

Original Article

Evaluation of the *excopula* ejaculatory potentials of *Bersama engleriana* in spinal male rats

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Abstract

The aim of this study was to investigate the effects of *Bersama engleriana* and its potential mechanism on fictive ejaculation in spinal male rats. The electromyographic activities of the bulbospongiosus muscles were recorded in spinal cord transected and urethane-anesthetized rats treated intravenously with aqueous (100 mg kg⁻¹) and methanolic (100 mg kg⁻¹) extracts from the dried leaves of *B. engleriana* in the absence and presence of dopamine (0.1 µmol kg⁻¹) or oxytocin (0.5 UI kg⁻¹). Mechanical stimulations of the urethra were also carried out 5 min after the sequential treatments. A single intravenous administration of aqueous (100 mg kg⁻¹) and methanolic (100 mg kg⁻¹) extracts of *B. engleriana* did not activate fictive ejaculation. The electromyography recorded after the application of the plant extract was similar to that obtained after intravenous saline injection (200 G l min⁻¹) with no contraction of the bulbospongiosus muscles. Dopamine (0.1 µmol kg⁻¹) and oxytocin (0.5 UI kg⁻¹) induced rapid rhythmic contractions ($P < 0.001$) of the bulbospongiosus muscles accompanied by penile erection and sometimes with expulsion of the seminal plugs. Pre-treatment of rats with the two plant extracts completely abolished the occurrence of ejaculation induced by dopamine (0.1 µmol kg⁻¹) and oxytocin (0.5 UI kg⁻¹). Mechanical stimulation of the urethra carried out 5 min after the sequential treatments always induced penile movements and erections. The inhibitory effect of *B. engleriana* extracts on the expression of fictive ejaculation in spinal male rat is mediated through dopaminergic and oxytocinergic pathways. This prolonged ejaculatory latency caused by *B. engleriana* could support its potential use in patients with rapid ejaculation.

Asian Journal of Andrology (2009) 11: 533–539. doi: 10.1038/aja.2009.41; published online 3 August 2009

Keywords: *Bersama engleriana*, dopamine, ejaculation, inhibition, oxytocin, spinal rat

1 Introduction

Medicinal plants have become a great source of

relief for more than two out of three of the population in developing countries, where the access to modern medicine is very limited. Some of these plants are used as aphrodisiac agents [1–3]. Aphrodisiacs refer to substances of various origins that can be categorized according to their mode of action into three groups: those that increase libido (that is, sexual desire), those that increase potency (that is, effectiveness of erection) and those increasing sexual pleasure (that is, arouse sexual instinct) [4]. The aphrodisiacs act at the level of the central nervous system by altering specific neu-

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Received: 29 January 2009

Accepted: 23 June 2009

Revised: 5 April 2009

Published online: 3 August 2009

rotransmitters or specific sex hormone concentrations. *Bersama engleriana* of the Melianthaceae family is one such aphrodisiac plants. It is a small or medium sized tree of 6–9 m, rarely exceeding 25 m in height. It is wide spread throughout tropical Africa, preferring higher rainfall or evergreen forests. It is distributed from Senegal to Zaire, and in parts of Southern Africa. In the south-west region of Cameroon, the leaves have been extensively used over the years for the treatment of diabetes and also as an aphrodisiac [5, 6]. Antitumor, antioxidant and antimicrobial activities of the plant have also been reported [7]. Oral administration of aqueous and methanolic extracts of the leaves for 21 days to adult male rats increased the frequencies of erection and intromission [8]. As erection and intromission are dependent on intrinsic and extrinsic signals, it was thought that the active components contained in the extracts of *B. engleriana* could probably act by inducing changes in the levels of some central neurotransmitters, by modulating some actions on their target cells or by increasing androgen levels, and might be useful in delaying ejaculation [9]. To the best of our knowledge, no work has been previously reported on the effect of this medicinal plant on ejaculation, another centrally controlled sexual response. The present study was, therefore, undertaken to evaluate the pharmacological actions of *B. engleriana* on male rat ejaculation. To this purpose, we analysed the effect of a single intravenous administration of aqueous and methanolic extracts of the leaves of *B. engleriana* on the expression of the rhythmic genital motor pattern of ejaculation. As oxytocin plays a capital role in ejaculation, and also, as oxytocinergic receptors can be activated by dopamine during the induction of male sexual behavior [10, 11], the effects of *B. engleriana* on dopamine and oxytocin-induced ejaculation were also investigated. We used the fictive ejaculation model, which permits the recording and visualization of the rhythmic motor pattern of ejaculation and autonomic events such as penile erections and penile movements that accompany ejaculation, and that can be induced by sensory and pharmacological means [12].

2 Materials and methods

2.1 Collection of plant material and extraction

The leaves of *B. engleriana* (Melianthaceae) were collected in July in Kumba, south-west province,

Cameroon. Botanical identification was carried out at the National Herbarium, Yaoundé, Cameroon (HNC) where a voucher specimen no. 32427/HNC has been deposited. The leaves were shade-dried and reduced to powder.

2.1.1 Aqueous extract

A total of 400 g of the powder of *B. engleriana* leaves were extracted in 5l of distilled water for 1 h and boiled for 30 min. The heated decoction was taken and allowed to cool at room temperature ($22 \pm 2^\circ\text{C}$). The decoction was filtered and the filtrate was oven dried (45°C). The resulting material was found to weigh 112 g (28% yield, w/w based on the dried starting weight). The working solution was obtained by dissolving 1 g of the residue in a known volume of distilled water and the final volume adjusted to 10 mL.

2.1.2 Methanolic extract

Ground leaves (600 g) of *B. engleriana* were macerated with methanol (3L; $2 \times$) for 72 h to yield, after solvent evaporation under reduced pressure, 16.5 g of brownish extract corresponding to an extraction yield of 14.29% (w/w based on the dried starting weight). The working methanolic extract was obtained by dissolving 1 g of the residue in a known volume of distilled water and the final volume adjusted to 10 mL.

2.2 Preliminary phytochemical screening

The freshly prepared aqueous and methanolic extracts of the leaves of *B. engleriana* were qualitatively tested for the presence of chemical constituents by using standard procedures [13, 14].

2.3 Animals

Healthy adult male Wistar rats (> 90 days, 250–300 g body weight) were obtained from the animal house of the Laboratorio de Comportamiento of the Facultad de Agrobiología y Medicina Veterinaria of the Universidad Autónoma de Tlaxcala, México. They were housed in groups (four rats per cage), under an inverted LD cycle 12 : 12 h, at 22°C and with free access to food and water. The Local Committee of Ethics on Animal Experimentation approved all experimental procedures, which followed the regulations established in the Mexican official norm for the use and care of laboratory animals “NOM-062-ZOO-1999”. All males were trained for sexual experience, as previously described [15] and only those exhibiting good copulatory behavior were

selected for the study.

2.4 Drugs

Dopamine (Tecnofarma, México City, México), urethane and oxytocin (Sigma Chemicals, St Louis, USA) used in the present study were of analytical grade.

2.5 Surgical preparation

All animals were urethane-anesthetized (0.7 g kg^{-1} intraperitoneally), and by performing a surgical incision on the perineum, the bulbospongiosus genital muscles were identified. Two platinum wires (Grass Technologies) were inserted into the muscles to record electromyographic (EMG) activity, which was registered on a polygraph (Grass M6). For a better visualization of the motor genital activity associated with the ejaculation, an additional surgery was performed to expose the bulbar portion of the penis and its anatomical connections with the striated bulbospongiosus muscles. At the end of the surgical approach, the spinal cord was blunt transected at T6 spinal level and prepared for recording [16]. Treatments were administered by infusing the selected extracts and compounds into the femoral vein.

2.6 Experimental treatment

Animals were randomly divided into nine groups of five rats each and treated as follows: Group 1, saline solution (1 mL kg^{-1} , control); Group 2, aqueous extract of *B. engleriana* (100 mg kg^{-1}); Group 3, methanolic extract of *B. engleriana* (100 mg kg^{-1}); Group 4, oxytocin (0.5 UI kg^{-1}); Group 5, dopamine ($0.1 \text{ } \mu\text{mol kg}^{-1}$); Group 6, aqueous extract (100 mg kg^{-1}) plus oxytocin (0.5 UI kg^{-1}); Group 7, aqueous extract (100 mg kg^{-1}) plus dopamine ($0.1 \text{ } \mu\text{mol kg}^{-1}$); Group 8, methanolic extract (100 mg kg^{-1}) plus oxytocin (0.5 UI kg^{-1}); Group 9, methanolic extract (100 mg kg^{-1}) plus dopamine ($0.1 \text{ } \mu\text{mol kg}^{-1}$). In the sequential treatment (Groups 7–9), the standard drug was administered 3 min after injecting the plant extract. The doses of plant extracts and standard drugs were chosen on the basis of our pilot studies.

2.7 Activation of the rhythmic genital motor pattern of ejaculation

Immediately after spinal cord transection, ejaculatory motor patterns could be reflexively expressed and recorded in the genital muscles of all animals. To establish the capacity of the spinal apparatus to produce the genital rhythmic pattern after spinalization, two to three consecutive ejaculatory motor patterns were

repeatedly evoked at 3-min intervals by the injection of saline solution ($200 \text{ } \mu\text{L min}^{-1}$) through a PE-50 catheter (0.965 mm o.d.) inserted into the pelvic urethra through a bladder incision. Thereafter, one of the selected treatments (Groups 2–9) was intravenously applied and the number and frequency of contractions of the striated bulbospongiosus muscles obtained under their influence were recorded for 60 s. A similar protocol was considered for animals receiving saline solution (Group 1, control) [12, 6]. At 5 min after recording the EMG in each sequential treatment (Groups 7–9), three additive urethral stimulations were monitored at 3-min intervals, as described above. The frequency of contractions of the bulbospongiosus muscles was calculated by dividing the number of contractions by its duration.

2.8 Statistical analysis

The parameters recorded for each ejaculatory motor pattern were the number and frequency of contractions. Values were expressed as mean \pm SEM. Mean values were calculated for each animal and quantitative comparisons between groups were established from those means. One-way analysis of variance (ANOVA) followed by Tukey's test was carried out using SPSS for Windows version 10.0. Comparisons with $P < 0.05$ were considered to be statistically significant.

3 Results

3.1 Phytochemical screening

The samples were screened for the presence of sterol, triterpens and saponins.

3.2 Activation of the ejaculatory motor response by mechanical stimulation of the urethra

In all spinal cord transected and urethane-anesthetized rats, injection of saline solution ($200 \text{ } \mu\text{L min}^{-1}$) into the pelvic urethra (urethral stimulation) before each drug administration provoked rapid rhythmic contractions of the striated bulbospongiosus muscles with an average mean of all sensory induced-contractions of 5.75 ± 0.87 (Figure 1A, Table 1). In some cases, an expulsion of the urethral content was observed and the contractions were always accompanied by penile movements and penile erections.

3.3 Effects of aqueous and methanolic extracts of *B. engleriana* on fictive ejaculation

Single intravenous administration of either the

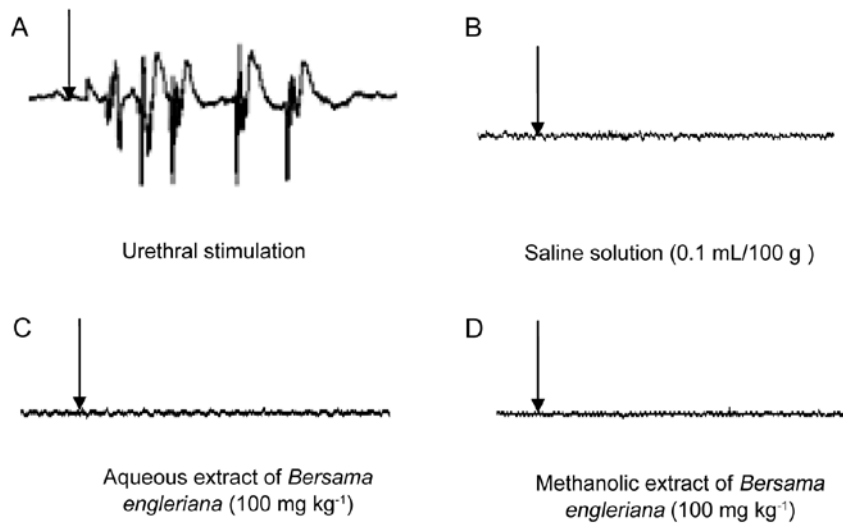


Figure 1. Original electromyographic (EMG) tracings showing the effects of urethral stimulation (A) and intravenous injection of saline solution (B), aqueous (C) and methanolic (D) extracts from the leaves of *Bersama engleriana* on the ejaculatory rhythmic motor pattern in sexually experienced spinal male rats. Arrows indicate the moment of injection. Calibration bar = 100 mv, 60s.

Table 1. Effect of urethral stimulation and intravenous administration of drugs on the number and frequency of contractions of bulbospongiosus muscles in spinal cord transected and urethane-anesthetized rats.

Treatment	Number of contractions (<i>N</i>)	Frequency of contractions (<i>N s</i> ⁻¹)
Urethral stimulation	5.75 ± 0.87	0.86 ± 0.16
Saline solution (0.1 mL per 100 g) (<i>n</i> = 5)	0.00 ± 0.00	0.00 ± 0.00
Aqueous extract of BE (100 mg kg ⁻¹) (<i>n</i> = 5)	0.00 ± 0.00	0.00 ± 0.00
Methanolic extract of BE (100 mg kg ⁻¹) (<i>n</i> = 5)	0.00 ± 0.00	0.00 ± 0.00
Oxytocin (0.5 UI kg ⁻¹) (<i>n</i> = 5)	7.33 ± 1.45 ^a	0.51 ± 0.09 ^a
Aqueous extract (100 mg kg ⁻¹) plus oxytocin (0.5 UI kg ⁻¹) (<i>n</i> = 5)	0.00 ± 0.00 ^b (5.67 ± 0.88)	0.00 ± 0.00 ^b (1.10 ± 0.34)
Methanolic extract (100 mg kg ⁻¹) plus oxytocin (0.5 UI kg ⁻¹) (<i>n</i> = 5)	0.00 ± 0.00 ^b (7.33 ± 0.88)	0.00 ± 0.00 ^b (0.85 ± 0.17)
Dopamine (0.1 μmol kg ⁻¹) (<i>n</i> = 5)	10.67 ± 2.40 ^a	0.82 ± 0.19 ^a
Aqueous extract (100 mg kg ⁻¹) plus dopamine (0.1 μmol kg ⁻¹) (<i>n</i> = 5)	0.00 ± 0.00 ^c (6.00 ± 1.00)	0.00 ± 0.00 ^c (1.28 ± 0.03)
Methanolic extract (100 mg kg ⁻¹) plus dopamine (0.1 μmol kg ⁻¹) (<i>n</i> = 5)	0.00 ± 0.00 ^c (10.00 ± 0.58)	0.00 ± 0.00 ^c (1.31 ± 0.04)

Abbreviations: BE, *Bersama engleriana*; *N*, number of rats per group. All values are expressed as mean ± SEM. Urethral stimulation represents the mean value of all urethral stimulations carried out in this study (three stimulations per rat). ^a*P* < 0.001 significantly different compared with saline solution. ^b*P* < 0.001 significantly different compared with oxytocin. ^c*P* < 0.001 significantly different compared with dopamine. Values in parenthesis represent mean value of urethral stimulation carried out 5 min after application of the corresponding sequential treatment. For each rat, the frequency of contractions was calculated by dividing the number of contractions (*n*) by its duration (s).

aqueous extract (100 mg kg⁻¹) or the methanolic extract (100 mg kg⁻¹) of *B. engleriana* in spinal cord transected and urethane-anesthetized rats did not activate the spinal pattern of ejaculation as evidenced by the absence of contraction of the striated bulbospongiosus muscles. These effects were similar to that obtained after intravenous saline injection (0.1 mL per 100 g) (Figure 1B, C, D and Table 1).

3.4 Effects of dopamine and oxytocin on fictive ejaculation

The effects of dopamine and oxytocin on spinal cord transected and urethane-anesthetized rats are outlined in Figure 2A, B and Table 1. Dopamine (0.1 μmol kg⁻¹) and oxytocin (0.5 UI kg⁻¹) provoked rhythmic contractions (*P* < 0.001) of the bulbospongiosus muscles. These rapid contractions were accompanied by sustained erection of the penis and sometimes with expulsion of the urethral

contents. The number and frequency of contractions were high after dopamine administration (number of contractions: 10.67 ± 2.40 ; frequency of contractions: 0.82 ± 0.19) compared with those in oxytocin-treated rats (number of contractions: 7.33 ± 1.45 ; frequency of contractions: 0.51 ± 0.09).

3.5 Effects of *B. engleriana* aqueous and methanolic extracts on the expression of dopamine and oxytocin-induced fictive ejaculation

The facts that the aqueous and methanolic extracts of *B. engleriana* possess inhibitory properties on fictive ejaculation on the one hand, and that urethral stimulation as well as dopamine and oxytocin intravenous injections provoke fictive ejaculation on the other hand were shown (Figure 1A, C, D and Figure 2A, B). Figure 2 (C, D, E, F) also clearly describes the effects of *B. engleriana* on the expression of dopamine and oxytocin-induced fictive ejaculations. It can be observed that the treatment of spinal cord transected and urethane-anesthetized rats with *B. engleriana* extracts before the intravenous injection of dopamine ($0.1 \mu\text{mol kg}^{-1}$) and oxytocin

(0.5 UI kg^{-1}) completely abolished the occurrence of ejaculation induced by these standard drugs. It is noteworthy mentioning that after subjecting the same animals to three consecutive urethral stimulations 5 min later, there were contractions of the ejaculatory muscles that were always accompanied by penile movements and erections (Figure 3). An increasing trend was also noticed in the number and frequency of contractions of the bulbospongiosus muscles when compared with the initial urethral stimulation (not shown). The rhythmic contractions of the ejaculatory muscles were sometimes followed by an expulsion of the urethral content.

4 Discussion

Results of the present study support the hypothesis that natural compounds contained in *B. engleriana* crude extracts might be useful in preventing ejaculatory response in a rat model for the study of ejaculation. In agreement with Sandroni [4], substances that increase sexual potency are considered as aphrodisiac compounds [17] and its influence is directly observed

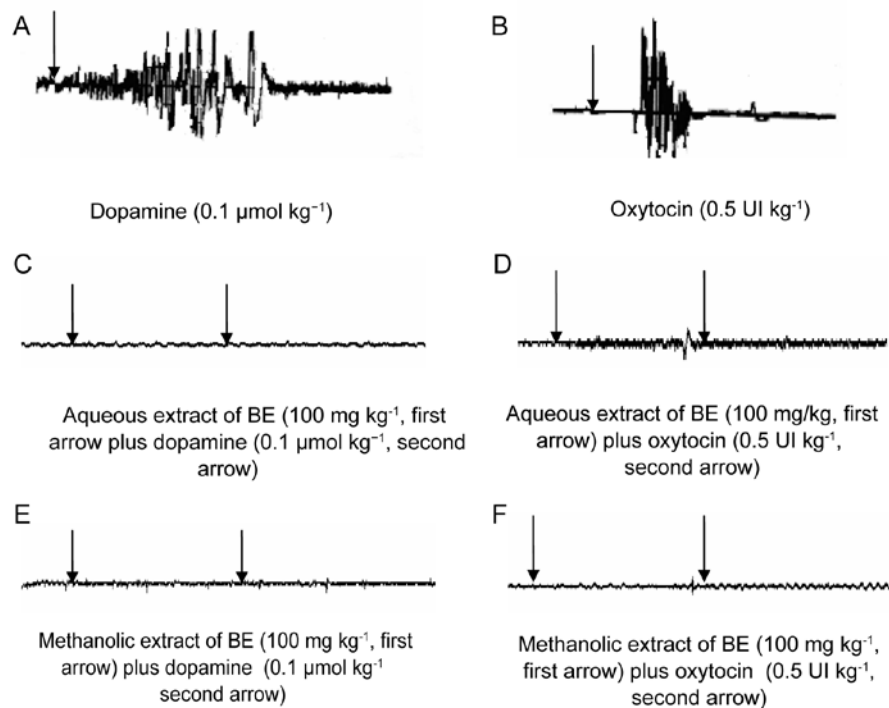


Figure 2. Original electromyographic (EMG) tracings showing the effects of dopamine ($0.1 \mu\text{mol kg}^{-1}$) (A), oxytocin (0.5 UI kg^{-1}) (B) and the sequential treatments (C, D, E, F) on the ejaculatory rhythmic motor pattern in sexually experienced spinal male rats. Arrows indicate the moment of injection of drugs. BE, *Bersama engleriana*. Calibration bar = 100 mv, 60 s.

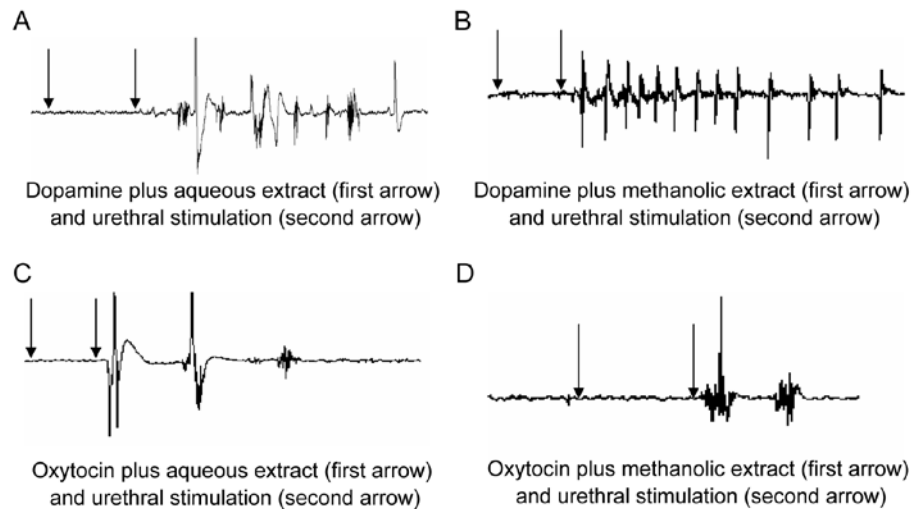


Figure 3. Original electromyographic (EMG) tracings showing the effects of sensory stimulation (second arrow) applied 5 min after the sequential treatments (first arrow) (A, B, C, D) on the ejaculatory rhythmic motor pattern in sexually virgin spinal male rats. Calibration bar = 100mv, 60 s.

on male sexual reflexes, including ejaculation. An increase in the sexual potency by the administration of aphrodisiac substances may comprise sustained penile erections, expression of ejaculation or its interruption. The inhibition of ejaculation provoked by the aqueous and methanolic extracts of *B. engleriana* could be considered as a substantial part into the categorization of aphrodisiacs that increase sexual activity by delaying ejaculation [4]. According to the folkloric claims, the leaves of *B. engleriana* are orally consumed by men seeking an increase in their sexual activities [5]. In connection with this belief, we recently showed that the oral administration of aqueous and methanolic extracts of *B. engleriana* leaves for 21 days to adult male rats increased the mount and intromission frequencies [8]. From these observations, it could be well understood that the kinetics of intravenous administration differs from that following oral administration, wherein the bioactive compounds present in the extracts may undergo some chemical and/or biochemical transformations in the digestive tract before entering the blood system. In this regards, the intravenous treatment carried out in the present work may lead to a direct contact of the extracts with the spinal and extra-spinal circuits of ejaculation, a centrally controlled sexual response in which the bulbospongiosus muscles play a primary role by way of contracting in a rhythmic manner [18–20]. It could then be thought that the biological activity of *B. engleriana* may be because of one or

more of the phytochemicals found in its extracts and which may interact directly with the elements of the spinal circuits in charge of ejaculation, leading to its inhibition. In an attempt to determine the possible mechanism(s) of action of *B. engleriana* in the blockade of ejaculation, the ejaculation-preventing effects of *B. engleriana* were evaluated on dopamine and oxytocin-induced ejaculation. As expected, dopamine and oxytocin significantly induced the expression of the ejaculatory motor pattern [11, 21]. The ability of the extracts to completely block the pro-ejaculatory effects of dopamine and oxytocin denotes the potential involvement of dopaminergic and oxytocinergic receptors. On the contrary to this inhibitory effect of *B. engleriana* on fictive ejaculation, the pro-ejaculatory and oxytocin-like properties of Cihuapatli (*Montanoa tomentosa*) have been reported [16]. Thus, it seems that the aphrodisiac plants have different effects on the expression of fictive ejaculation in spinal animals, but the clear participation of oxytocinergic system mediating pro-sexual actions is observed. Moreover, recording of the rhythmic contractions of the striated bulbospongiosus muscles in animals receiving the sequential treatments and submitted to further mechanical stimulations of the urethra suggests a short-lasting inhibitory effect of *B. engleriana* on the spinal center of ejaculation. Results of the present work also confirm the fact that in animals with transected spinal cords, ejaculatory responses remain intact and can be activated by

sensory or pharmacological means [20, 22–24]. Taken together, these results clearly show that the aphrodisiac properties of *B. engleriana* can be extended to ejaculatory function. In conclusion, inhibition of the expression of ejaculation by *B. engleriana* extracts can account for the increase in the ejaculatory latency and, hence, to an extended sexual activity, as claimed by traditional beliefs [5, 8]. Finally, results of the study give additive value to the aphrodisiac claim regarding *B. engleriana*.

Acknowledgment

One of the authors, Dr. Watcho Pierre, would like to thank the Academy of Science for Developing World (TWAS) for the “TWAS Fellowship for Research and Advanced Training”; Professor Ortiz Alberto Zamora, Director of the “Facultad of Agrobiología y Medicina Veterinaria” of the “Universidad Autónoma de Tlaxcala-México”, for financial support and the University of Dschang, Cameroon for his research leave at the “Universidad Autónoma de Tlaxcala, Mexico”.

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