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·*Complementary Medicine*·

Effects of *Boesenbergia rotunda* (L.) Mansf. on sexual behaviour of male rats

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Abstract

Aim: To study the effects of *Boesenbergia rotunda* (Krachai) on sexual behaviour in male albino rats. **Methods:** Thirty-two male Wistar rats were equally divided into four groups: experimental groups were gavaged with the ethanolic extract of the rhizome of *B. rotunda* at doses of 60, 120 and 240 mg/kg and a control group received distilled water, for 60 days. Sexual behaviour, reproductive organs, diameter of seminiferous tubule, epididymal sperm density, and androgenic hormones were evaluated. **Results:** Within 30-min observation, there was no significant difference of courtship behaviour, mount frequency (MF), intromission frequency (IF), mount latency (ML), intromission latency (IL), copulatory efficiency or intercopulatory interval in male rats. In three 10-min intervals over a 30-min period, courtship behaviour and MF during the first 10-min were significantly higher than those in the second and third 10-min observation in all groups, whereas IF had no significant difference. All doses of *B. rotunda* extract significantly increased the relative testicular weight and the diameter of the seminiferous tubules. The dose of 60 mg/kg also significantly increased the relative weight of the seminal vesicle. Nevertheless, the sperm density, serum testosterone and androstenedione levels were not affected by the *B. rotunda* extract. **Conclusion:** *B. rotunda* does not affect sexual behaviour nor serum androgenic levels. *(Asian J Androl 2007 Nov; 9: 849–855)*

Keywords: *Boesenbergia rotunda*; sexual behaviour; testosterone; androstenedione; reproductive organ; sperm density

1 Introduction

Boesenbergia rotunda (L.) Mansf., called "Krachai" in Thai, is a herb of the Zingiberaceae family. The fresh rhizomes are commonly used in Southeast and South Asia as a food ingredient. In Thailand, they are also used as a folk medicine for health-promotion, antiflatulence, stomach discomfort, diuresis, leucorrhea, treatment of oral diseases and antidysentery [1] without acute toxicity [2]. The rhizomes of *B. rotunda* were found to contain 1,5-Cineol, Boesenbergin A, dl-Pinostrobin Corphor, flavonoid, Chromene [1], panduratin C, panduratin A, hydroxypanduratin A, helichrysetin, 2', 4',6'-trihydroxyhydrochalcone and uvangoletin [3]. In addition to the purposes of primary health care, the rhizomes have been reported as having aphrodisiac properties [4–7]. It is sometimes referred to as "Thai ginseng" [4] and has long been used among Thai men for sexual enhancement by using it as an ingredient of traditional remedies for impotency [5] and a sexual enhancing herb [4–8]. Nevertheless, there is no animal model to con-

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firm or support these properties.

In the present study, we investigated the effect of ethanolic extract from the rhizomes of *B. rotunda* on sexual behaviour, reproductive organs, diameter of seminiferous tubule, sperm density and sex hormones in male albino rats to evaluate its properties.

2 Materials and methods

2.1 Animals

Thirty-two male and 20 female Wistar rats (*Rattus norvegicus*), weighing 200–240 g and 6 weeks of age were purchased from the National Laboratory Animal Centre, Salaya, Nakhorn Pathom, Thailand. The animals were housed in groups (two rats per cage) under standard conditions (inverted 12 h : 12 h light : dark cycle) at 25 ± 2ºC and with free access to pelleted food and water at the Animal Facility Unit, Department of Biology, the Faculty of Science, Chiang Mai University. They were accustomed to the housing conditions for at least 1 week before studying. All experimental procedures were in accordance with institutional regulations for Animal Care and Use (No. RE 001/06), Department of Biology, Faculty of Science, Chiang Mai University.

2.2 Plants and extract preparation

The rhizomes of *B. rotunda* were collected from Chiang Mai Province, Thailand and identified by Dr Charun Maknoi, the Botany Section, Queen Sirikit Botanic Garden, Ministry of Natural Resources and Environment, Mae Rim, Chiang Mai, Thailand (voucher specimen No. 06-051718). They were sliced, dried in an oven at 60ºC, ground, extracted with 50% ethanol in a soxhlet apparatus and evaporated by rotary evaporation. This extract was stored at 4ºC till use. All doses of *B. rotunda* extract were diluted in distilled water at a low dosage (60 mg/kg BW), at the amount equivalent to the quantity of daily traditional use in humans (120 mg/kg BW) and at a high dose (240 mg/kg BW). The amount of 120 mg/kg BW was used by referring to Hem-hongsa and Chaophya Abhaibhubejhr Hospital (Muang, Prachinburi, Thailand) [1, 9].

2.3 Experimental design

The animals were divided into three groups (n = 8) of male rats that were given the *B. rotunda* extract at doses of 60, 120 and 240 mg/kg BW by oral administration for 60 days and a control group (n = 8), which received distilled water at 1 mL/day. Sexual behaviours were tested twice in each of the 32 male rats between days 26–30 and between days 56–60 of the *B. rotunda* application by introducing a receptive female with estrous phase into the observation cage. At day 61, the animals were anesthetized and blood was collected for hormone assay by cardiac puncture from the left ventricle. The testis, cauda epididymis, seminal vesicle and prostate gland were removed, trimmed of fat, and weighed. After weights were recorded, the cauda epididymis was cut into small pieces, homogenized in 10 mL of 0.9% NaCl and the sperm number was estimated in duplicate using a hemocytometer (Improved Neubauer, Boeco, Germany).

2.4 Measurement of seminiferous tubule diameter

The left testis from each animal was fixed in Bouin's solution for paraffin work and sectioned at 6 μm, stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS), and examined with a light microscope. Approximately 20 (per testis) cross-sections of seminiferous tubules with an apparent lumen were sampled. The diameter of round tubules or the short axis of elliptical tubules was measured using an ocular micrometer coupled to a 40 × objective lens.

2.5 Sexual behaviour testing procedure

The totals of 32 male rats were individually tested for sexual behaviour in a rectangular glass observatory cage and were given a 10-min accommodation for adaptation period. Thereafter, an estrous female was introduced and sexual behaviour was recorded along 30 min. Video recordings were made throughout the whole period for following behavioural parameters: courtship behaviour, sniffing, rubbing against or moving under or over the female's torsal [10]; mount frequency (MF), the number of mounts; intromission frequency (IF), the number of intromissions; mount latency (ML), time from introduction of the female to the first mount with pelvic thrusting; intromission latency (IL), time from introduction of the female to the first mount with pelvic thrusting and vaginal penetration (intromission) [11]. Courtship behaviour and latencies were recorded in seconds.

The following parameters were calculated: % courtship behaviour, % mount, % intromission, copulatory efficiency (number of intromissions/number of mounts) and intercopulatory interval (average time between intromissions) [12].

2.6 Hormone tests

Serum was prepared for the electrochemiluminescence immunoassay (ECLIA) and the radioimmunoassay (RIA). The testosterone levels were determined by using Elecsys Testosterone reagent kit (Roche Diagnostics GmbH, Indianapolis, IN, USA) for the Elecsys 2010 immunoassay analyzer and androstenedione was determined by using a Coat-A-Count Direct Androstenedione kit (Diagnostic Products Corporation, Los Angeles, CA, USA) for Gamma counter with the cooperation of the Reproductive Medicine Laboratory, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University.

2.7 Data analysis

Data were expressed in mean ± SD. The mean values of sexual behaviour for both doses and durations were statistically analyzed by two-way analysis of variance (ANOVA) layout completely randomized design, followed by one-way ANOVA and subsequently least significant difference (LSD) multiple comparisons test. Mean values of other parameters were statistically analyzed by one-way ANOVA followed by LSD. The percentages were analyzed using Chi-squared test. The SPSS 6.0 and 10.0 (SPSS Inc., Chicago, IL, USA) was employed for all statistical analyses. Significance was inferred when $P \leq 0.05$.

3 Results

3.1 Sexual behaviour

There were no significant changes in the whole 30-min period of % courtship behaviour, % mounting, % intromission, the time of courtship behaviour, IF, ML, IL, copulatory efficiency and intercopulatory interval in male rats (Tables 1, 2). However, the study of three 10-min intervals over a 30-min period revealed that the control and treated groups of rats had significantly higher courtship behaviour and MF during the first 10-min observation than in the second and third 10-min observation (Tables 3, 4), whereas both treated and control groups had no significant difference in three 10-min periods of IF during 30 min and they showed consistent frequency along 30 min (Table 5).

3.2 Organ weights

All groups of male rats receiving *B. rotunda* extract had significantly higher relative testis weight than the control group. *B. rotunda* extract at the dose of 60 mg/kg BW also significantly induced the relative weights of the seminal vesicles of the male rats (Figure 1). There were no significant differences in the caudal epididymis or prostate gland weights when compared with the control group.

3.3 Diameter of seminiferous tubule

For all groups of male rats receiving *B. rotunda* extract, the diameters of seminiferous tubule were larger than that the control group (Figure 2).

3.4 Sperm density

All doses of the ethanolic extract of *B. rotunda* did not significantly affect the sperm density when compared with the control (Figure 3).

3.5 Hormone levels

Serum testosterone and androstenedione levels were not affected by *B. rotunda* extract (Figure 4).

4 Discussion

This study used different doses and durations of *B. rotunda* extract to determine their effects on sexual behaviour in male rats. The results showed clearly that *B. rotunda* extract in all doses and durations did not affect sexual behaviour when compared with the control groups. Consequently, this research did not support the belief that *B. rotunda* could enhance sexual behaviour.

Nevertheless, *B. rotunda* extract caused an increase in the testicular weight and seminiferous tubular diameter of all treated groups and in the seminal vesicle weight of the lowest treated group. These weight gains may signify a selective effect of *B. rotunda* as reported in *Lepidium meyenii* [13]. Because *B. rotunda* extract did not change the weights of the other sexual organs, sperm density, serum testosterone or androstenedione levels after being treated for 60 days, it is difficult to conclude that ethanolic extract from *B. rotunda* could enhance sexual activity. This plant may have an opportunity to make itself fully apparent in other conditions as reported in the experiments with other plant species in various doses and times [14, 15], in various solvent extractions or with methods of extraction [16], or in sexual condition of male rats [11, 16–17]. Likewise, Watcho *et al.* [14, 15] found that the rats treated with *Mondia whitei* for 55 days and 1, 2, 4 and 6 h after treatments showed no changes in testosterone concentration, but an applica-

Table 1. Percentages of sexual behaviour of male rats treated with *B. rotunda* (60, 120 and 240 mg/kg) and control groups between days 26–30 and between days 56–60. There were no significant differences (Chi-squared test). Data are expressed as median and interquartile range. MF, mount frequency; IF, intromission frequency.

Parameter	Days 26–30				Days 56–60			
	Control (n = 8)	60 mg/kg (n = 8)	120 mg/kg (n = 8)	240 mg/kg (n = 8)	Control (n = 8)	60 mg/kg (n = 8)	120 mg/kg (n = 8)	240 mg/kg (n = 8)
Courtship (%) (median; range)	100 (594.50; 538.75–616.25)	87.5 (582.50; 479.25–679.75)	100 (470.00; 380.00–613.00)	100 (625.00; 416.00–703.00)	100 (497.00; 416.50–595.25)	100 (492.50; 419.00–558.25)	100 (475.50; 366.50–581.50)	100 (462.00; 408.75–584.75)
MF (%) (median; range)	100 (9.00; 7.25–17.75)	87.5 (10.00; 2.75–12.00)	100 (10.00; 4.00–10.00)	100.0 (10.00; 8.00–20.00)	100.0 (11.50; 5.00–17.00)	100.0 (11.50; 6.25–19.00)	100.0 (13.00; 6.25–22.00)	87.5 (7.00; 2.50–20.25)
IF (%) (median; range)	50.0 (0.00; 0.00–29.75)	62.5 (4.00; 0.00–14.75)	37.5 (0.00; 0.00–5.00)	100 (18.00; 0.00–30.00)	62.5 (1.00; 0.00–16.50)	37.5 (0.00; 0.00–30.70)	62.5 (4.00; 0.00–17.00)	37.5 (0.00; 0.00–4.75)

Table 2. Mean ± SD of sexual behaviour of male rats treated with *B. rotunda* (60, 120 and 240 mg/kg) and control groups between days 26–30 and days 56–60. There were no significant differences (two-way ANOVA). MF, mount frequency; IF, intromission frequency; ML, mount latency; IL, intromission latency; ICI, intercopulation interval.

Parameter	Days 26–30				Days 56–60			
	Control (n = 8)	60 mg/kg (n = 8)	120 mg/kg (n = 8)	240 mg/kg (n = 8)	Control (n = 8)	60 mg/kg (n = 8)	120 mg/kg (n = 8)	240 mg/kg (n = 8)
Courtship	586.62 ± 73.32	570.12 ± 134.29	487.00 ± 127.45	589.57 ± 170.07	503.12 ± 129.70	485.00 ± 86.83	462.75 ± 131.51	511.25 ± 155.62
MF	11.88 ± 5.18	9.00 ± 6.19	8.14 ± 4.14	12.57 ± 7.34	11.62 ± 6.50	13.00 ± 8.09	13.50 ± 8.72	12.50 ± 14.36
IF	11.11 ± 16.15	9.00 ± 12.14	8.00 ± 18.18	14.71 ± 14.14	10.88 ± 20.62	10.50 ± 8.55	8.25 ± 4.18	6.75 ± 5.59
ML	51.00 ± 97.92	284.25 ± 613.74	98.88 ± 104.44	34.12 ± 32.62	41.38 ± 44.28	92.12 ± 80.35	189.25 ± 208.49	334.38 ± 606.39
IL	943.62 ± 916.15	852.12 ± 842.14	1202.75 ± 830.22	813.00 ± 864.00	938.50 ± 921.56	1141.00 ± 909.61	827.88 ± 848.40	1143.75 ± 905.89
IF/MF	0.86 ± 1.48	1.02 ± 1.54	0.79 ± 1.82	1.18 ± 1.47	0.68 ± 1.24	1.07 ± 2.38	0.96 ± 1.83	1.70 ± 3.92
ICI	69.96 ± 48.08	87.65 ± 51.53	90.58 ± 82.27	51.27 ± 18.87	55.77 ± 36.53	86.89 ± 75.45	81.10 ± 68.92	78.86 ± 78.40

Table 3. Period of courtship behaviour (second) of male rats receiving *B. rotunda* extract at different doses for 26–30 and 56–60 days, in three 10-min observations over a 30-min period (two-way ANOVA followed by one-way ANOVA and LSD). Data were expressed as mean ± SD. Different letters denote significant mean differences between groups at $P < 0.05$. In general, the three 10-min intervals of *B. rotunda* treatment had significant effects, while the doses of *B. rotunda* had no significant differences.

Groups	Days 26–30			Days 56–60		
	1st 10-min	2nd 10-min	3rd 10-min	1st 10-min	2nd 10-min	3rd 10-min
Control (n = 8)	303.38 ± 34.76^{a}	152.12 ± 35.95^{b,c}	131.12 ± 57.46^{b,c,d}	282.12 ± 78.06^{a}	148.88 ± 59.78^{b,c}	83.38 ± 37.83^{d}
60 mg/kg (n = 8)	281.00 ± 74.52^{a}	139.38 ± 31.88^{b,c,d}	149.75 ± 57.58^{b,c}	263.00 ± 65.29^{a}	141.50 ± 43.53^{b,c,d}	96.25 ± 44.42^{d,c}
120 mg/kg (n = 8)	257.12 ± 94.48^{a}	154.38 ± 74.46^{b,c}	100.00 ± 37.84^{c,d}	283.12 ± 82.65^{a}	114.50 ± 50.83^{b,c,d}	79.88 ± 43.21^{d}
240 mg/kg (n = 8)	277.62 ± 81.84^{a}	163.80 ± 61.12^{b}	163.80 ± 49.56^{b,c,d}	275.75 ± 56.82^{a}	150.25 ± 104.75^{b,c}	97.75 ± 50.06^{dc}

Table 4. Mount frequency (MF) of male rats receiving *B. rotunda* extract at different doses for 26–30 and 56–60 days, in the three 10-min observations over a 30-min period (two-way ANOVA followed by one-way ANOVA and LSD). Data were expressed as mean ± SD. Different letters denote significant mean differences between groups at $P < 0.05$. In general, the three 10-min intervals of *B. rotunda* treatment had significant effects, while the doses of *B. rotunda* had no significant differences.

Groups	Days 26–30			Days 56–60		
	1st 10-min	2nd 10-min	3rd 10-min	1st 10-min	2nd 10-min	3rd 10-min
Control (n = 8)	8.00 ± 4.31^{a,b}	5.00 ± 2.14^{f}	1.38 ± 1.68^{f}	6.88 ± 3.52^{a,b,c,d}	1.75 ± 1.58^{f}	3.00 ± 4.41^{d,e,f}
60 mg/kg (n = 8)	4.38 ± 3.34^{b,c,d,e,f}	2.75 ± 2.87^{e,f}	1.88 ± 2.42^{f}	7.50 ± 4.63^{a,b,c}	3.62 ± 2.62^{c,d,e,f}	1.88 ± 3.04^{f}
120 mg/kg (n = 8)	6.50 ± 5.32^{a,b,c,d,e}	3.25 ± 2.92^{d,e,f}	0.86 ± 1.22^{f}	7.75 ± 5.80^{a,b}	2.75 ± 2.82^{e,f}	3.00 ± 3.42^{d,e,f}
240 mg/kg (n = 8)	9.38 ± 6.00^{a}	1.86 ± 2.26^{f}	2.14 ± 2.12^{f}	7.75 ± 8.83^{a,b}	3.75 ± 4.71^{c,d,e,f}	1.00 ± 1.60^{f}

Table 5. Intromission frequency (IF) of male rats receiving *B. rotunda* extract at different doses for 26–30 and 56–60 days, in the three 10-min intervals over a 30-min period. There were no significant differences (two-way ANOVA).

Groups	Days 26–30			Days 56–60		
	1st 10-min	2nd 10-min	3rd 10-min	1st 10-min	2nd 10-min	3rd 10-min
Control (n = 8)	5.75 ± 8.43	2.00 ± 3.34	3.25 ± 4.98	5.38 ± 9.58	3.25 ± 6.39	2.00 ± 4.28
60 mg/kg (n = 8)	5.00 ± 5.56	2.88 ± 4.94	1.12 ± 2.47	5.38 ± 9.10	2.38 ± 4.47	2.75 ± 5.20
120 mg/kg (n = 8)	2.62 ± 5.88	2.00 ± 4.57	2.71 ± 7.18	4.62 ± 5.29	1.62 ± 2.72	2.00 ± 4.28
240 mg/kg (n = 8)	9.62 ± 9.38	5.43 ± 5.71	1.86 ± 2.67	3.75 ± 7.26	1.88 ± 5.30	1.12 ± 3.18

tion of *M. whitei* for 8 days induced an increase in the testicular weight, testicular testosterone level and sperm density without affecting the accessory gland weights [15]. Another study in aphrodisiac herbs, *Tribulus terrestris* extract did not affect the sexual behaviours or intracavernous pressure in normal rats, but it improved those parameters in the castrated rats [17]. The study of *T. terrestris* was followed in humans and showed a lack of effect on testosterone and androstenedione levels in young men receiving the extract at 1, 3, 8, 20 and 24 days from the beginning of the supplementation [18]. Thus, *B. rotunda* will be needed to demonstrate the course and dose response relationship, animal conditions or ultrastructural changes of testis.

In conclusion, the ethanolic extract of *B. rotunda,* does not affect sexual behaviour or serum androgen levels, but it enhances seminiferous tubule, testis and seminal vesicle.

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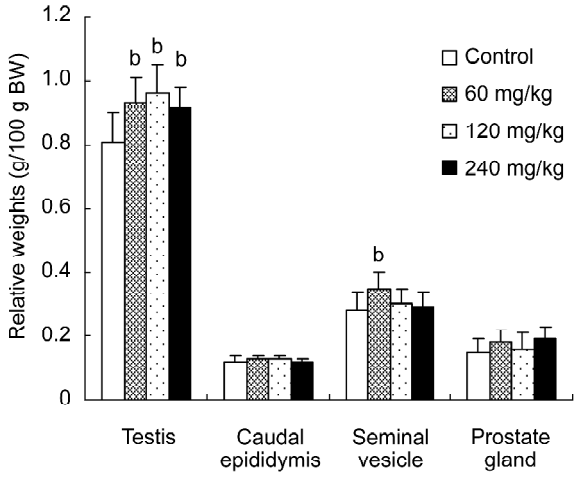

Figure 1. Relative reproductive organ weights (g/100 g body weight) of male rats treated with *B. rotunda* extract for 60 days (one-way ANOVA followed by LSD; $^{b}P < 0.05$, compared with the control group).

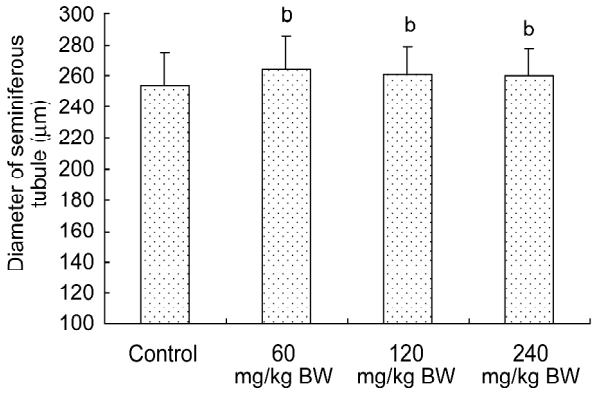

Figure 2. Diameter of seminiferous tubule of male rats treated with *B. rotunda* extract for 60 days (one-way ANOVA followed by LSD; $^{b}P < 0.05$, compared with the control group). Cross-sections of seminiferous tubules were measured in each of 32 animals using a calibrated ocular micrometer under 40 × objective lens (control, n = 160 tubes; 60 mg/kg, n = 158 tubes; 120 mg/kg, n = 148 tubes; and 240 mg/kg, n = 139 tubes).

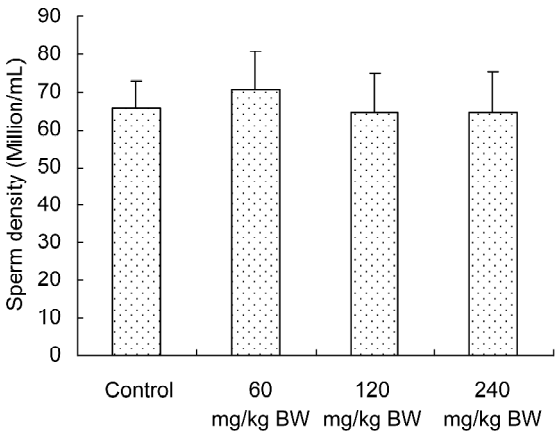

Figure 3. Sperm density of male rats treated with *B. rotunda* extract for 60 days (one-way ANOVA; there were no significant differences).

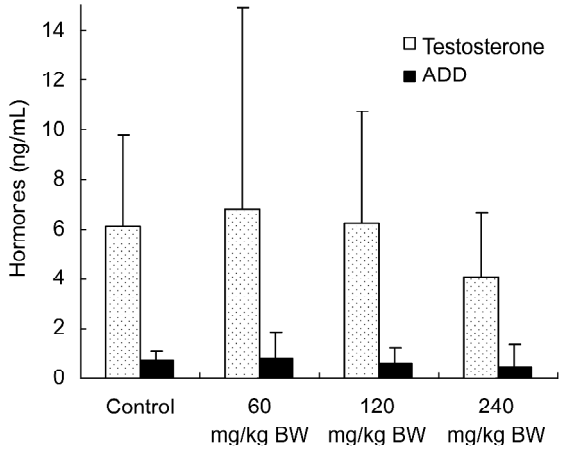

Figure 4. Testosterone and androstenedione (ADD) levels of male rats treated with *B. rotunda* extract for 60 days (one-way ANOVA; there were no significant differences). The data were expressed as mean ± SD.

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