

SEXUAL STIMULANT EFFECTS OF THE AQUEOUS AND METHANOLIC EXTRACTS FROM THE LEAVES OF *Bersama engleriana* IN ADULT MALE RATS

Pierre WATCHO^{*}, Amie MEKEMDJIO, Téléphore Benoît NGUELEFACK AND Albert KAMANYI

Animal Physiology and Phytopharmacology Laboratory, Faculty of Science, University of Dschang, Cameroon, P.O. BOX 67 Dschang

Summary

The present investigation was designed to determine the effects of *Bersama engleriana* on sexual behaviour and some biochemical parameters of adult male rats. Animals (5 per dose) were gavaged daily with the aqueous and methanolic extracts from the leaves of *Bersama engleriana* at doses of 300 mg/kg and 600 mg/kg for 21 days. The control animals received distilled water (10 mL/kg). On day 22, the animals were sacrificed after chloroform anaesthesia. Sperm density, total cholesterol and total protein levels as well as globular counts and haematocrit were determined. In another series of experiment, 25 rats were treated in a similar manner and 30 minutes after the last dose of the plant extracts (day 21), the sexual behaviour of each rat was monitored by measuring the frequencies of erection, mount and intromission in three phases of 30 minutes each. *Bersama engleriana* significantly increased ($p < 0.05-0.001$) the sperm density, relative weights of the testes, epididymis, ventral prostate, seminal vesicles, intratesticular and epididymal total protein concentration. The treatment had no significant effect on the relative weights of vas deferens, RBC and WBC counts, haematocrit, cholesterol contents of testes, total serum proteins when compared to control rats. Administration of *Bersama engleriana* extracts for 21 consecutive days significantly increased ($p < 0.05$) the sexual behaviour of rats during the first 30min of observation and the dose 600 mg/kg appeared to be the most active. No statistical change was recorded during the second and third phases of observation. Sterol and triterpens revealed in the extracts of *Bersama engleriana* may account for the recorded sexual effects. Results of the present study also support the use of the plant as an aphrodisiac.

Key Words: *Bersama engleriana*, androgenic, sexual behaviour, rat.

Introduction

Bersama engleriana (Melianthaceae) is a small or medium size tree of 6-9m, rarely exceeding 25m in height. It is wide spread throughout tropical Africa, preferring higher rainfall or evergreen forests. It is distributed from Senegal to Zaire, and parts of southern Africa. In the South-West province of Cameroon, the leaves are used for the treatment of many ailments including diabetes and male impotence (1). In our earlier findings, we demonstrated the anti-hyperglycaemic effect of the aqueous and methanolic extracts from the leaves of *Bersama engleriana* in adult male Wistar rats (2). To the best of our knowledge, no work has been done to verify the aphrodisiac claims of *Bersama engleriana*. The present study was therefore undertaken to determine the effects of the aqueous and methanolic extracts from the leaves of *Bersama engleriana* on sexual behaviour, body and relative sexual organ weights, sperm density, haematological (WBC, RBC, hemaetocrit) and biochemical parameters (total proteins and total cholesterol) of adult male Wistar rats in order to validate its reputation as male sexual stimulant.

Materials and Methods

Animals

Adult Wistar rats of either sex (50 males and 25 females) weighing 100-185g were used in this study. The animals were maintained at room temperature (22-23°C) with a reverse natural light-dark cycle in the animal house of the Faculty of Science, University of Dschang, Cameroon. Food and tap water were available *ad libitum*. The ethic committee of the Cameroon Ministry of Scientific Research and Technology which has adopted the guidelines established by the European Union on Animal Care and Experimentation (CEE Council 86/609), approved all experimental procedures. Female rats were ovariectomized (3) and brought later into estrus by a sequential subcutaneous injection of 30 µg of estradiol benzoate (Sigma Chemicals, USA) and 600 µg of progesterone (Sigma Chemicals, USA), 48 h and 6 h respectively

before testing. In ovariectomized rat, it was shown that estradiol benzoate induced a specific urge to seek contact with a sexual active male (4). Furthermore, they were screened with non-experimental vigorous males and only those exhibiting good sexual receptivity and no rejection behaviour were employed in the test.

Plant material

The leaves of *Bersama engleriana* were collected in Kumba (South-West province of Cameroon). Botanical identification was performed at the Cameroon National Herbarium (HNC) in Yaounde, Cameroon in comparison with the existing specimen number 32427/HNC, collected by MBENKUM. The leaves were dried at room temperature and reduced to powder.

Aqueous extract preparation

Four hundred grams of *Bersama engleriana* powder was soaked in 5L of distilled water for one hour and boiled for 30 minutes. The heated decoction was taken and allowed to cool at room temperature ($22\pm 2^{\circ}\text{C}$). The decoction was filtered twice and the filtrate was oven-dried (56°C) for 3 days. The resulting material was found to weigh 112g (28% yield). The working solution was obtained by dispersing 1g of the residue in a known volume of distilled water and the final volume adjusted to 10 mL (100 mg/mL).

Methanolic extract preparation

Seven hundred grams of *Bersama engleriana* powder were soaked in 3L of methanol and occasionally shaken. After 3 days, the decoction was filtered and the filtrate was concentrated (70°C) to obtain a residue (10g; 14.29% yield). The working methanolic extract was obtained by suspending 1g of the residue in a known volume of distilled water and the final volume adjusted to 10 mL (100 mg/mL).

Phytochemical screening

The test of Libermann Buchard was used to determine the presence of sterol and triterpens (5) while saponins were revealed as described by Hostettmann et al (6).

Experimental design

Androgenic study

Twenty five male rats were divided into 5 groups of 5 animals each and orally treated as follows: Group 1 received distilled water (10 ml/kg) and served as control. Groups 2 and 3 were treated with the aqueous extract of *Bersama engleriana* at doses of 300 mg/kg and 600 mg/kg respectively. Groups 4 and 5 were administered with the methanolic extract of *Bersama engleriana* at doses of 300 mg/kg and 600 mg/kg respectively. After 24h of the last dose (day 22), animals were sacrificed under chloroform anesthesia; blood was collected from heart and serum was separated, and the reproductive organs were removed, cleared of fat and weighed. Whole blood was analysed for the red blood cell (RBC) and white blood cell (WBC) counts, and haematocrit (7, 8). Total proteins and total cholesterol were determined in the serum (9). Tissues from each rat were kept at -20°C until assayed for total protein (testes, epididymis) (10) and cholesterol (testes) (using a commercial kit of Human Gesellschaft für biochemica und Diagnostica, mbh, Germany) estimations. In the epididymal cauda, sperm density was assessed using Bükér's counting chamber (11).

Sexual behaviour study

Twenty five rats (n=5/dose) (different from those used in the androgenic study) were orally treated with the above doses of *Bersama engleriana* extracts 2 h after the onset of darkness for 21 consecutive days. 30 min after the last dose application (day 21), the sexual behaviour of each male was tested during three periods of 1h each in a quiet room after dropping a stimulus-receptive ovariectomised female in the copulation cage. During each hour, the observations were recorded only in the first 30 min and the female withdrawn during the last 30 min. The following sexual performance parameters were recorded according to standard methods (12, 13): mount frequency (MF), the number of mounts preceding ejaculation; intromission frequency (IF), the number of intromissions preceding ejaculation; penile erection (PE), the number of times the rat bent down to lick the penis.

Statistical analysis

Data are expressed in mean \pm SEM. The influence of the period of observation on each copulatory parameter was analysed using ANOVA Repeated Measures followed by Wilcoxon test. Mean values of the control and treated groups in the androgenic study and in the sexual behaviour work within the same period of observation were compared using ANOVA 1-way with post-hoc Student-Newman-Keuls. The value of $p < 0.05$ was considered to be statistically significant. All statistical analysis were performed using SPSS for Windows version 10.0.7.

Results

Body and organ weights

After 21 days of oral administration of the plant extracts (aqueous and methanolic extracts), a trend to an increase in the body weight was observed in all *Bersama engleriana* treated groups compared to control animals. Relative weights of testes, ventral prostate, epididymis and seminal vesicles were significantly increased ($p < 0.05-0.001$) whereas the treatments had no statistical effect ($p > 0.05$) on the relative weights of vas deferens (Table 1).

Table 1: Body weight and relative organ weights of rat after *Bersama engleriana* treatment for 21 days

Groups	Body weight (g)		Relative organ weights (mg/100g)				
	Initial	Final	Testes	Epididymis	Vas deferens	Ventral prostate	Seminal vesicles
Control	118.30±2.23	162.56±3.43	557.48±20.18	144.30±10.05	27.41±3.12	35.07±5.44	219.46±24.05
<i>Bersama engleriana</i>							
Aqueous extract							
300 mg/kg	131.82±3.41	175.56±6.78	558.57±20.20*	140.47±11.90	25.20±1.47	46.07±12.57	253.12±24.66
600 mg/kg	176.70±2.09	205.80±8.34	614.93±15.15*	171.01±6.58	31.27±2.97	85.46±15.52*	279.22±25.12*
Methanolic extract							
300 mg/kg	169.20±1.97	194.06±5.91	641.55±21.17*	178.50±7.20	32.16±2.60	91.41±7.81**	326.64±20.75***
600 mg/kg	148.70±2.28	178.20±5.11	707.11±29.21**	183.81±3.29***	32.68±1.29	82.33±8.34**	288.93±19.23**

All values: Mean ± SEM Number of rats per group = 5

*: p<0.05; **: p<0.01; ***: p<0.001 compared to control (ANOVA 1-way, Student-Newman-Keuls)

Serum analysis

Red blood cells, white blood cells and haematocrit were found to be within the normal range. Level of serum proteins was statistically unchanged ($p>0.05$) in *Bersama engleriana*-treated rats compared to control animals (Table 2).

Biochemical changes in the testes and epididymis

Total protein contents of testes and epididymis increased significantly ($p<0.05$) whereas the intratesticular cholesterol level was unchanged compared to rats treated with distilled water. Aqueous and methanolic extracts from the leaves of *Bersama engleriana* significantly increased ($p<0.05-0.001$) the number of spermatozoa in the cauda of the epididymis (Table 2).

Sexual performance

The sexual behaviour of the male rats treated with *Bersama engleriana* extracts is outlined in Table 3. During the first period of observation, a significant increase ($p<0.05$) of IF (600 mg/kg, percentage of increase: 398.33%) and PE (300 and 600 mg/kg, percentage of increase: 190.27% and 212.39% respectively) was noticed in animals receiving the methanolic extracts from the leaves of *Bersama engleriana* and the dose of 600 mg/kg appeared to be the most active when compared to both control and *Bersama engleriana*-treated groups. On the contrary, the second and third periods of observation of the sexual performance parameters did not bring any significant change ($p>0.05$) when compared to respective control. Change of the period of observation did not statistically influence the copulatory parameters in the distilled water and 600 aqueous extract groups whereas the FM, FI and PE of the other plant extract-treated animals were modified and the effects were more expressed in rats gavaged with 600 mg/kg of the methanolic extract of *Bersama engleriana*.

Pharmacologyonline 1 : 464-476 (2007) Watcho et al.

Table 2: Effect of the aqueous and methanolic extracts of *Bersama engleriana* on sperm density, total cholesterol, total and haematological parameters of rats.

Groups	Sperm density (10 ⁶)	Total cholesterol Testes (µg/mg)	Total proteins			Globular counts		Haematocrit (%)
			Serum (mg/ml)	Testes (µg/mg)	Epididymis (µg/mg)	RBC (10 ⁶ /mm ³)	WBC (10 ³ /mm ³)	
Control	0.48±0.05	3.20±0.40	121.55±7.07	23.78±1.67	10.68±0.77	7.31±0.29	5.38±0.55	40.03±2.26
<i>Bersama engleriana</i>								
Aqueous extract								
300 mg/kg	0.76±0.04**	2.85±0.35	109.97±1.52	23.67±0.63	13.91±0.42*	6.33±0.26	4.75±0.27	42.30±14.1
600 mg/kg	1.26±0.05***	1.84±0.39	112.05±7.36	29.65±1.28*	12.92±0.56	6.71±0.50	4.37±0.34	46.64±1.70
Methanolic extract								
300 mg/kg	1.33±0.02***	2.18±0.07	164.61±34.29	28.83±0.83*	12.02±0.83	6.70±0.60	4.52±0.21	44.22±1.24
600 mg/kg	1.83±0.07***	2.56±0.42	102.46±9.16	30.00±0.69*	13.86±0.63*	6.94±0.14	4.68±0.54	48.95±2.32

All values: Mean ± SEM Number of rats per group = 5

*: p<0.05; **:p<0.01; ***:p<0.001 compared to control (ANOVA 1-way, Student-Newman-Keuls)

Pharmacologyonline 1 : 464-476 (2007) Watcho et al.

Table 3: Effects of *Bersama engleriana* on MF, (IF) and [PE] in adult male rat

	Period of observation (30 min each)		
	1 st hour	2 nd hour	3 rd hour
Control (Distilled water, 10 mL/kg)	30.20±4.52 ^a (12.00±0.00) ^a [22.60 5.60] ^a	25.40±4.99 ^a (22.20±5.25) ^a [23.80 5.07] ^a	15.20± 3.54 ^a (14.2±3.07) ^a [13.80±2.87] ^a
<i>Bersama engleriana</i> Aqueous extract			
300 mg/kg	39.2±7.31 ^a (12.00±0.00) ^a [29.40±7.11] ^{αa} 36.2±7.45 ^a	22.80±12.85 ^{ab} (16.00±9.40) ^a [18.80 9.89] ^{ab} 20.00±6.32 ^a	5.00± 4.29 ^b (4.40±4.15) ^a [4.20±3.97] ^b 20.00±10.68 ^a
600 mg/kg	(12.00±0.00) ^a [32.20±6.54] ^{αa}	(17.40±5.07) ^a [17.00 4.87] ^a	(18.00±9.64) ^a [18.20±9.59] ^a
Methanolic extract			
300 mg/kg	48.0±10.70 ^a (12.00±0.00) ^a [43.00±9.22] ^{*αa}	41.80±16.07 ^{ab} (36.20±12.75) ^a [37.40±13.55] ^a	27.20±11.74 ^b (23.00±9.46) ^a [23.60±10.40] ^a
600 mg/kg	50.40±5.77 ^a (47.80±5.40) ^{*a} [48.00±5.30] ^{*a}	39.20±7.68 ^{ab} (33.60±5.97) ^a [34.20±6.54] ^{ab}	21.40±4.57 ^b (18.40±3.27) ^b [18.60±2.84] ^b

All values: Mean ± SEM n=number of rats per group

a,b: For each copulatory parameter, values with the same superscript letter in the same line do not differ significantly (p>0.05) (ANOVA Repeated Measures, Wilcoxon test)

*: p<0.05 compared to respective control (ANOVA 1-way, Student-Newman-Keuls)

α: p<0.05 compared to 600mg/kg of the methanolic extract of *Bersama engleriana* (ANOVA 1-way, Student-Newman-Keuls)

Values within () and [] represent intromission frequency (IF) and penile erection [PE] respectively

Discussion

Animals treated with the aqueous and methanolic extracts from the leaves of *Bersama engleriana* for 21 consecutive days showed an increase in the relative weights of testes, epididymis, ventral prostate and seminal vesicles. This increase in the relative weights of the accessory sex glands denotes direct androgenic effect of *Bersama engleriana* (14). Androgenic steroids are essential for male development and the major androgen is testosterone secreted from the testes. It has been demonstrated that sex differentiation, growth and maintenance of the epididymis, ventral prostate and seminal vesicles are androgen-dependent processes (15, 16, 17). In rats, any increase in serum testosterone or treatment with androgens are associated with increased secretory activity and increased weight of these organs (18, 19). The accessory sex organs possess 5 alpha-reductase activity, which converts testosterone to dihydrotestosterone, the active hormone (20). The observed increase in tissular total proteins suggests an important anabolic effect of the plant (21). Although non significant, the decrease in the intratesticular cholesterol level of the treated animals is of physiological importance. Cholesterol is the major substrate for steroidogenesis and its testicular drop may reflect a conversion into testosterone under the control of luteinising hormone (LH) (22). This supposed androgenic effect of *Bersama engleriana* could also be justified in the present study by the marked increase in sperm concentration in the cauda of the epididymis of all plant extract-treated animals compared to control. It has been observed that blood parameters remained within the normal range after *Bersama engleriana* administration indicating a non-toxic nature of the plant.

The present investigation was also undertaken in order to validate the folkloric claim of the plant as an aphrodisiac. The copulatory behaviour of normal male rat when tested with estrus female consists of repeated series of mounts and intromissions culminating with ejaculation (23). Long term administration of the aqueous and methanolic extracts from the leaves of *Bersama engleriana* significantly increased the sexual behaviour of the animals during the first period of observation. Increase in penile erection and intromission frequency during the first 30 minutes of observation implies a dose-dependent aphrodisiac activity of *Bersama engleriana* with the methanolic extract (600 mg/kg) being

the most active. Mount, intromission and erectile frequencies constitute the real criteria for the determination of libido in males (24). Sterol and triterpens revealed in the extracts of *Bersama engleriana* may account for the enhancement of the sexual performance of the treated animals. Male sexual behaviour being dependent on intrinsic and extrinsic signals (25), it could be proposed that these active principles probably act by inducing changes in levels of neurotransmitters, modulating the action of these neurotransmitters on their target cells or by increasing androgen levels (26). Lack of significant effects during the second and third periods of observation compared to respective control in the one hand and the trend to decrease of the mount frequency, intromission frequency and penile erection observed in the second and third periods of investigation compared to the first 30 minutes of observation in the other hand, suggest a short-lasting effect of the plant.

In conclusion, results of the present work highlight the sex-stimulant effects of *Bersama engleriana* in adult male Wistar rats and therefore give value to its aphrodisiac reputation.

Acknowledgments

The authors thank Mr Ngoula Ferdinand for his excellent technical assistance.

References

- 1 Iwu MM. Handbook of African medicinal plants Ed. CRC. Press Boca Raton (Florida-USA) 1993;435p.
- 2 Nyah Njike G, Watcho P, Nguenefack TB, Kamanyi A. Hypoglycaemic activity of the leaves extracts of *Bersama engleriana* in rats. Afr. J. Trad. CAM 2005;2:215–221.
- 3 Cariton AE. Experimental surgery of the genital system. In: William I. Gay and James E. Heavner ed. Methods of Animal experimentation: Research surgery and care of the research animal; Part B Surgical approaches to organ systems. Academic Press, Inc., Orlando, Florida, 1986;Vol 7:p1991.
- 4 Meyerson BJ, Lindstrom L. Sexual motivation in the female rat. Acta Physiol Scand Suppl 1973;389:1-80.

5 Klyne W. Quimica de los esteroides. Compania editorial continental SA, Barcelona, Spain 1970;pp 126-149.

6 Hostettmann K, Hostettmann M, Marston A. Saponins. In: Dey PM and Harbonne ed. Methods in plant biochemistry, Academic, New York, 1991: pp435-471.

7 Benson J, Williams P, Cales B. Animal Anatomy and Physiology. Laboratory text book. Wm. Brown Communication, Dubuque, 1992: p325.

8 Theml H. Atlas de poche d'hématologie. Flammarion. Médecine Science pour la traduction française. ISBN, Paris, 2000:pp 2-21.

9 Gornall AG, Bardwil GS, David MM. Determination of serum proteins by the biuret reactions. J Biol Chem 1949;177: 751-766.

10 Bradford MM. A rapid and sensitive method for the quantitation microgram quantities of proteins utilizing the principle of protein dye binding. Anal Biochem 1976;72:248-254.

11 Chinoy MR, Chinoy NJ. Effect of cyproterone acetate on the vas deferens of albino rat. Ind J Exp Biol 1979;17:1176-1181.

12 Ageel AM, Islam MW, Ginawi OT, Al-yahya MA. Evaluation of the aphrodisiac activities of *Litsea chinensis* (Lauraceae) and *Orchis maculata* (Orchidaceae) extracts in rats. Phytother Res 1994;8:103-105.

13 Carro-Juarez M, Cervantes E, Cervantes-Mendez M, Rodriguez-Manzo M. Aphrodisiac properties of *Montano tomentosa* aqueous crude extract in male rats. Pharmacol Biochem Behav 2004;78:129-134.

14 Kamtchouing P, Mbongue FGY, Dimo T, Jatsa BH. Evaluation of androgenic activity of *Zingiber officinale* and *Pentadiplandra brazzeana* in male rats. Asian J Androl 2002;4: 299-301.

15 De Kretser DM. The testis. In: Austin CR, Short Frs RV, editors Hormonal Control of Reproduction. Cambridge University Press 1987:pp76-90.

16 Patil SR, Patil SR, Londonkar R, Patil SB. Effect of pathidine on spermatogenesis in albino rats. Ind J Pharmacol 1998;30:249-253.

17 Ang HH, Cheang HS, Yusof APEffects of *Eurycoma longifolia* Jack (Tongkat Ali) on the initiation of sexual performance of inexperienced castrated male rats. Exp Anim 2000;49:35-38.

18 Dewan ZF, Morris ID, Lendon RG. Administration of exogenous testosterone in the adult rat and its effects on reproductive organs, ex hormones and body-weight. Bangladesh Med Res Counc Bull 2000;26:48-55.

19 Gonzales GF. Function of seminal vesicles and their role in male fertility. Asian J Androl 2001;3:251-258.

20 Hiort O. Androgens and Puberty. Best Pract Res Clin Endocrinol Metab 2002;16:31-41.

21 Watcho P, Donfack MM, Zelefac F, Nguelefack TB, Wansi S, Ngoula F, Kamtchouing P, Tsamo E, Kamanyi A. Effects of the hexane extract of *Mondia whitei* on the reproductive organs of male rat. Afr. J. Trad. CAM 2005;2:302 –311.

22 Bhasin S, Bross R, Storer TW, Casaburi R. Androgens and Muscles. In Nieschlag E., Behre H.M., Editors. Testosterone: Action, Deficiency, Substitution. Springer-Verlag-Berlin Heidelberg 1998:pp209-227.

23 Slob AK, Van der Werff ten Bosch JJ. The fundamental role of gonadal steroids in sexual behaviour. Baillière's Clin Psych 1997;3:1-24.

24 Kamtchouing P, Mbongue GYT, Dimo T, Watcho P, Jatsa HB, Sokeng SD. Effects of Aframomum melegueta and Piper guineense on sexual behaviour of male rats. Behav Pharmacol 2002;13:243-247.

25 Hull E, Meisel R, Sachs B. Male sexual behaviour. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Rubin R ed. Hormones, Brain and Behavior, New York Academy Press 2002, pp3-137.

26 Suresk Kumar PK, Subramaniam A, Pushpangadan P. Aphrodisiac activity of *Vanda tessellate* (Robx) Hook; Ex Don extract in male mice. Ind J Pharmacol 2000;32:300-304.

*** Corresponding author: pwatcho@yahoo.fr**