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# Vasodilatory and hypoglycaemic effects of two pyrano-isoflavone extractives from *Eriosema kraussianum* N. E. Br. [Fabaceae] rootstock in experimental rat models

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**Abstract**

Zulu traditional health practitioners have claimed that the roots of *Eriosema kraussianum* N. E. Br. (Fabaceae) and other *Eriosema* species (Zulu indigenous umbrella name of "*uBangalala*") are effective remedies for the treatment of erectile dysfunction (ED) and/or impotence. In order to scientifically appraise the significance and contribution of *Eriosema kraussianum* to its ethnomedical use as "*uBangalala*" and "VIAGRA™ substitute", the present study was undertaken to investigate the vasodilatory and hypoglycaemic properties of the two main bioactive chemical compounds [Kraussianone-1 (K1), and Kraussianone-2 (K2), Drewes, S.E., Horn, M.M., Munro, O.Q., Dhlamini, J.T.B., Meyer, J.J.M., Rakuambo, N.C., 2002. Pyrano-isoflavones with erectile-dysfunction activity from *Eriosema kraussianum*. Phytochemistry 59 739–747.] obtained from *E. kraussianum*, in experimental rat models, using sildenafil citrate (VIAGRA™) as the reference drug for comparison. The two *E. kraussianum* rootstock constituents (K1 and K2, 20–80 mg/kg p.o.) caused dose-dependent and significant ($P < 0.05$–$0.001$) hypoglycaemia in rats. Relatively low to high concentrations of the plant's extracts (K1 and K2, 100–2000 μg/ml) always produced biphasic effects on rat isolated portal veins. K1- and K2-provoked responses of the isolated portal veins always consisted of concentration-related initial transient, but significant ($P < 0.05$), contractions of the venous muscle preparations, followed by secondary, longer-lasting, highly significant ($P < 0.01$–$0.001$) relaxations of the venous muscle strips. Sildenafil citrate (VIAGRA™, 5–100 μg/ml) always produced concentration-related and highly significant relaxations of the rat isolated portal veins. Unlike K1 and K2 (20–80 mg/kg p.o.), however, sildenafil citrate (VIAGRA™, 100 mg/kg p. o.) only caused slight and insignificant ($P > 0.05$) reductions in the blood glucose levels of the experimental animals used. On the other hand, glibenclamide (10 mg/kg p.o.) induced highly significant ($P < 0.05$–$0.001$), marked reductions in the blood glucose concentrations of the rats. The findings of this laboratory animal study indicate that the two hydro-ethanol extractives of *E. kraussianum* (K1 and K2) possess hypoglycaemic and secondary, vasorelaxant effects in the experimental paradigms used.


*Keywords:* *Eriosema kraussianum*; Pyrano-isoflavones; K1 and K2; Hypoglycaemic and vasodilatory properties; Rat model

## 1. Introduction

In South Africa, the genus *Eriosema* consists of annual herbs or shrublets of 10–18 cm in height, and the plants in this genus are found mainly in grassland areas of the country. The species in the genus possess well-developed root system, and it is this morphological part of the plants that is mainly used in South African traditional medicine.

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The Zulu people of South Africa traditionally use the roots of the genus *Eriosema* for a variety of human ailments, including management, control and/or treatment of impotence and urinary disorders (Hulme, 1954; Bryant, 1966; Hutchings et al., 1996). The plant's roots are also used as expectorants and diuretics (Watt and Breyer-Brandwijk, 1962). Generally, the genus *Eriosema* contains plants which come under the isiZulu indigenous umbrella name of "*uBangalala*", and most of the plant species listed under this name ("*uBangalala*") are used mainly for the purpose of curing or alleviating impotence (Bryant, 1966; Hutchings et al., 1996). In this regard, hot milk infusions of the plant's roots and/or pounded boiled root decoctions are taken in small doses in the morning and at night for impotence (Hulme, 1954; Bryant, 1966).

Previous studies in our laboratories (Drewes et al., 2002) have shown that *Eriosema kraussianum* N. E. Br. (Fabaceae) is one of the frequently used *Eriosema* species for the treatment of impotence, an ailment otherwise frequently referred to as "erectile dysfunction" (ED). Erectile dysfunction has been described as "*a consistent inability to achieve and maintain an erection sufficient for satisfactory sexual activity*" (Goldstein et al., 1998). Drewes et al. (2002, 2003) have shown in a rabbit experimental model, the beneficial effects of bioactive compounds of *E. kraussianum* in the management of erectile dysfunction. Cardiovascular disorders and diabetes mellitus are known to contribute significantly to ED of organic origin (Zusman et al., 1999; Rendell et al., 1999). Since men with ED of organic, psychogenic and mixed aetiologies are known to benefit from VIAGRA™ therapy, it is speculated that *E. kraussianum* extractives may also be effective as "VIAGRA™ substitutes" in the treatment of ED of organic, psychogenic and mixed origins. Although Drewes et al. (2002) used sildenafil citrate (VIAGRA™) as the reference compound for comparison in their ED studies on the two new pyrano-isoflavones, unlike sildenafil citrate, the exact mechanisms of action of the two extractives from *E. kraussianum* still remain unknown. The two new compounds tested positive in experimental rabbit ED treatment, and attained values of 85% and 65%, respectively, compared with VIAGRA™, in relaxing rabbit *corpus cavernosum* smooth muscles (Drewes et al., 2002, 2003). The Zulu people of South Africa traditionally employ hot milk infusions and pounded decoctions of the roots of *E. kraussianum* and other species of *Eriosema* as substitutes for VIAGRA™ in the treatment of erectile dysfunction (ED) and/or impotence. For this reason, we have compared the effects of *E. kraussianum* extractives (K1 and K2) with that of VIAGRA™ in the experimental animal models used in the present study. In order to throw some light on the plausible mechanisms of action of the extractives, the present study was undertaken to investigate the hypoglycaemic and vasodilatory properties of the two new pyrano-isoflavones [Kraussianone-1 (K1) and Kraussianone-2 (K2)] from the roots of *E. kraussianum* in experimental rat models, using sildenafil citrate (VIAGRA™) as the reference sexual stimulant drug for comparison (see Fig. 1).

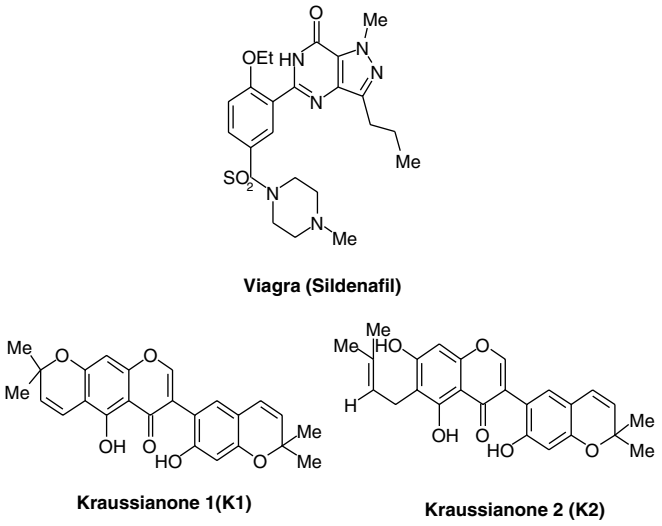


Fig. 1. Structural formulae of Viagra™ (sildenafil citrate), Kraussianone-1 (K1) and Kraussianone-2 (K2).

## 2. Results and discussion

### 2.1. Effects of K1 and K2 on blood glucose levels of healthy, normal rats

In a separate set of experiments involving 16-h fasted healthy, normal, male rats, the baseline blood glucose levels were found to vary between $4.10 \pm 0.13$ and $4.36 \pm 0.14$ mmol/l. In our 'control' set of experiments, acute treatment of the animals with the hydro-ethanol vehicle (3 ml/kg p.o.) alone did not significantly modify ($P > 0.05$) the blood glucose concentrations of the fasted normal rats. In these animals, pretreatment with the hydro-ethanol vehicle (3 ml/kg p.o.) for 1, 2, 4 and 8 h 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 vehicle-induced changes in the blood glucose levels of the fasted rats varied by values ranging between 0.1% and 1.0% of the mean baseline values (Table 1). Similarly, pretreatment of the normal rats with sildenafil citrate (VIAGRA™, 100 mg/kg p.o.) for 1, 2, 4 and 8 h did not reduce the baseline blood glucose levels significantly ($P > 0.05$) after 21/2 h. However, compared with the vehicle-treated 'control' rats, pretreatment of the fasted rats with relatively moderate to high doses of K1 or K2 extract (20, 40 and 80 mg/kg p.o.) for 1, 2, 4 and 8 h produced significant reductions ($P < 0.05$–$0.001$) in the blood glucose concentrations of the fasted normal rats (Table 1). Maximal reductions in the blood glucose concentrations of the fasted 'test' rats occurred at the plant's extract (K1 or K2) dose of 80 mg/kg (p.o.). Compared with the vehicle-treated fasted 'control' rats, pretreatment of fasted normal rats with glibenclamide (10 mg/kg p.o.) for 1, 2, 4 and 8 h also produced significant reductions ($P < 0.05$–$0.001$) in the blood glucose concentrations of the animals (Table 1). The hypoglycaemic effects of the plant's extracts (K1 and K2) became significant

Table 1
Effects of Krausianone-1 (K1, 80 mg/kg p.o.), Krausianone-2 (K2, 80 mg/kg p.o.), VIAGRA™ (SC, 100 mg/kg p.o.) and glibenclamide (GBC, 10 mg/kg p.o.) on blood glucose concentrations (mmol/l) of normal (normoglycaemic) rats

| Treatment | Before treatment | After treatment | | | | Maximal reduction | % Maximal reduction |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 2 h | 4 h | 8 h | | |
| Control (3 ml/kg hydro-ethanol vehicle p.o.) | $4.35 \pm 0.11$ | $4.34 \pm 0.12$ | $4.36 \pm 0.10$ | $4.35 \pm 0.13$ | $4.34 \pm 0.10$ | 0.00 | 0.23 NS |
| Kraussianone-1 (K1, 80 mg/kg p.o.) | $4.28 \pm 0.15$ | $4.03 \pm 0.13$ | $3.67 \pm 0.12^{*}$ | $3.18 \pm 0.14^{*}$ | $3.84 \pm 0.11^{*}$ | 0.26 | 25.70* |
| Kraussianone-2 (K2, 80 mg/kg p.o.) | $4.30 \pm 0.12$ | $4.01 \pm 0.10$ | $3.54 \pm 0.13^{*}$ | $3.08 \pm 0.11^{*}$ | $3.71 \pm 0.10^{*}$ | 0.28 | 28.37* |
| VIAGRA™ (SC, 100 mg/kg p.o.) | $4.12 \pm 0.13$ | $4.06 \pm 0.12$ | $4.03 \pm 0.10$ | $4.01 \pm 0.14$ | $4.06 \pm 0.13$ | 0.03 | 2.67 NS |
| Glibenclamide (GBC, 10 mg/kg p.o.) | $4.18 \pm 0.14$ | $3.53 \pm 0.12^{*}$ | $3.04 \pm 0.10^{**}$ | $2.24 \pm 0.12^{***}$ | $3.08 \pm 0.12^{**}$ | 0.46 | 46.41*** |

Values given represent the mean (±SEM) of 8 observations. NS = $P > 0.05$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ vs control.

($P < 0.05$) 2 h following oral administration, reaching the peak of their hypoglycaemic effects 4 h after administration. However, the hypoglycaemic effects of the extracts were still significant 8 h after oral administration (Table 1). Thereafter, the blood glucose concentrations of the animals gradually returned to baseline levels at the end of the 24th h.

### 2.2. Effects K1 and K2 on oral glucose tolerance test (OGTT) in normal rats

The effects of K1, K2 (80 mg/kg p.o.), VIAGRA™ (100 mg/kg p.o.) and glibenclamide (10 mg/kg p.o.) on blood glucose levels of rats following oral glucose load are shown in Fig. 2. This figure shows that following oral glucose load, the blood glucose concentrations of the 16-h fasted normal rats increased to $8.7 \pm 0.6$ mmol/l from a baseline value of $4.36 \pm 0.14$ mmol/l; before gradually declining to $5.8 \pm 0.6$ mmol/l after 21/2 h. Pretreatment of the normal rats with K1 or K2 (80 mg/kg p.o.) for 20 min prior to oral glucose load, significantly reduced ($P < 0.05$) the peak blood glucose levels from $8.7 \pm 0.6$ to $5.1 \pm 0.6$ and $5.3 \pm 0.7$ mmol/l, respectively, after 21/2 h. This observation would appear to suggest that the plant's extracts (K1 and K2) facilitate or promote the clearance of postprandial blood glucose in rats. The effects of K1 and K2 on the blood glucose concentrations of the rats were not significantly different ($P > 0.05$) after 21/2 h. Pre-

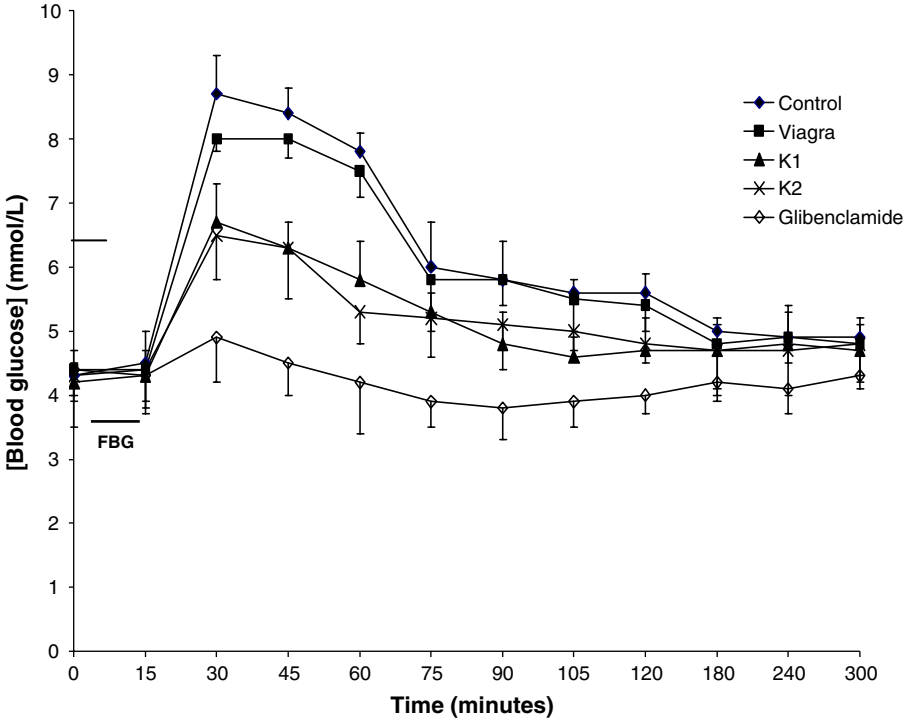


Fig. 2. Effects of Kraussianone-1 (K1, 80 mg/kg p.o.), Kraussianone-2 (K2, 80 mg/kg p.o.), Viagra™ (100 mg/kg p.o.) and glibenclamide (10 mg/kg p.o.) on blood glucose concentrations (mmol/l) of normal rats following oral glucose load (2 g/kg p.o.). Each point represents the mean (±SEM) of eight determinations, while the vertical bars represent standard errors of the means. FBG denotes 'Fasting Blood Glucose'.

treatment of the normal rats with glibenclamide (10 mg/kg p.o.) for 20 min prior to oral glucose load, also significantly reduced ($P < 0.001$) the peak blood glucose levels from $8.7 \pm 0.6$ mmol/l to $4.8 \pm 0.7$ mmol/l after 21/2 h. However, pretreatment of the normal rats with sildenafil citrate (VIAGRA™, 100 mg/kg p.o.) for 20 min prior to oral glucose load, did not reduce the baseline blood glucose concentrations significantly ($P > 0.05$) after 21/2 h.

### 2.3. Effects of K1 and K2 on rat isolated portal vein

Relatively low to high concentrations of K1 or K2 (100–2000 μg/ml) always produced biphasic effects on rat isolated portal veins. The K1- and K2-induced responses of the isolated portal veins always consisted of dose-related initial transient, but significant ($P < 0.05$) contractions of the venous muscle preparations, followed by secondary, longer-lasting, highly significant ($P < 0.01$–$0.001$) relaxations of the muscle strips. During the initial transient, contractile phase, K1 or K2 (100–2000 μg/ml) usually increased the contractile frequency, and inhibited the amplitude of the spontaneous, myogenic contractions of the isolated portal veins in a concentration-dependent manner. Fig. 3 summarizes the results obtained with K1 and K2 (1000 μg/ml) and VIAGRA™ (500 μg/ml). Sildenafil citrate (VIAGRA™, 5–1000 μg/ml) always produced concentration-related, significant relaxations ($P < 0.05$–$0.001$) of the isolated portal veins. The possibility that the K1, K2 and VIAGRA™–induced responses of the isolated portal veins might involve interaction with $Ca^{2+}$ at the cell membrane was also investigated. In these experiments, the concentration of $Ca^{2+}$ in the bathing normal Krebs–Henseleit physiological solution [of composition, in g/l: NaCl, 6.92; KCl, 0.34; $NaH_2PO_4$, 0.15; $NaHCO_3$, 2.10; $MgCl_2$, 0.11; $CaCl_2$, 0.26; and glucose, 1.00 – pH adjusted to 7.4 maintained at $34 \pm 1$ °C and continuously aerated with carbogen (i.e., 95% $O_2$ + 5% $CO_2$ gas mixture)] was either reduced from 0.26 to 0.13 g/l, or raised from 0.26 to 0.52 g/l, respectively. The initial transient, contractile responses of the isolated muscle strips induced by relatively low to high concentrations K1 and K2 (100–2000 μg/ml) were reduced and/or abolished in the presence of low calcium concentration [$Ca^{2+} = 0.13$ g/l] in the bathing Krebs–Henseleit physiological solution. However, the secondary, relaxant responses of the isolated venous muscle strips produced by low to high concentrations of the extracts (K1 and K2, 100–2000 μg/ml) increased as the concentration of the external $Ca^{2+}$ was reduced. Raising the bathing fluid $Ca^{2+}$ concentration from 0.26 to 0.52 g/l increased and/or enhanced K1 and K2 (100–2000 μg/ml)–induced initial transient, contractile responses of the isolated venous muscle preparations. However, the secondary, relaxant responses of the isolated venous muscle strips induced by low to high concentrations of the plant's extracts (K1 and K2, 100–2000 μg/ml) decreased as the $Ca^{2+}$ concentration of the external bathing fluid was increased. In all cases, washing of the isolated venous muscle preparations with fresh, normal Krebs–Henseleit physiological solution 3–5 times usually restored physiological activities of the isolated muscle strips to control, baseline values.

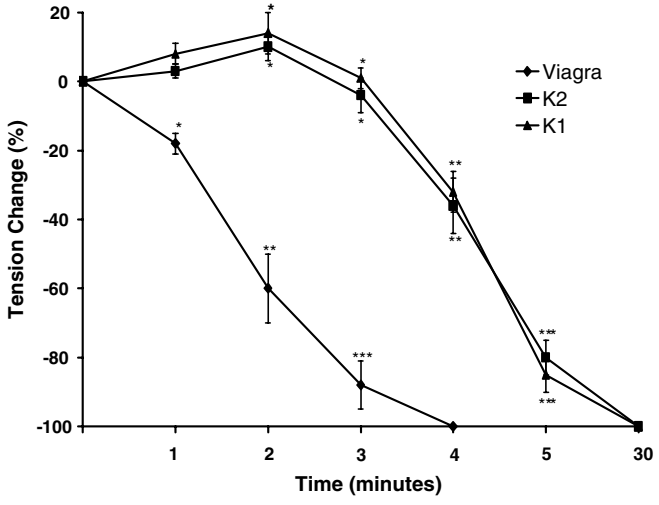


Fig. 3. Effects of Krausianone-1 (K1, 1000 μg/ml), Krausianone-2 (K2, 1000 μg/ml) and sildenafil citrate (VIAGRA™, 500 μg/ml) on spontaneous contractions of rat isolated portal veins. Each point represents the mean (±SEM) of 8–10 preparations, while the vertical bars denote standard errors of the means. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. control.

### 2.4. Possible mechanisms of action

Experimental evidence obtained in the present study show that the hydro-ethanol rootstock extractives of *E. kraussianum* (K1 and K2, 20–80 mg/kg p.o.) dose-dependently and significantly ($P < 0.05$–$0.001$) reduced blood glucose levels in rats. While sildenafil citrate (VIAGRA™) only produced slight and non-significant ($P > 0.05$) hypoglycaemic effects in the experimental animal model used, glibenclamide induced highly significant hypoglycaemia ($P < 0.01$–$0.001$) in the experimental animals.

The major classes of synthetic oral hypoglycaemic agents currently available for the management and/or control of adult-onset, type-2, non-insulin-dependent diabetes mellitus (NIDDM) include the sulphonylureas, biguanides, thiazolidinediones, α-glucosidase inhibitors, and so on. Glibenclamide, used as the reference hypoglycaemic agent in this study, is a member of the 'first generation' sulphonylureas. As a class, sulphonylureas enhance 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*; (ii) *potentiation of insulin's action on target tissues and increased glucose removal from the blood*, and (iii) *reduction of blood glucagon levels*. Thus, any plant secondary metabolite or chemical compound which 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. Although the precise mechanisms of the hypoglycaemic actions of K1 and K2 remain speculative at present, experimental evidence obtained in this study tends to suggest that the plant's extracts dose-dependently enhance or promote the clearance of postprandial blood glucose in rats. Therefore, the possibility exists that the plant's extracts (K1 and K2) may act like sulphonylureas, and mimic or improve insulin action at cellular level.

VIAGRA™ always produced concentration-dependent, highly significant ($P < 0.001$) relaxations of the rat isolated portal veins. However, K1 and K2 always caused concentration-related, initial slight contractions, followed by secondary, longer-lasting, significant ($P < 0.05$–$0.001$) relaxations of the isolated portal veins. The initial transient, contractile effects of K1 and K2 may be due to the ability of the extracts to transiently release noradrenaline (NA) from NA tissue stores. The precise mechanism of the secondary, pronounced vasorelaxant effects of the plant's extractives (K1 and K2) on the rat isolated portal veins is unknown at the moment. However, because the secondary venous relaxant effects of the plant's extracts were resistant to blockade by standard, receptor specific antagonists such as atropine sulphate (2 μg/ml) and mepyramine maleate (2 μg/ml) in all the muscle preparations tested, it is speculated that the secondary, longer-lasting venorelaxant effects of K1 and K2 on the isolated venous muscle strips may be non-specific in nature. Furthermore, the finding that changes (decrease or increase) in calcium ion concentrations [$Ca^{2+}$] of the bathing physiological solution modified the responses of the isolated venous tissue preparations to bath-applied concentrations of *E. kraussianum* extracts (K1 and K2) would appear to suggest that the extracts affected calcium mobilization and/or sequestration, and possibly also, calcium release from its various tissue stores (Webb et al., 1999). Further studies are certainly needed to shed more light on the plausible mechanisms of action of the two *E. kraussianum* extracts.

The data obtained in this study do not allow any definite conclusions to be drawn on the mechanisms of hypoglycaemic and vasorelaxant actions of *E. kraussianum* root hydro-alcohol extracts in the experimental animal models used. However, a number of investigators have shown that tannins and other polyphenolic compounds (e.g., coumarins), flavonoids, triterpenoids, and a host of other secondary plant metabolites possess anti-inflammatory, hypoglycaemic, spasmolytic and analgesic 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; Li et al., 2003; Marles and Farnsworth, 1995).

We have compared the effects of *E. kraussianum* extractives (K1 and K2) with that of VIAGRA™ in the experimental animal models used in the present study. Although biomedical literature is limited to two publications (Drewes et al., 2002, 2003) on the extractives of *E. kraussianum* as effective remedies for erectile dysfunction, literature abounds on the effectiveness of VIAGRA™ in the treatment of erectile dysfunction. Erectile dysfunction (ED) is a common medical condition in men with cardiovascular disorders such as ischaemic heart disease, hypertension and peripheral vascular diseases (Feldman et al., 1994; Conti et al., 1999), and is also common in men with diabetes mellitus (Rendell et al., 1999), probably because of the shared factors that impair haemodynamic mechanisms in the penile and ischaemic vasculature. Erectile dysfunction is also caused by spinal cord injury [cord level range, T6–L5] (Derry et al., 1998), and other factors such as radical prostatectomy, long-term use of certain medications (e.g., antidepressants, antipsychotics, antihypertensives and diuretics), indeces of anger and depression, and cigarette smoking (Feldman et al., 1994).

Sildenafil citrate (VIAGRA™) has been widely studied for its efficacy in the treatment of erectile dysfunction (ED) in a variety of patient populations. In men, oral sildenafil citrate is generally known to be effective in erectile dysfunctions of organic, psychogenic or mixed origins. However, the aetiology of erectile dysfunction has been shown to have a significant impact on treatment success and satisfaction rates, with neurogenic causes of erectile dysfunction (e.g., diabetes mellitus, prostate surgery, and so forth) having significantly lower treatment success rates than psychogenic or vasculogenic erectile dysfunction (Jarow et al., 1999). Although VIAGRA™ has transient vasodilatory properties in vivo, it has no direct relaxant effect on isolated human *corpus cavernosum* in vitro (Conti et al., 1999). The main mechanism of action of Viagra™ in erectile dysfunction is well-known and, therefore, needs no repetition here. Suffice it to say that it facilitates the patient's *ability to achieve and maintain an erection for satisfactory sexual activity after penetration*. This it does by its ability to selectively inhibit cyclic guanosine monophosphate (cGMP)-specific phosphadiesterase type 5 (PDE5). According to Pfizer's data on file, "VIAGRA™ is rapidly absorbed from gastro-intestinal smooth muscles following oral administration, with an absolute bioavailability of about 40%. Its pharmacokinetics are dose-related over recommended dose ranges (25, 50 or 100 mg/kg p.o.). It is eliminated predominantly by hepatic metabolism (mainly by cytochrome $P_{450}$ 3A4), and is converted to an active metabolite with properties similar to that of the parent, sildenafil. Both sildenafil and its metabolite have terminal half-lives of about 4 h. Maximum observed plasma concentrations are reached within 30–120 min (median 60 min) of oral dosing in the fasted state. When VIAGRA™ is taken with a high fat meal, the rate of absorption is reduced with a mean delay in $T_{max}$ of 60 min, and a mean reduction in $C_{max}$ of 29%".

K1 and K2 caused significant hypoglycaemia in rats, and like VIAGRA™, produced relaxations of isolated portal veins. Unlike VIAGRA™, however, human knowledge on the benefit, usefulness and/or efficacy of the extractives of *E. kraussianum* (and other species of *Eriosema*) in the

treatment of erectile dysfunction is limited. Nonetheless, as with oral VIAGRA™ and fat meal, oral administration of infusion and/or decoction of *Eriosema* species roots with milk probably delays and/or reduces the rate of absorption of the compounds from the patient's gastro-intestinal tract (GIT), and thereby prolongs the duration of action of the extractives. It has been reported that for maximum benefit, milk infusions and/or decoctions of the plant's roots are to be taken 2–4 h before any anticipated sexual intercourse, and the sexual activity effects (achievement and maintenance of penile erection sufficient for satisfactory sexual intercourse after penetration) of the extractives have also been reported to last for 4–6 h following oral dosing of the milk infusion or decoction of the root extracts (Drewes, Personal Communication). Unlike VIAGRA™, the bioavailability, half-life ($t_{1/2}$), $T_{max}$, $C_{max}$ and other pharmacokinetic parameters of the extracts are unknown at the moment. The effects of the plant's extracts (K1 and K2) on the biochemical activities of cGMP and PDE5 are also obscure at present. Further studies are certainly warranted to throw more light on the pharmacokinetic profiles of K1 and K2, as well as on the biochemical effects of the extractives on cGMP and PDE5.

The experimental evidence obtained in this laboratory animal study indicates that the two hydro-alcoholic extracts of *E. kraussianum* (K1 and K2) possess hypoglycaemic and secondary vasorelaxant effects. These pharmacological properties of the plant's extracts may contribute significantly to the effectiveness of the herb in the management and/or treatment of erectile dysfunction among the Zulu men of South Africa.

## 3. Experimental

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

### 3.1. Plant material

*E. kraussianum* N. E. Br. (Fabaceae) was collected in November 2004 from an open grassland on the northern boundary of the National Botanical Garden in Pietermaritzburg, South Africa. Identification of the plant was done by Professor Trevor Edwards (Curator of the Bews Herbarium at the University of KwaZulu-Natal, Pietermaritzburg). A voucher specimen of the plant (S.E.D. No. 7) has been deposited in the Herbarium.

### 3.2. Preparation of K1 and K2 hydro-ethanol solutions

The plant material, *E. kraussianum* rootstock (700 g), was milled and extracted with $CH_2Cl_2$ for 3 weeks to give a brown powder (7.1 g). On a TLC plate (with $CH_2Cl_2$ as solvent), five fluorescent bands were clearly seen. K1 and K2 migrated at Rf values of 0.65 and 0.20, respectively. Using the process described previously (Drewes et al., 2002), K1 (73 mg) and K2 (230 mg) were isolated in crystalline form from 6.2 g of the starting material. The structures of both compounds were verified by spectroscopic techniques (described earlier by Drewes et al., 2002). The hydro-ethanol solutions of the plant's extracts were prepared using 70:30 ethanol:water mixtures, and had an initial concentration of 1 mg/ml.

### 3.3. Animal material

Healthy, male, 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 plant extract- and drug-treated 'test', and hydroethanol-treated 'control' groups (of 8 animals per group) in the in vivo experiments. All the animals were fasted for 16 h, but still allowed free access to water, before the commencement of our experiments.

### 3.4. Assessment of hypoglycaemic activity

#### 3.4.1. Effects of K1 and K2 on blood glucose levels of normal rats

Seven groups of 8 rats each were used for this assessment. Group 1 rats served as 'untreated control' for the other six groups of rats. After a four-day acclimatization period, during which time the animals were allowed free access to food (standard pellet diet) and water ad libitum, all the rats were fasted for 16 h. Initial fasting blood glucose levels ($G_0$) of the Groups 1 to 7 rats were determined and recorded. Thereafter, Group 2 rats received 3 ml/kg (p.o.) of hydro-ethanol [distilled water:ethanol (3:7) mixture] vehicle, and Groups 3, 4 and 5 rats received graded doses of the plant's extracts (K1 or K2, 20, 40 and 80 mg/kg p.o., respectively). Group 6 rats received glibenclamide (10 mg/kg p.o.), while Group 7 rats received sildenafil citrate (VIAGRA™, 100 mg/kg) by gastric intubation. The effects of the hydro-ethanol vehicle, graded doses of the pure compounds (K1 and K2), sildenafil citrate (VIAGRA™) and glibenclamide on the fasting blood glucose concentrations of the animals were then monitored and measured hourly for eight hours. 1, 2, 4 and 8 h following administration of the 'test' compounds to the animals, blood glucose concentrations ($G_t$) were determined. In each case and for each dose, 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 needle 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:

$$\% \text{ glycaemic change} = G_0 - G_t \times 100/G_0,$$

where $G_0$ and $G_t$ represent glycaemic values before (i.e., zero time or 0 h glycaemic values), and glycaemic values at 1, 2, 4 and 8 h after, oral administrations of the 'test' compounds, respectively. Under the same experimental conditions, rats treated with hydro-ethanol vehicle (3 ml/kg p.o.) alone were used as 'control' animals.

#### 3.4.2. Oral glucose tolerance test (OGTT)

Seven Groups of healthy, 16-h fasted, normal, young adult, male Wistar rats (*R. norvegicus*) were used. After measuring the initial blood glucose levels ($G_0$) in all the animals in each of the seven groups, each of the rats in Group 7 received sildenafil citrate (VIAGRA™, 100 mg/kg p.o.), while each rat in Group 6 was treated with glibenclamide (10 mg/kg p.o.). Each rat in Groups 5, 4 and 3 received 80, 40 and 20 mg/kg (p.o.) of either K1 or K2, respectively, while each of the animals in Group 2 was treated with hydro-ethanol vehicle (3 ml/kg p.o.). Group 1 rats were not treated with anything at all, and the 8 rats in this Group 1 served as 'untreated control' for the rats in Groups 2–7. Twenty minutes following pretreatment with either VIAGRA™ (100 mg/kg p.o.), glibenclamide (10 mg/kg p. o.), K1 or K2 (20, 40 and 80 mg/kg p.o.) or the vehicle (3 ml/kg p.o.), glucose (2 g/kg body weight) was orally administered (by gastric intubation) into each of the rats in Groups 2 to 7. Postprandial (i.e., post-glucose administration) blood glucose levels ($G_t$) were then monitored in, and recorded for, each of the rats in Groups 2–7 at 15 min-intervals for the first two-and-a-half hours, and hourly thereafter for another 2 h following the oral glucose load. In each case and for each treatment dose, 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 needle 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:

$$\% \text{ glycaemic change} = G_0 - G_t \times 100/G_0$$

where $G_0$ and $G_t$ represent glycaemic values before (i.e., zero time or 0 h glycaemic values), and glycaemic values at 1/4, 1/2, 3/4, 1, 11/4, 11/2, 13/4, 2, 4 and 8 h after, oral glucose load. Under the same experimental conditions, rats treated with hydro-ethanol vehicle (3 ml/kg p.o.) alone were used as 'control' animals.

### 3.5. Evaluation of vasodilatory activity

#### 3.5.1. Effects of K1 and K2 on rat isolated portal vein

The experimental procedure used for the rat isolated portal vein was adopted from that described in detail by Ojewole (1977). Healthy, male, Wistar rats weighing 200–350 g were used. Each rat was sacrificed by decapitation. 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 (of composition, in g/l: NaCl, 6.92; KCl, 0.34; $NaH_2PO_4$, 0.15; $NaHCO_3$, 2.10; $MgCl_2$, 0.11; $CaCl_2$, 0.26; and glucose, 1.00 – pH adjusted to 7.4) maintained at $34 \pm 1$ °C and continuously aerated with carbogen (i.e., 5% carbon-dioxide + 95% oxygen gas mixture). Two isolated portal veins (one used as 'control' and the other one used as K1-, K2- or sildenafil citrate-treated 'test' 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 min under an applied resting tension of 0.5 g, before it was challenged with graded concentrations of K1, K2 or sildenafil citrate. Stepwise concentrations of K1, K2 or sildenafil citrate were applied to the bath-fluid either cumulatively or sequentially, and washed out 3–5 times after the maximum responses of the tissues were attained. Concentrations of K1, K2 or sildenafil citrate were repeated where appropriate and/or possible, at regular intervals of 20–30 min after the last washing. Because acetylcholine and histamine have been shown to relax vascular blood vessels through endothelium-dependent nitric oxide (NO) production and release (Yin et al., 2005; Luscher and Vanhoutte, 1986; Zawadzki et al., 1980), the effects of atropine sulphate (2 μg/ml) and mepyramine maleate (2 μg/ml) were examined on K1- and K2-induced secondary relaxant responses of the isolated portal veins. The amplitude and frequency (rate) of the spontaneous, myogenic contractions, as well as the K1-, K2- or sildenafil citrate-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-writing microdynamometers (model 7070).

### 3.6. Data analysis

Experimental data obtained from 'test' rats treated with the two pure compounds (K1 and K2, isolated from *E. kraussianum*), glibenclamide and VIAGRA™, as well as those obtained from hydroethanol-treated 'control' rats, or isolated portal vein preparations, were pooled and expressed as means (±SEM). Differences between the plant's extract-, sildenafil citrate (VIAGRA™)- or glibenclamide-treated 'test' means, and the hydroethanol-treated 'control' means, were analysed statistically by one-way analysis o f variance (ANOVA), followed by Scheffe's multiple comparison test. Values of $P \leqslant 0.05$ were taken to imply statistical significance.

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