

EFFECTS OF AQUEOUS EXTRACT OF *ALPINIA CALCARATA* RHIZOMES ON REPRODUCTIVE COMPETENCE OF MALE RATS

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This study examined the effects of rhizomes of *Alpinia calcarata* Roscoe (Zingiberaceae) on male sexual competence and fertility, using a hot water extract (HWE) and rats. Different doses of HWE (150, 250 and 500 mg/kg) were orally administrated to male rats and their sexual behaviour was monitored (for 15 min) 3 h later using receptive females. Fertility was determined in a separate group (with the highest dose) using a noncompetitive copulation test. In the sexual behaviour study, the HWE impaired the number of rats ejaculating and markedly prolonged the latency for ejaculation. Further, the number of rats mounting and intromitting, and the latencies for mounting and intromission were inhibited. Collectively, these observations indicate a strong aphrodisiac action. The other parameters remained unchanged indicating non-impairment in libido, sexual arousability, sexual vigour and sexual performance or penile erectile ability. However, a slight impairment was evident in sexual motivation (with the highest dose) in a partner preference test.

In the fertility test, HWE induced profound oligozoospermia but fertility was uninhibited. The highest dose of HWE also elevated the serum testosterone level and the number of spontaneous penile erections rapidly and markedly. Further, the HWE was nontoxic. It is concluded that *A. calcarata* rhizomes possess a strong and safe oral aphrodisiac activity.

Keywords: *Alpinia calcarata* – aphrodisiac – oligozoospermia – fertility

INTRODUCTION

The rhizomes of *Alpinia galanga* Roscoe (Zingiberaceae) are often recommended as an orally active natural aphrodisiac in traditional medicine in many parts of the world [13, 25]. Experimental studies have confirmed the presence of aphrodisiac activity of this plant [12]. On the other hand, some Sri Lankan traditional medical practitioners

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claim that the rhizomes of a closely related species, *A. calcarata* Roscoe, possesses strong aphrodisiac activity: it is recommended that a piece of fresh rhizome be kept in the oral cavity during sexual intercourse to improve sexual performance [25]. Further, Arambewela et al. [3] have shown the presence of essential oils with 18 volatile constituents in the rhizome of *A. calcarata*. Herbal preparations of plants, which are applied to the penis to delay ejaculation, are known to contain essential oils that possess an aphrodisiac activity [23]. Thus, a strong possibility exists that *A. calcarata* rhizomes may also have this aphrodisiac activity. However, as yet, the effects of *A. calcarata* rhizomes on sexual behaviour has not been scientifically validated. The main aim of this study was to investigate the effects of *A. calcarata* rhizomes on male sexual competence. The other aim was to ascertain its effects on male fertility. These aspects were tested in rats using a hot water extract (HWE) of the rhizome with a view to extrapolate the data obtained to humans.

A. calcarata, known as Heen-aratha in Sinhala and Amkoliniji in Tamil is a rhizomatous perennial herb. The mature rhizomes are branched and dense with a light to dark brown colour. The leaves of the plant are simple and alternate. The flowers are irregular, bisexual and pedunculate [8, 13]. *A. calcarata* is cultivated in tropical countries including Sri Lanka, India and Malaysia. Especially, decoctions are made from the rhizomes of *A. calcarata* are used for medicinal purposes [13]: in the treatment of bronchitis, cough, respiratory ailments, diabetes, asthma [25] and arthritis [2, 25]. Experimentally, extracts of *A. calcarata* have shown to possess antibacterial [11], antifungal [24], antihelmintic [14] and antinociceptive activities [4]. Further, essential oil of *A. calcarata* is shown to have repellent activity against the common cockroach *Periplaneta americana* [20].

Phytochemical screening have shown the presence of alkaloids, steroidal, coumarins, reducing sugars and flavonoids in hot water and hot ethanolic extracts of the rhizomes of Sri Lankan *A. calcarata* [4].

MATERIALS AND METHODS

Plant material

Fresh *A. calcarata* rhizomes were collected from home gardens in the Kalutara district, Sri Lanka between August–November 2002. The plant material was identified and authenticated by Mr. D. H. P. Peramunagama, Curator of the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (AS 01) was deposited in the Industrial Institute, Colombo 7, Sri Lanka.

Animals

Adult Wistar rats of proven fertility (males 225–250 g and females 200–225 g) kept under standardised animal house conditions (temperature: 28–31 °C, photoperiod:

approximately 12 hours with natural light per day, relative humidity: 50–55%) were used. They had free access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and tap water.

Preparation of A. calcarata HWE

Fresh *A. calcarata* rhizomes were cut into small pieces and air-dried for 5–6 days in the shade. Five hundred grams of dried rhizomes were boiled with 2.5 L (to cover the raw material) of distilled water (DW) [4] for 4 hours. The hot water extract was concentrated under vacuum at 60 °C and freeze-dried at –20 °C (yield 15.6% w/w dry weight basis) and stored at 4 °C until use.

Administration of extracts

Doses of 150, 250 and 500 mg/kg of HWE were made in 1 ml DW [4] and were given orally to separate groups of rats. One millilitre of DW was given orally as a control.

Effects on sexual behaviour

Sexual behaviour was examined in a noncompetitive copulation test. Twenty-five male rats were assigned into four groups and orally administrated with HWE in the following way: (1) HWE, 150 mg/kg, n = 6, (2) HWE, 250 mg/kg, n = 6, (3) HWE, 500 mg/kg, n = 7 and (4) vehicle control, 1 ml of DW, n = 6. The treated rats were placed individually in cages for 3 hours following administration of vehicle or HWE. Following a 10-min adaptation period, a female that had been brought into oestrous (oestradiol benzoate 12 µg in olive oil injected intramuscularly 56 hours plus 0.5 mg progesterone in olive oil 8 hours prior to pairing) was placed in the cage. The pre-coital behaviour was observed for 15 min or until ejaculation. During this period the mount latency (the time from the introduction of the receptive female to the first mount): intromission latency (the time from the introduction of the receptive female to the first intromission) and ejaculation latency (the time from the introduction of the receptive female to ejaculation) and the number of mounts and number of intromissions displayed by the rats were recorded. When there is no intromission and ejaculation during the observation period their latencies were taken as 15 min. Using these data the following parameters were computed, % mounted, % intromitted, % ejaculated, copulatory efficiency [(number of intromissions/number of mounts) × 100], intromission ratio (number of intromission/number of intromission + number of mounts) and intercopulatory interval (average time between intromissions) [27].

Partner preference test

Twelve male rats were assigned into two equal groups ($n = 6/\text{group}$). One group was orally treated with 500 mg/kg of HWE and the other group with 1 ml of DW. These rats were then individually caged. Sixty minutes later, one oestrous (receptive) and one dioestrous (nonreceptive) female were introduced to the cage of each of the treated males. The cumulative time spent by a male in physical contact with each of the females was recorded for 15 min. Partner preference index was calculated by subtracting the time spent with dioestrous female from the time spent with oestrous female. A positive score indicates preference for the sexually receptive female while a negative score indicates its preference for the nonreceptive female [10].

Determination of serum testosterone level

Twelve male rats were randomly divided into two groups ($n = 6/\text{group}$). One group was orally treated with 500 mg/kg/day of HWE and the other with 1 ml of DW/day for 3 consecutive days. On day 1 post-treatment, these rats were anaesthetised with ether and 1 ml of blood was removed from the heart of each rat using a syringe. The blood was stored overnight at 4 °C and serum was separated on the following morning (8.00–9.00 h). Testosterone level was measured using an immunometric assay with IMMULITE analyser (Diagnostic Products Corporation, California, USA), as per instructions given by the manufacturer.

Determination of serum prolactin level

Twelve male rats were randomly divided into two equal groups ($n = 6/\text{group}$). One group was orally treated with 500 mg/kg/day of HWE for 3 consecutive days and the other with 1 ml/day of DW. On day 1 post-treatment, these rats were anaesthetised with ether and 1 ml of blood was removed from each rat through a cardiac puncture using a syringe. The blood was stored overnight at 4 °C and on the following morning serum was separated carefully using a micropipette. Prolactin level was determined using an immunometric assay with IMMULITE analyser, as per instructions given by the manufacturer.

Effects on penile erections

Twelve male rats were assigned into two equal groups ($n = 6/\text{group}$). One group was orally treated with 500 mg/kg of HWE and the other group with 1 ml of DW. Shortly after treatment, these rats were placed individually in plexiglass cages and the number of penile erections (characterized by penile body elongation and its dorsiflection, reddening and engorgement of glans and its flaring) were recorded for 30 min as described in detail by De Mateos-Verchere et al. [9].

Effects on fertility

Fifteen rats were divided into two groups. One group ($n = 9$) was orally treated with 500 mg/kg of HWE and the other group ($n = 6$) with 1 ml of DW at 16.00 h. Each male was then individually paired overnight with a pro-oestrous female at 16.30–17.00 h. Vaginal smears of the paired females were taken on the following morning (at 8.00–8.30 h) and examined microscopically ($\times 100$). If spermatozoa were present, their numbers and gross morphology were noted and this was taken as the onset of pregnancy. If spermatozoa were absent then daily vaginal smearing was undertaken for at least 14 days to determine the appearance or pregnancy or pseudo-pregnancy. At 14 days after mating, the females were subjected to uterotomy under ether anaesthesia to determine the number and viability of fetuses and number of resorption sites. The gross appearance of corpora lutea was also recorded. The following reproductive indices were computed: index of libido [(number mated/number paired) $\times 100$], quantal pregnancy [(number pregnant/number mated) $\times 100$], fertility index [(number pregnant/number paired) $\times 100$], implantation index [(total number of implantation/number mated) $\times 100$], pre implantation loss [(number of corpora lutea-number of implants)/number of corpora lutea] $\times 100$, and postimplantation loss [(number of implants-number of viable implants)/number of implants] $\times 100$ [27].

Effects of sperm number in urine

This was done as described by Ratnasooriya et al. [26]. Briefly, 18 male rats were randomly assigned into two groups. One group ($n = 9$) was orally treated with 500 mg/kg of HWE and the other with 1 ml of DW. These rats were individually placed in wire-meshed cages and urine was collected for 24 h. These were centrifuged at approximately 1600 g for 5 min and the pellet was resuspended in saline. Then the sperm numbers were estimated in duplicate using improved Neubaur type haemocytometer.

Effects on muscle strength and co-ordination

Twelve rats were randomly divided into two equal groups ($n = 6$ /group). One group was orally treated with 500 mg/kg of HWE and other with 1 ml of DW. One hour post-treatment, each of these rats was subjected to bar holding test [to evaluate muscle strength [22] and the time taken (in sec) for the rat to fall from the bar was determined. Immediately following this test, these rats were subjected to Bridge test [to evaluate muscle co-ordination [22] and the latency to slide off (in sec) were recorded.

Chronic toxicity

Male rats were randomly divided into two equal groups ($n = 9/\text{group}$). Group 1 received 1 ml of HWE orally at the dose of 500 mg/kg and group 2 received 1 ml of DW per day for 42 consecutive days (between 9.00–11.00 h). Rats were checked twice daily (12.00 h and 16.00 h) for overt signs of toxicity (salivation, diarrhoea, teeth exposure, lacrimation, ptosis, stupor, tremors, ataxia, rolling, jumping, yellowing of hair, loss of hair, postural abnormalities and behavioural changes), stress (fur erection or exophthalmia) and aversive behaviours (biting of paw and penis, intense grooming behaviour, scratching behaviour, licking of tail or vocalization), morbidity and mortality. The consistency of the faeces and colour of urine were noted daily.

On day 1 post-treatment, blood was taken from the tail under ether anaesthesia and serum was separated. The serum activities of glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), urea and creatinine were determined using Randox enzyme kits (Randox Laboratories Ltd., Antrim, UK) and a spectrophotometer (Jasco V 500, Jasco Corporation, Tokyo, Japan), as per instructions given by the manufacturer.

At the same time anticoagulant blood samples were analyzed for red blood cell (RBC) counts, white blood cells (WBC) counts and hemoglobin (Hb) concentration.

Statistical analyses

Data are given as mean $\pm$ SEM. Statistical comparisons were made using the G-test and Mann-Whitney U-test as appropriate. $p < 0.05$ was accepted as indicating significance.

RESULTS

Effect on sexual behaviour

All the three doses of the HWE significantly ($p < 0.05$) and dose-dependently ($r^2 = 0.80$, $p < 0.05$) reduced the number of rats that ejaculated (low dose, 3 of 6, mid dose, 1 of 6 and high dose 0 of 7) within the test period (see Table 1 and Fig. 1). In addition, the time required for the HWE treated rats to ejaculate was greatly (low dose, by 48.4%; mid dose, by 48.6%; and high dose, by 55.3%) and significantly ($p < 0.05$) prolonged (Table 1 and Fig. 2).

On the other hand, all the HWE treated rats mounted and intromitted without displaying aberrant sexual behaviour, as the vehicle treated rats. Furthermore, the times required to mount and intromit, intromission ratio, copulatory efficiency and inter-copulatory interval of treated rats were not significantly ($p > 0.05$) altered (Table 1).

Effect on partner preference test

The high dose of HWE moderately (by 68%) and significantly ($p < 0.05$) impaired the partner preference index (control vs. treatment: 59.9 ± 5.0 vs 19.1 ± 2.9 sec).

Table 1
Effect of oral treatment of hot water extract of *Alpinia calcarata* (150, 250 or 500 mg/kg) on the sexual behaviour of male rats ($n = 6-7$, $\pm$ SEM; ranges in parentheses)

Parameter	Control	150 mg/kg	250 mg/kg	500 mg/kg
% mounted	100	100	100	100
% intromitted	100	100	100	100
% ejaculated	100	50.0*	0.0**	0.0**
No. of intromissions	15.5 ± 2.4 (11–27)	18.3 ± 2.0 (13–25)	14.5 ± 1.3 (11–19)	18.2 ± 2.0 (11–26)
Mount latency (sec)	57.7 ± 18.5 (17–130)	56.3 ± 22.3 (20–165)	74.5 ± 17.0 (18–136)	47.4 ± 1.3 (8–87)
Intromission latency (sec)	57.3 ± 18.5 (17–130)	56.3 ± 22.3 (20–165)	78.5 ± 16.1 (65–136)	64.1 ± 18.2 (8–150)
Intromission ratio	0.5 ± 0.0 (0.5–0.5)	0.5 ± 0.0 (0.5–0.5)	0.49 ± 0.0 (0.49–0.5)	0.49 ± 0.0 (0.44–0.5)
Copulatory efficiency	100 ± 0.0 (100–100)	100 ± 0.0 (100–100)	98.8 ± 1.2 (92.8–100)	97.3 ± 3.0 (97.3–100)
Intercopulatory interval (sec)	39.1 ± 3.7 (28.5–52.3)	51.2 ± 5.0 (38.4–69.2)	$61.8 \pm 6.3^*$ (47.4–81.8)	53.3 ± 6.4 (36.0–1.8)
Ejaculatory latency (sec)	579.5 ± 62.3 (428–787)	$860.3 \pm 22.2^*$ (769–900)	$861.2 \pm 39.0^{**}$ (667–900)	$900.0 \pm 0.0^{**}$ (900–900)

* $p < 0.05$, ** $p < 0.01$, compared with the control (Mann–Whitney U-test and G-test)

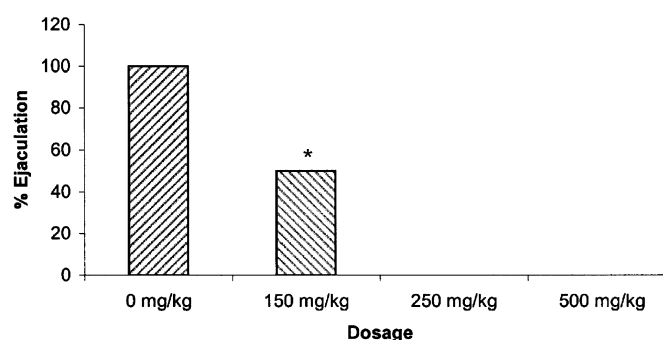


Fig. 1. Effect of oral treatment of hot water extract of *Alpinia calcarata* (150, 250 and 500 mg/kg) on % ejaculation of male rats. $n = 6-7 \pm$ SEM. * $p < 0.05$, compared with the control (Mann–Whitney U-test)

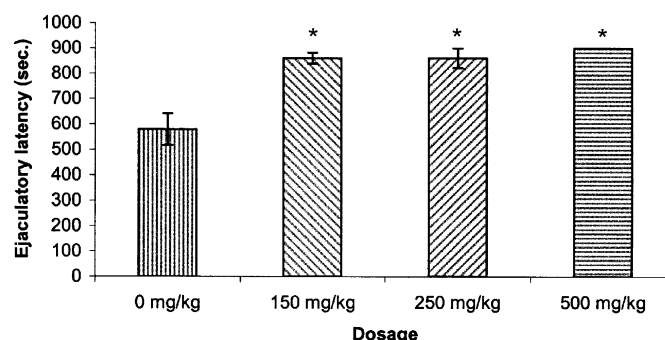


Fig. 2. Effect of oral treatment of hot water extract of *Alpinia calcarata* (150, 250 and 500 mg/kg) on ejaculatory latency of male rats. $n = 6-7 \pm \text{SEM}$. * $p < 0.05$, compared with the control (Mann-Whitney U-test)

Effects on serum testosterone level

Inter- and intra-assay coefficient of variation for testosterone was 6.8% and 9.1%, respectively. The high dose of HWE markedly (by 122%) and significantly ($p < 0.05$) increased the serum testosterone level (control vs. treatment: 46.6 ± 8.6 vs. 103.5 ± 30.4 ng/dl).

Effect on serum prolactin level

Inter- and intra-assay coefficient of variation for prolactin were 5.5% and 7.4%, respectively. The high dose of HWE did not significantly ($p > 0.05$) alter the serum prolactin level (control vs. treatment 0.50 ± 0.01 vs. 0.52 ± 0.03 mg/ml).

Effect on penile erection

The high dose of HWE provoked significant ($p < 0.05$) increases in spontaneous erections (control vs. treatment: 0.0 ± 0.0 vs. 2.9 ± 0.2). Further, there were no milky exudates evident at the penises of both HWE and control rats.

Effect on fertility

In the fertility study, all the rats treated with high doses of HWE ejaculated but the number of vaginal sperms was significantly ($p < 0.05$) and markedly (by 89%) reduced (control vs. treatment 27.7 ± 1.5 vs. $2.9 \pm 0.3 \times 10^6/\text{ml}$) (see Fig. 3). However, these ejaculated sperms were not decapitated and were devoid of any gross morphological abnormalities. On the other hand, despite this lowering of vaginal

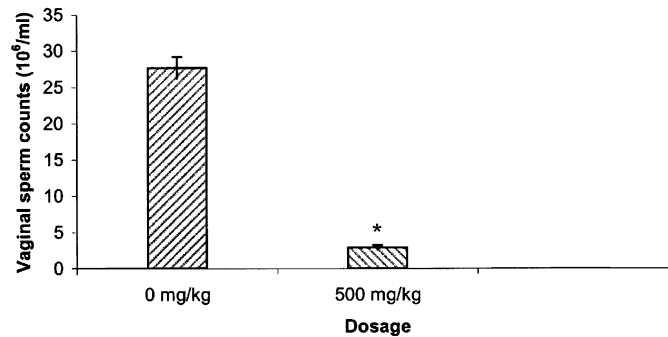


Fig. 3. Effect of oral treatment of hot water extract of *Alpinia calcarata* (500 mg/kg) on vaginal sperm counts of female rats. $n = 6$, $\pm$ SEM. * $p < 0.05$, compared with the control (Mann–Whitney U-test)

sperm numbers none of the fertility parameters investigated, namely, quantal pregnancy, fertility index, implantation index, number of implants, pre implantation loss and post implantation loss were significantly inhibited ($p > 0.05$) (Table 2).

Table 2
Effect of oral treatment of high dose (500 mg/kg) of *Alpinia calcarata* hot water extract on some fertility parameters of female rats (means $\pm$ SEM)

Parameter	Control	Treated
Index of libido %	100	100
Quantal pregnancy %	100	100
Fertility index %	100	100
Implantation index %	916	777
Number of implants	9.16	7.77
Pre-implantation loss	26.0 $\pm$ 4.0	34.1 $\pm$ 1.8
Post-implantation loss	0.0	3.75 $\pm$ 1.24
Vaginal sperm counts (10 ⁶ /ml)	27.7 $\pm$ 1.5	2.9 $\pm$ 0.33**

** $p < 0.01$, compared with the control (Mann–Whitney U-test and G-test)

Sperm in urine

No sperms were detected in the urine of both control and HWE-treated rats.

Effect on muscle strength and co-ordination

The HWE failed to alter the latency to fall significantly ($p > 0.05$) in the bar holding test (control vs. treatment: 60.0 ± 0.0 vs. 60 ± 0.0 sec) and the latency to the slide off in the Bridge test (control vs. treatment: 40.9 ± 9.6 vs. 48.8 ± 4.3 sec).

Effect of chronic toxicity

There were no treatment-related deaths or morbidity evident. Further, the HWE did not induce any overt signs of toxicity, stress or aversive behaviour. The consistency of faeces and colour of urine of HWE treated rats were essentially similar to that of the control. The HWE did not significantly ($p > 0.05$) induce toxicity in terms of liver SGOT (control vs. treatment: 37.0 ± 2.3 vs. 41.1 ± 3.5 U/l) and SGPT (control vs. treatment: 16.8 ± 1.0 vs. 17.5 ± 1.1 U/l) levels and kidney serum urea (control vs. treatment: 32.0 ± 2.7 vs. 34.7 ± 4.0 mg/dl) and creatinine (control vs. treatment: 0.74 ± 0.05 vs. 0.71 ± 0.04 mg/dl).

DISCUSSION

In this study we examined the effects of HWE of *A. calcarata* rhizome on male reproductive competence in rats. Rats were used as subjects as they are one of the most widely used animals in reproductive studies [33] as there are obvious homologies between the copulatory behaviour of humans and rats [21]. The results show that the oral administration of HWE of *A. calcarata* rhizomes markedly prolonged the ejaculatory latency without concurrently altering other parameters of male sexual behaviour and producing unacceptable side-effects. This indicates strong and safe aphrodisiac action. Further, the HWE neither induced anejaculation nor interrupted orgasm. Collectively, these observations also indicate that the HWE may be effective in the treatment of premature ejaculation. Premature ejaculation is the most common sexual dysfunction [18] affecting the sexual well-being of both partners.

All the HWE treated rats mounted and intromitted without any impairment of mount and intromission frequencies, copulatory efficiency or intercopulatory interval. This suggests that the sexual arouseability [6], libido, sexual vigour and sexual performance [27] were uninhibited during the induction of the aphrodisiac action. Interestingly, HWE did not suppress intromission ratio, suggesting an unimpaired penile erectile function [31]. In addition, HWE provoked spontaneous noncontact erections. These are analogous and perhaps homologous to human psychogenic penile erections [31]. This also indicates that HWE may be useful in the treatment of at least some forms of erectile dysfunction. In contrast, several herbal extracts such as *Terminalia catappa* seeds [27], *Mucuna prurita* seeds [28], *Piper betle* leaves [30], *Artocarpus heterophyllus* seeds [29] although prolong time to ejaculation severely depresses other aspects of sexual behaviour. Thus, the aphrodisiac action of *A. cal-*

carata is superior to these plant extracts. However, in the partner preference test, although HWE displayed a positive score, the partner preference index was moderately reduced. This suggests that the HWE exert mild depressive action on sexual motivation [10]. This is a negative effect of HWE on sexual function, which is noteworthy.

Male libido is androgen-dependent and antiandrogens suppress masculine sexual behaviour of rats [19]. There is evidence that hyperprolactinaemia in males inhibit libido [7]. HWE elevated the serum testosterone level rapidly and markedly without changing the prolactin level. This indicates that the HWE may facilitate overall male sexual function. Furthermore, male sexual function decreases profoundly with ageing [16]. Because HWE has serum testosterone elevating activity with no alterations in the liver function tests, HWE may be useful in improving sexual function of ageing men and men with impaired libido.

The onset of prolongatory effect on ejaculation was quite rapid. This suggests an action on the central nervous system. Several neurotransmitter systems in the central nervous system are implicated with male sexual behaviour [31]. Dopamine and dopamine agonist facilitate male sexual behaviour [31]. Since HWE lacks dopaminergic activity [4] prolongation of ejaculatory latency is unlikely to be mediated via dopaminergic system. On the other hand, HWE may prolong ejaculatory latency via opioid mechanisms: opioids facilitate masculine sex behaviour [31] and HWE is shown to have opiomimetic activity [4]. The prolongation of the ejaculatory latency could also be due to essential oils reported to be present in the rhizomes [3] as herbal preparations with essential oils are shown to delay ejaculation [23]. On the other hand, the effect of HWE on the other neurotransmitter systems in the mediation of this aphrodisiac action is unknown at present. Interruption of penile sensitivity can prolong latency to ejaculation [31]. But such a mechanism is unlikely as there was no aberrant sexual behaviour [31] and because intromission ratio of HWE rats remained unaltered [31]. Motoric incoordination can prolong time to ejaculation but this mechanism seems unlikely since the latencies of both bar and Bridge tests remained unchanged.

In the fertility study, rats treated with HWE produced oligozoospermic ejaculates without teratozoospermia. Since the onset of this effect was extremely quick it cannot be attributed to inhibition of spermatogenesis [17], production of sperm granulomas [15] or the enhanced epididymal sperm resorption [5]. Because there were no overt signs of stress oligozoospermia cannot be due to stress either [15]. Spermatorrhoea can reduce sperm numbers in ejaculates [15] but this mechanism is also unlikely as there were no sperms in the urine of HWE-treated rats. Production of spontaneous ejaculations is yet another mechanism that can induce oligozoospermia [1]. But this too is unlikely as there were no milky exudates evident in HWE-treated rats. So, it is likely that HWE induced oligozoospermia via inhibition of ejaculatory contraction of vas deferens and epididymis [32]. In spite of the marked oligozoospermia HWE failed to impair fertility: rats are known to be fertile even after 90% depletion of sperm counts [17]. In contrast, human fertility is impaired by even a small reduction of sperm counts [17]. Thus, if HWE can induce oligozoospermia

in a magnitude comparable to that in rats, fertility in humans would be seriously impaired.

In conclusion, this study scientifically demonstrates, for the first time, aphrodisiac potential and the ability of HWE of *Alpinia calcarata* rhizomes to produce oligozoospermic ejaculates.

REFERENCES

1. Agmo, A. (1976) The number of spermatozoa in spontaneous ejaculates of rats. *J. Reprod. Fertil.* 48, 405–407.
2. Arambewela, L. S. R., Basnayake, C. S., Serasinghe, P., Tissera, M. S. A., Dias, S., Weerasekera, D. R. (1995) *Traditional Treatment in Sri Lanka for Chronic Arthritis*. NARESA Printing unit, Colombo, Sri Lanka, pp. 1–10.
3. Arambewela, L. S. R., Kumarasinghe, A., Arawwawala, M., Owen, N. L., Du, L. (2004) Volatile oils of *Alpinia calcarata* grown in Sri Lanka. *J. Essent. Oil Res.* (in press)
4. Arambewela, L. S. R., Arawwawala, L. D. A. M., Ratnasooriya, W. D. (2004) Antinociceptive activities of aqueous and ethanolic extracts of *Alpinia calcarata* rhizomes in rats. *J. Ethnopharmacol.* 95, 311–316.
5. Barrat, C. L. R., Cohen, J. (1987) Quantification of sperm disposal and phagocytic cells in the tract of short and long-term vasectomized mice. *J. Reprod. Fertil.* 48, 405–407.
6. Benelli, A., Arletti, R., Poggioli, R., Cavazzati, E., Mameli, M., Bertolini, A. (1997) Effect of potassium channel modulators on male sexual behaviour. *Life Sci.* 60, 263–267.
7. Chambers, K. C., Phoenix, C. H. (1992) Sexual behaviour and serum levels of prolactin, testosterone and oestradiol in young and old *Rhesus* males. *Physiol. Behav.* 52, 13–16.
8. Dassanayake, D., Fosberg, F. R. (1981) *A Revised Hand Book to the Flora of Ceylon*. Amreind, New Delhi, India 4, pp. 517–518.
9. De Mateos-Verchere, J. G., Leprince, J., Tonson, M. C., Vaudry, H., Costetin, J. (1998) The octadecanuropeptide ODN inhibits apomorphine-induced yawning in rats. *Eur. J. Pharmacol.* 357, 121–126.
10. Edwards, D. A., Griffis, K. T., Tardivel, C. (1990) Olfactory bulb removal: effect on sexual behaviour and partner preference in male rats. *Physiol. Behav.* 48, 447–450.
11. George, M., Panalai, K. M. (1949) Investigations on plant antibiotics. *Ind. J. Med. Res.* 37, 169–181.
12. Islam, M. W., Zakaria, M. N. M., Radhakrishnan, R., Liu, X. M. (2000) Galangal (*Alpinia galanga* Willd.) and black seeds (*Nigella sativa* Linn) and sexual stimulation in male mice. *J. Pharm. Pharmacol.* 52, 278–280.
13. Jayaweera, D. M. A. (1982) *Medicinal Plants Used in Ceylon*. Vol. V. National Science Council of Sri Lanka, Colombo, p. 213.
14. Kaleysa, R. R. (1975) Screening of indigenous plants for antihelmintic activity against human *Ascaris lumbricoides*. *Ind. J. Physiol. Pharmacol.* 19, 47–49.
15. Koeman, M. (2001) Male fertility and subfertility: etiology and treatment considerations. *Aust. J. Med. Herbalism* 13, 85–94.
16. Lunenfeld, B. (2001) Aging men—challengers ahead. *Asian J. Androl.* 3, 161–168.
17. Mangelsdorf, I., Buschmann, J., Orthen, B. (2003) Some aspects relating to the evaluation of the effects of chemicals on the male fertility. *Regulatory Toxicol. Pharmacol.* 37, 356–369.
18. Metz, M. E., Pryor, J. L., Nesuacil, L. J., Abuzzahab, T., Koznar, J. (1997) Premature ejaculation: a psychophysiological review. *J. Sex Marital Therap.* 23, 3–23.
19. Neumann, F. (1994) The antiandrogen cyproterone acetate: discovery, chemistry, basic pharmacology, clinical use and tool in basic research. *Expt. Clin. Endocrinol.* 102, 1–32.

20. Paranagama, P. A., Ekanayake, E. M. D. S. (2004) Repellant properties from essential oil of *Alpinia calcarata* Rosc. against the American cockroach, *Periplaneta americana*. *J. Nat. Sci. Foundation Sri Lanka* 32, 1–12.
21. Pfaus, J. G. (1996) Homologies of animal and human sexual behaviours. *Hormones Behav.* 30, 187–200.
22. Plaznik, A., Stefanski, R., Palejko, W., Kotawski, W. (1993) The role of accumbens GABA-B receptors in the regulation of rat behaviour. *Neurosci. Res. Comm.* 12, 23–30.
23. Puri, H. S. (1971) Vegetable aphrodisiacs of India. *Quarterly J. Crude Drug Res.* 11, 1742–1748.
24. Pushpangadan, P., Atal, C. K. (1984) Ethno-medico-botanical investigations in Kerala. *J. Ethnopharmacol.* 111, 59–77.
25. Ramanayake, L. (1994) *Osu Visituru*. Publication of Department of Ayurveda, Colombo, pp. 68–71.
26. Ratnasooriya, W. D., Dharmasiri, M. G. (1998) Effect of water extract of small unripe *Areca catechu* nuts on sperm output in rats. *Med. Sci. Res.* 26, 263–266.
27. Ratnasooriya, W. D., Dharmasiri, M. G. (2000) Effects of *Terminalia catappa* seeds on sexual behaviour and fertility of male rats. *Asian J. Androl.* 2, 213–219.
28. Ratnasooriya, W. D., Dharmasiri, M. G., Sirisena, D. C. (1999) Effects of *Mucuna prurita* seeds on sexual behaviour of male rats. *Med. Sci. Res.* 27, 375–378.
29. Ratnasooriya, W. D., Jayakody, J. R. A. C. (2002) *Artocarpus heterophyllus* seeds inhibits sexual competence but not fertility of male rats. *Ind. J. Expt. Biol.* 40, 304–308.
30. Ratnasooriya, W. D., Premakumara, G. A. S. (1996) Piper belt leaves impairs masculine sexual behaviour of rats. *Med. Sci. Res.* 24, 303–306.
31. Sachs, B. D. (2002) Contextual approaches to the physiology and classification of erectile function, erectile dysfunction and sexual arousal. *Neurosci. Biobehav. Rev.* 24, 541–560.
32. Thomas, A. J. (1983) Ejaculatory dysfunction. *Fertil. Steril.* 39, 445–454.
33. Yonezawa, A., Watanabe, C., Ando, R., Furata, S., Sakurda, S., Yoshimara, H., Iwanaga, T., Kimura, Y. (2000) Characterization of p-chloroamphetamine-induced penile erection and ejaculation in anaesthetized rats. *Life Sci.* 67, 3031–3039.