling in the rat. Ren. Fail. 27: 1–8.

Ojewole, J.A.O. (1977). Studies on the pharmacology of some antimalarial drugs. PhD Thesis, University of Strathclyde, Glasgow, Scotland, UK.

Ojewole, J.A.O. (2001). Traditional medicine and African indigenous plant remedies: evaluation of crude plant drugs used as antidiabetic remedies in zulu folk medicine. Curare 24: 143–160.

Ojewole, J.A.O. (2002). Anti-inflammatory properties of Hypoxis hemerocallidea corm (African potato) extracts in rats. Methods Find. Exp. Clin. Pharmacol. 24: 685–687.

Ojewole, J.A.O. (2003). Evaluation of the anti-inflammatory properties of Sclerocarya birrea (A. Rich.) Hochst. (family: Anacardiaceae) stem-bark extracts in rats. J. Ethnopharmacol. 85: 217–220.

Ojewole, J.A.O. (2004). Evaluation of the analgesic, anti-inflammatory and antidiabetic properties of Sclerocarya birrea (A. Rich.) Hochst. stem-bark aqueous extract in mice and rats. Phytother. Res. 18: 601–608.

Ojewole, J.A.O. (2005). Antinociceptive, anti-inflammatory and antidiabetic effects of Bryophyllum pinnatum (Crassulaceae) leaf aqueous extract. J. Ethnopharmacol. 99: 13–19.

Ojewole, J.A.O. (2006). Antinociceptive, anti-inflammatory and antidiabetic properties of Hypoxis hemerocallidea Fisch. & C. A. Mey. (Hypoxidaceae) corm ['African Potato'] aqueous extract in mice and rats. J. Ethnopharmacol. 103: 126–134.

Ojewole, J.A.O. (2008). Anticonvulsant activity of Hypoxis hemerocallidea Fisch. & C. A. Mey. (Hypoxidaceae) corm ['African potato'] aqueous extract in mice. Phytother. Res. 22: 91–96.

Owira, P.M. and Ojewole, J.A.O. (2009). ‘African Potato’ (Hypoxis hemerocallidea corm): a plant-medicine for modern and 21st century diseases of mankind?—a review. Phytother. Res. 23: 147–152.

Pujol, J. (1990). Naturafrica—The Herbalist Handbook, Jean Pujol Natural Healers’ Foundation, Durban, South Africa.

Steenkamp, V., Gouws, M.C., Gulumian, M., Elgorashi, E.E. and van Staden, J. (2006). Studies on antibacterial, anti-inflammatory and antioxidant activities of herbal remedies used in the treatment of benign prostatic hyperplasia and prostatitis. J. Ethnopharmacol. 103: 71–75.

Van Wyk, B-E., Van Oudtshoorn, B. and Gericke, N. (2002). Medicinal Plants of South Africa, 2nd ed., Briza Publications, Pretoria, South Africa, pp. 156–157.

Watt, J.M. and Breyer-Brandwijk, M.G. (1962). Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed., E. & S. Livingstone Ltd., Edinburgh and London, pp. 39–41.