PRO-SEXUAL EFFECTS OF DRACAENA ARBOREA (WILD) LINK (DRACAENACEAE) IN SEXUALLY EXPERIENCED MALE RATS

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Summary

The objective of this work was to investigate the effects of the dried roots of *Dracaena arborea*, a medicinal plant, on the sexual behaviour of gonado-intact (normal) and castrated sexually experienced male Wistar rats. Animals were orally administered with 100 mg/kg and 500 mg/kg of either the aqueous or the ethanol extracts of *Dracaena arborea* whilst the neutral control group received in the same way 10 mL/kg of distilled water. The standard control group (positive control) was treated with a subcutaneous injection of testosterone propionate (20 mg/kg/day/3days) prior to the experiment. The sexual behaviour of all rats was monitored on days 0, 1, 7 and 14 of normal treatment

and 14 days post-treatment by measuring the latencies and frequencies of erection, mount, intromission and ejaculation. In a separate group of normal sexually experienced rats, the prosexual effects induced by a single dose of the ethanol extract (100 mg/kg) were measured after pre-treatment with either haloperidol (10 mg/kg), atropine (10 mg/kg) or L-omega-nitro-argininemethyl ester (Lu-NAME, 10 mg/kg). Results obtained showed a significant increase (p<0.05-0.01) in erection, mount and intromission frequencies of normal and castrated rats particularly in animals receiving 100 mg/kg of b.w of the ethanolic extract of Dracaena arborea after 7 and 14 days respectively. At all time the erection, mount, intromission points. and ejaculation latencies remained statistically unchanged (p>0.05) when compared to their respective control. The subcutaneous injection of testosterone propionate (20 mg/kg) to the castrated animals significantly (p<0.05-0.01) increased the sexual performance (erection, mount and intromission frequencies) and motivation reduced (p>0.05)sexual (mount, intromission and ejaculation latencies). After a washout period of 14 days, there was a decrease in the sexual performance of both normal and castrated rats compared to Day 14 data. Pre-treatment of rats with atropine (10 mg/kg) or haloperidol (10 mg/kg) completely abolished the sexual behaviour of the animals whereas pre-administration of L@-NAME (10 mg/kg) did not bring any change. Flavonoids and sterols revealed in the aqueous and ethanolic extracts of the roots of Dracaena arborea may account for the enhancement of sexual activity in experimental rats which could be expressed through dopaminergic and/or cholinergic receptor(s).

Key words: *Dracaena arborea*, sexual behaviour, normal and castrated rats.

Introduction

Male sexual behaviour is a motivated behaviour dependent on intrinsic and extrinsic signals (1). The copulatory behaviour of normal male rat when tested with estrus female consists of repeated series of mounts and intromissions culminating with ejaculation (2). A variety of pharmacological agents including dopaminergic, cholinergic, nitrergic, androgenic and many other agents are capable of modulating male sexual behaviour. It has been proven that dopaminergic agonists such as apomorphine can elicit erectile responses (3). The effect of dopamine upon erection is blocked by haloperidol (4). Activation of the parasympathetic system induces penile tumescence and erection (5, 6); the pro-erectile effect of acetylcholine, the main parasympathetic neurotransmitter, is blocked by atropine (7). Nitric oxide (NO) is involved in the neurotransmission processes that lead to smooth muscle relaxation in the corpus cavernosum permetting penile erection. This erectogenic effect of NO can be stopped after pre-treatment with Lω-NAME (8, 9). The importance of androgens on sexual behaviour is well established (10) and the proerectile effects of testosterone, the main androgen, is related to its interaction with the nitrergic system (11).

Despite the increasing availability of effective conventional medical treatments for male sexual disturbances, plant-derived and herbal remedies continue to provide a popular alternative for men seeking to improve their sexual life (12). A variety of plants are reported to possess aphrodisiac potentials (13, 14, 15, 16). Cameroonian traditional medicine indicates that *Dracaena arborea* (Wild) Link (Dracaenaceae) is one of such plants. It is a tall tree native to West Africa where it is used against gonorrhoea, small pox, malaria and leishmaniasis (17). In the western part of Cameroon, the mixture of the roots of this plant (also referred to as "keubgouh") with "palm wine" has gained reputation as male aphrodisiac (18). However, this report is based on subjective opinions rather than on its scientific verification. The present study was therefore undertaken to verify this folkloric claim and provide some rationale for its use.

Materials and Methods

Plant material and extraction

The plant material was harvested in Bagnoun, West Province of Cameroon and authenticated by Dr Pinta Jonas of the Botany Department, Faculty of Science, University of Dschang, Cameroon. The harvested fresh barks were cut into small pieces, dried at room temperature and ground into a fine powder.

Preparation of the aqueous extract

Six hundred grams (600 g) of the powdered roots was dissolved in 5 L of distilled water and kept for 72 h at 4°C, and occasionally stirred. After filtration, the filtrate was concentrated in an oven (55°C) to give 35.10 g of brownish residue corresponding to an extraction yield of 5.85 %. The aqueous extract used in the study was prepared at a final concentration of 100 mg/mL in distilled water and the doses used were 100 mg/kg and 500 mg/kg.

Preparation of the ethanolic extract

Ground root bark (1kg) of *Dracaena arborea* was macerated with ethanol (95%) (5L, 2x) for 72 hours to yield, after solvent evaporation under reduced pressure, 30 g of a brownish extract corresponding to an extraction yield of 3%. The ethanolic extract used in the study was prepared by dispersing 1 g of the residue in a known volume of distilled water and the final volume adjusted to 10 mL (100 mg/mL). The doses used in our study were 100 mg/kg and 500 mg/kg.

Phytochemical screening

The test of Shinoda was used to determine the presence of flavonoids (19), Libermann Buchard's test revealed the existence of sterols (20) while saponins were revealed as described by Hostettmann et al (21).

Animals

A total of 160 adult Wistar rats (160-200 g; > 90 days) of either sex (80 males and 80 females) were obtained from the animal house of the Faculty of Science of the University of Dschang, Cameroon. The animals were raised at room temperature (23°C) with a natural light-dark cycle (12/12h) and fed on standard Laboratory rat diet with tap water given *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.

Male rats were trained for sexual experience. To provide sexual experience, each male was allowed 1 h exposure to a female rat (used as mating stimulator) in behavioural estrus for 5 consecutive days and only those exhibiting good copulatory behaviour (active males) were selected.

Ovariectomy was performed following the technique of Cariton (22) with minor modifications. The animals were anaesthetised by intraperitoneal injection of Diazepam (10 mg/kg) followed 10 minutes later by Ketamine (50 mg/kg). The onset of anaesthesia was followed by the bilateral shaving of the lumbar dorsum and exposure of the skin in preparation for aseptic surgery (95 % alcohol wipe). For each ovary, a ³/₄ cm dorsal flank incision penetrating the abdominal cavity was made, the par ovarian fatty tissue identified and retracted, and the exposed ovary and associated oviduct severed and removed. This was followed by a ligature around the severed ovarian vasculature to maintain homeostasis and finally, an intramuscular injection of penicillin G (2000 IU/kg b.w/day/3days). One month later, they were brought into estrus by sequential subcutaneous injection of 30 µg of estradiol benzoate and 600 µg of progesterone, 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 (23). Furthermore, they were screened with non-experimental vigorous males and only those exhibiting good sexual receptivity and no rejection behaviour were employed in the tests.

Orchidectomy was performed following the technique of Roubinian et al (24) with minor modifications. Anaesthesia was induced with diazepam/ketamine as described above. The scrotal area was clipped and prepared aseptically. A 1/2 cm incision was made in the scrotal sac. Each testis was delivered separately through

the scrotal incision. Once exteriorized, the testis was removed by severing the vas deferens and spermatic artery. Haemostasis was achieved by hemostat pressure. The incision was sutured in an interrupted pattern and finally, an intramuscular injection of penicillin G (2000 IU/kg /day/3days). Two weeks later, the castrated animals were used for the copulatory experiments.

Drugs

Estradiol benzoate (Sigma, USA), progesterone (Sigma, USA), haloperidol (MP Biomedicals, Germany), L-omega-nitroarginine-methyl ester (L ω -NAME) (Sigma, USA), atropine sulphate (CC Pharma, Belgium), diazepam (Renaudin, France), ketamine (Rotex Medica, Germany), testosterone propionate (Scharing AG, Germany) and penicillin G (Clarion Medicals, Nigeria) were used in the study.

Groups

Twenty five (25)gonado-intact (normal) sexually experienced male rats were randomly assigned to one of the following groups (n=5, each): Group 1 received the vehicle orally (10 mL/kg of distilled water) and served as control. Groups 2 and 3 received 100 mg/kg and 500 mg/kg of the aqueous extract of Dracaena arborea whereas Groups 4 and 5 were treated with 100 mg/kg and 500 mg/kg of the ethanolic extract of Dracaena arborea respectively. To study the effect of Dracaena arborea on erectile function in sexually experienced rats with erectile dysfunction, 30 castrated rats were divided in 6 groups of 5 animals each. The control group consists of two subgroups: testes-removed rats receiving 10 mL/kg of distilled water (neutral control, Group 6) and testosterone propionate-treated rats (standard control, Group 7). Testosterone propionate (20 mg/kg) was subcutaneously injected daily for 3 consecutive days before the start of the experiment. Dracaena arborea aqueous and ethanolic extracts were administered orally at a dose of 100 or 500 mg/kg (groups 8-11). In normal and castrated animals, the vehicle (distilled water) and different doses of Dracaena arborea were given by gavage 2 h after the onset of darkness for 14 days. On days 0, 1, 7, 14 of treatment and 14 days post-treatment, animals were tested for male sexual behaviour after 30 min of the application of each dose.

In the last series of experiments, in order to identify the effect of *Dracaena arborea* on dopaminergic, cholinergic and nitrergic systems, the prosexual effect induced by a single oral dose of the ethanolic extract (100 mg/kg) (the most active extract) was monitored after 1h of pre-treatment of normal male rats with L ω -NAME (10 mg/kg, i.p, n=5) or haloperidol (10 mg/kg, i.p, n=5) or atropine (10 mg/kg, i.m, n=5). The control animals received 10 mL/kg of 0.25% Tween 80 in NaCl 0.9% (i.p, n=5) or 10 mL/kg b.w of distilled water (i.m, n=5). The doses of the standard antagonist drugs were chosen from a pilot study (unpublished data).

Sexual behaviour testing procedure

The sexual behaviour of all male rats was tested during the period of darkness (08:00 p.m local time) in a quiet room for a duration of 1h30. After 30 min of acclimatation in the copulation cage (rectangular glass cage), a stimulus-receptive female was presented to each male by dropping it gently into the cage. The following parameters were recorded according to standard methods (13, 15): penile erection (PE), the number of times the rat bent down to lick the penis; mount (ML) and intromission latency (IL), the time elapsed from the introduction of the female into a cage until the first mount and intromission respectively; mount (MF) and intromission frequency (IF), the number of mounts and intromission preceding ejaculation respectively; ejaculation latency (EL), the time from the first intromission until ejaculation; ejaculation frequency (EF), the number of ejaculation during the study.

Statistical analysis

Data are expressed in mean \pm SEM. The effect of length of treatment on each copulatory parameter during normal treatment (day 0 to day 14) and wash-out study (Day14 to Day 28) was analysed using ANOVA Repeated Measures followed by Wilcoson test when necessary. Within the same day of treatment, Kruskal-Wallis with post-hoc Mann Whitney when necessary was applied to compare the treated groups to control animals (distilled water). P values of 0.05 or less were taken to imply statistical significance between the means. All statistical analysis were performed using SPSS for Windows version 10.0.7.

Results

Effects of Dracaena arborea on mounting

The influence of Dracaena arborea extracts on mounting behaviour is indicated in Tables 1, 2, 3 & 4. As the treatment was increasing, a trend to a decrease in ML (p>0.05) was observed in both normal and castrated rats receiving the plant extracts whereas testosterone group exhibited a significant increase (p<0.05) when compared to day 14 data. Throughout the study, no significant difference (p>0.05) was revealed between the test groups and their respective control. Administration of Dracaena arborea to rats increased the mount frequency and the effect was more expressed (p<0.05) on day 7 of treatment of normal animals (percentage of increase, 299.24%) and on day 14 in castrated rats (percentage of increase, 308.97%) receiving both the ethanolic extract at the dose of 100 mg/kg compared to their respective control (distilled water group). Testosterone group showed a significant increase of the MF with the increase of the treatment (from day 0 to day 14). The washout period of 14 days brought no significant change (p>0.05) in the mount latency of all groups whilst the MF was significantly reduced in normal (ethanolic extract: 100 and 500 mg/kg; p<0.05) and castrated animals (control (distilled water), ethanolic extract: 100 and 500 mg/kg; p < 0.05) when compared to D14 data.

Effects of Dracaena arborea on intromission

In animals receiving 100 mg/kg of the ethanolic extract of *Dracaena arborea*, there was a significant increase (p<0.05-0.01) in the number of intromission after 7 and 14 days of treatment in normal (percentage of increase, 400.00%) and castrated rats (percentage of increase, 623.91%) respectively. Testosterone treatment also induced a significant increase (p<0.01) of the IF after 14 days of treatment (percentage of increase, 584.78%). In the post-treatment study, a significant increase of IL was observed in normal animals treated with the extracts of *Dracaena arborea* when compared to D14 (ethanolic extract: 100 and 500 mg/kg; p<0.05) and

control data (aqueous extract: 100 mg/kg; ethanolic extract: 500 mg/kg; p<0.05). The IF was significantly reduced (p<0.01) in both normal (ethanolic extract: 100mg/kg; p<0.01) and castrated rats (aqueous extract: 500 mg/kg; p<0.05) when compared to D14 values. In addition, there was an increase of the MF in the testosterone (p<0.05) and ethanolic extract of *Dracaena arborea* (100 mg/kg) (p<0.01) groups when compared to control group (distilled water) (Tables 1, 2, 3, 4).

Effects of Dracaena arborea on ejaculation

The treatments had no significant effect on ejaculation latency of both normal and castrated rats when compared to their respective control (distilled water group; p>0.05). EF was increased in normal rats after 1 (aqueous extract: 100 and 500 mg/kg; p<0.05-0.01) and 14 days (aqueous extract: 100 mg/kg; p<0.05; ethanolic extract: 500 mg/kg; p<0.01) of treatment compared to respective control. Castrated animals (testosterone and 100 mg/kg of the ethanolic extract of *Dracaena arborea*) significantly ejaculated on days 7 (p<0.05-0.01), 14 (p<0.01) and after the wash-out period (p<0.05) (Tables 1, 2, 3, 4).

Effects of Dracaena arborea on erection

Throughout the treatment, a trend to an increase in the PE was observed. Significant increase of the erectile frequency was recorded on days 7 and 14 of treatment in gonado-intact [aqueous extract: 100 mg/kg, percentage of increase 223.26%; ethanolic extract: 100 mg/kg (379.07%) and 500 mg/kg (283.72%)] and castrated rats [testosterone (603.64%); aqueous extract: 100 mg/kg (361.82%); ethanolic extract: 100 mg/kg (643.55%)] respectively and the dose 100 mg/kg of the ethanolic extract of *Dracaena arborea* appeared to be the most active. Withdrawal of the treatment for two weeks resulted in a decrease in the PE of testosterone-treated (p<0.05) and *Dracaena arborea*-treated animals (p<0.05-0.001) compared to respective D14 values (Tables 3& 4).

Effects of haloperidol, atropine or L ω -NAME pre-treatment upon the prosexual effect induced by the ethanolic extract (100 mg/kg) of *Dracaena arborea*.

L ω -NAME (10 mg/kg) failed to influence the sexual behaviour of the normal rats induced by the ethanolic extract (100 mg/kg) of *Dracaena arborea*. The sequential administration of atropine (10 mg/kg) or haloperidol (10 mg/kg) plus the plant extract (100 mg/kg) completely blocked the expression of *Dracaena arborea*-induced erection, intromission and ejaculation (Figure 1).

Table 1: Effects of aqueous and ethanolic extracts from the roots of Dracaena arborea on [ML], (IL) and {EL} of normal sexually experienced male rats

Groups	Period of treatment				
_	D0	D1	D7	D14	D28
Control: distilled water	[483.00±219.26] ^a	[455.40±153.82] ^a	[319.80±230.11] ^a	$[45.80\pm26.07]^{a}$	[60.20±23.42]
10 mL/kg b.w/day, n=5	(488.20±337.85) ^a	(393.40±126.54) ^a	(417.00±331.92) ^a	(151.60±73.94) ^a	(90.60±20.38)
	$\{135.20\pm84.61\}^{a}$	$\{495.00\pm250.98\}^{a}$	$\{1112.20\pm514.79\}^{a}$	$\{1160.60 \pm 489.68\}^{a}$	$\{622.80\pm270.97\}$
Dracaena arborea:	[214.60±118.30] ^a	$[242.60\pm169.43]^{a}$	[157.00±112.78] ^a	$[108.80\pm71.52]^{a}$	[121.80±56.04]
aqueous extract	(737.40±330.05) ^a	(299.60±163.04) ^a	(439.80±343.15) ^a	(229.40±54.64) ^a	$(269.60 \pm 70.63)^*$
100 mg/kg b.w/day, n=5	$\{1718.00\pm 645.75\}^a$	$\{1475.60 \pm 420.80\}^{a}$	$\{1422.80\pm282.74\}^a$	${977.80\pm203.48}^{a}$	$\{1017.20\pm194.68\}$
	[189.40±109.32] ^a	[176.20±129.42] ^a	$[70.40\pm46.35]^{a}$	$[121.00\pm109.95]^{a}$	[41.40±34.39]
500 mg/kg b.w/day, n=5	(259.80±81.85) ^a	(511.20±283.58) ^a	(353.60±231.33) ^a	(306.60±214.57) ^a	(120.20±76.85)
	${939.00\pm540.75}^{a}$	${765.40\pm233.28}^{a}$	$\{1829.80\pm700.83\}^{a}$	${794.20\pm503.95}^{a}$	{726.00±491.73}
Dracaena arborea:	[312.20±224.42] ^a	$[49.20\pm4.49]^{a}$	$[44.40\pm34.35]^{a}$	[55.40±32.25] ^a	[658.00±235.05]
ethanolic extract	(587.60±396.72) ^a	(102.60±32.20) ^a	(100.00±29.86) ^a	(68.80±41.54) ^a	$(899.60 \pm 470.77)^{\alpha}$
100 mg/kg b.w/day, n=5	$\{699.60 \pm 438.99\}^{a}$	$\{893.80\pm640.89\}^{a}$	${933.40\pm466.33}^{a}$	${496.80\pm239.23}^{a}$	{642.20±530.70}
	[143.80±105.46] ^a	$[198.80 \pm 128.70]^{a}$	[14.40±5.90] ^a	[72.40±24.39] ^a	[509.40±223.35]
500 mg/kg b.w/day, n=5	(559.00±340.35) ^a	(208.80±136.71) ^a	(86.80±34.03) ^a	(193.40±92.77) ^a	$(1160.40\pm310.43)^{\alpha^*}$
	${298.20 \pm 184.79}^{a}$	${307.20\pm226.16}^{a}$	$\{827.20\pm244.33\}^{a}$	$\{886.20\pm257.51\}^{a}$	{518.60±181.74}

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

a: For the period of treatment varying from D0 to D14, values with the same superscript letter in the same line (for each copulatory parameter) do not differ significantly (p>0.05) (ANOVA Repeated Measures, Wilcoson test)

*: p<0.05 compared to respective control (Kruskal-Wallis, Mann Whitney test)

 α : p<0.05 compared to day 14 of treatment (ANOVA Repeated Measures, Wilcoson test)

D28 designs rats treated for 14 days and allowed a wash-out period of 14 days.

Values within [], () and {} represent mount latency [ML], intromission latency (IL) and ejaculation latency {EL} respectively

Table 2: Effects of aqueous and ethanolic extracts from the roots of Dracaena arborea on [ML], (IL) and {EL} of castrated sexually experienced male rats

Groups	Period of treatment					
	D0	D1	D7	D14	D28	
Control: distilled water	[38.80±19.09] ^a	[269.80±233.15] ^a	[395.20±221.46] ^a	[307.40±227.19] ^a		
10 mL/kg b.w/day, n=5	(393.80±295.15) ^a	(936.80±599.90) ^a	$(1001.20\pm255.77)^{a}$	(511.60±259.36) ^a	[508.40±228.92]	
	$\{174.80\pm174.80\}^{a}$	${633.60\pm333.10}^{a}$	$\{493.00\pm493.00\}^{a}$	$\{626.20\pm391.25\}^{a}$	(ND)	
					{ND}	
Testosterone propionate	$[959.80 \pm 176.85]^{a}$	$[34.80\pm7.68]^{a}$	$[32.20\pm16.92]^{a}$	$[70.60\pm16.74]^{a}$	[89.60±59.86]	
20 mg/kg b.w/day/3days	(1354.00±235.68) ^a	$(478.40 \pm 435.73)^{a}$	(100.40±43.94) ^a	$(86.00 \pm 15.69)^{a}$	(272.00±163.84)	
	$\{ND\}$	${761.00\pm275.53}^{a}$	$\{462.80\pm205.14\}^a$	$\{431.40\pm119.54\}^{a}$	$\{980.20 \pm 462.97\}^*$	
Dracaena arborea:	$[502.60\pm284.84]^{a}$	[331.40±210.67] ^a	$[173.60\pm87.38]^{a}$	$[38.00\pm14.10]^{a}$	[316.80±214.68]	
aqueous extract	(940.00±700.99) ^a	$(1105.40\pm500.75)^{a}$	(675.20±513.35) ^a	$(104.00\pm 26.12)^{a}$	(278.60±278.60)	
100 mg/kg b.w/day, n=5	$\{576.40\pm576.40\}^{a}$	{ND}	${462.80\pm205.14}^{a}$	${431.40 \pm 119.54}^{a}$	{ND	
	$[439.40\pm215.88]^{a}$	$[310.80\pm216.53]^{a}$	$[147.00\pm99.58]^{a}$	$[81.20\pm63.13]^{a}$	[64.00±48.44]	
500 mg/kg b.w/day, n=5	$(864.80\pm620.57)^{a}$	(684.40±530.91) ^a	(704.60±302.94) ^a	$(145.40\pm46.94)^{a}$	(76.80±60.83)	
	${360.60 \pm 360.60}^{a}$	{ND}	${226.20\pm226.20}^{a}$	$\{185.80\pm185.80\}^{a}$	{ND}	
Dracaena arborea:	[344.40±209.03] ^a	$[244.20\pm226.49]^{a}$	$[78.80\pm26.81]^{a}$	[154.20±74.71] ^a	[64.60±26.50]	
ethanolic extract	(602.60±252.23) ^a	(255.40±98.30) ^a	$(102.00\pm35.02)^{a}$	(238.20±82.25) ^a	(275.00±146.80)	
100 mg/kg b.w/day, n=5	$\{601.40\pm293.41\}^{a}$	$\{1101.20\pm548.92\}^{a}$	$\{1253.60\pm123.50\}^{a}$	$\{542.40\pm 360.92\}^a$	$\{813.40\pm336.60\}^*$	
	$[69.60\pm20.39]^{a}$	[13.20±7.79] ^a	$[62.40\pm42.83]^{a}$	$[92.20\pm74.67]^{a}$	[216.60±137.93]	
500 mg/kg b.w/day, n=5	(198.00±131.21) ^a	(415.80±232.89) ^a	(125.80±103.99) ^a	(361.40±323.36) ^a	(56.80±42.10)	
	${271.00\pm271.00}^{a}$	{ND}	{ND}	$\{115.80\pm115.80\}^{a}$	{ND}	

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

a: For the period of treatment varying from D0 to D14, values with the same superscript letter in the same line (for each copulatory parameter) do not differ significantly (p>0.05) (ANOVA Repeated Measures, Wilcoson test)

*: p<0.05 compared to respective control (Kruskal-Wallis, Mann Whitney test).

D28 designs rats treated for 14 days and allowed a wash-out period of 14 days.

ND: Not determined

Values within [], () and {} represent mount latency [ML], intromission latency (IL) and ejaculation latency {EL} respectively.

Groups	Period of treatment				
	D0	D1	D7	D14	D28
Control: distilled water	$[32.60\pm5.64]^{a}(24.00\pm4.53)^{a}$	$[25.00\pm3.35]^{a}$ $(18.40\pm2.01)^{a}$	[26.20±4.47] ^a (14.00±2.28) ^a	$[27.20\pm8.62]^{a}$ $(15.00\pm5.08)^{a}$	[17.20±3.32] (12.40±2.69)
10 mL/kg b.w/day, n=5	$<\!\!<\!\!26.40{\pm}4.84 \\ >\!\!^{a} \{1.80{\pm}0.80\}^{a}$	${\scriptstyle (19.00\pm2.17)}^{a} \ {\scriptstyle (0.80\pm0.49)}^{a}$	$(17.20\pm2.25)^{a} \{1.60\pm0.40\}^{a}$	$(18.00\pm4.05)^{a}$ $(1.20\pm0.20)^{a}$	«20.80±5.26» {1.20±0.37}
Dracaena arborea:					
aqueous extract	$[43.00\pm4.75]^{a}$ $(20.20\pm3.69)^{a}$	$[34.20\pm6.71]^{a}$ $(19.00\pm5.54)^{a}$	$[53.60\pm6.04]^{a}$ $(28.00\pm4.51)^{a}$	$[45.60\pm10.35]^{a}$ (26.40±7.24) ^a	[50.00±8.62] (24.00±5.01)
100 mg/kg b.w/day, n=5	$(34.80\pm7.26)^{a}$ $\{2.00\pm0.55\}^{a}$	${\scriptstyle {\rm \ll}31.40\pm8.04}{\scriptstyle {\rm \gg}^{a}} {\scriptstyle {\rm (2.60\pm0.51)}^{a^{*}}}$	$(38.40\pm5.52)^{a^{*}}$ $(3.20\pm0.37)^{a}$	${\scriptstyle {\rm (40.20\pm8.96)}^a} {\scriptstyle \{2.80\pm0.49\}}^{a^*}$	«29.40±6.40» {3.40±0.40}
500 mg/kg b.w/day, n=5	$\begin{array}{l} [29.40{\pm}7.98]^a & (16.00{\pm}3.74)^a \\ {\approx}24.80{\pm}8.51{\gg}^a & \{1.40{\pm}0.40\}^a \end{array}$	$[57.60\pm12.33]^{b}$ $(39.60\pm7.39)^{b}$ $(63.00\pm18.73)^{b}$ $\{2.80\pm0.20\}^{b^{**}}$	$\begin{array}{l} [61.00{\pm}15.33]^{ab} \left(33.00{\pm}8.63\right)^{ab} \\ {}^{\ast}51.00{\pm}13.03{}^{ab} \left\{2.20{\pm}0.80\right\}^{ab} \end{array}$	$\begin{array}{l} [65.20{\pm}18.65]^{ab} \ (35.40{\pm}9.80)^{ab} \\ \\ \ \ \ \ \ \ \ \ \ \ \ \ $	[32.80±13.65] (11.40±4.87) «22.40±10.46» {1.40±0.75}
Dracaena arborea: ethanolic extract 100 mg/kg b.w/day, n=5	$\begin{array}{l} [43.00{\pm}11.54]^a ~(15.60{\pm}3.08)^a \\ {\color{red} {\scriptstyle \ll 25.20 \pm 5.10 }}^a ~\{0.80{\pm}0.37\}^a \end{array}$	$[55.80\pm19.99]^{ab}(34.40\pm14.24)^{ab}$ «48.20±16.79» ^a {1.60±0.40} ^a	$ [78.40 \pm 16.67]^{b} (56.00 \pm 16.46)^{b} \\ \ll 65.20 \pm 15.90 \\ \gg^{a^{*}} \{2.20 \pm 0.58\}^{a} $	$\begin{array}{l} [43.00{\pm}6.65]^{ab} & (28.60{\pm}4.17)^{ab} \\ & \ \ \ \ \ \ \ \ \ \ \ \ \$	$\begin{array}{l} [25.60{\pm}5.34]^{\delta} \ (11.20{\pm}3.75)^{\beta} \\ \\ \ \ \ \ \ \ \ \ \ \ \ \ $
500 mg/kg b.w/day, n=5	[31.80±6.41] ^a (11.60±3.47) ^a «16.60±4.95» ^a {0.80±0.37} ^a	$ \begin{array}{ll} [50.40{\pm}10.59]^{ab} & (20.60{\pm}4.98)^{ac} \\ & (29.80{\pm}7.43)^{a} & \{1.00{\pm}0.63\}^{ab} \end{array} $	$[67.60\pm8.94]^{b}$ (41.40±6.05) ^b «48.80±6.26» ^{b**} {2.60±0.68} ^{ab}	$[73.40\pm15.97]^{b}$ (43.20±9.61) ^c «59.80±12.37» ^{c**} {3.20±0.37} ^{b**}	$[26.40\pm4.60]^{\delta}$ (22.20±2.82) «19.60±4.00» ^{δ} {2.00±0.45}

Table 3: Effects of aqueous and ethanolic extracts from the roots of Dracaena arborea on [MF], (IF), «PE » and {EF} of normal sexually experienced male rats

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

a,b,c: For the period of treatment varying from D0 to D14, values with the same superscript letter in the same line (for each copulatory parameter) do not differ significantly (p>0.05) (ANOVA Repeated Measures, Wilcoson test)

*: p<0.05 compared to respective control (Kruskal-Wallis, Mann Whitney test).

 β : p<0.01; δ : p<0.001 compared to day 14 of treatment (ANOVA Repeated Measures, Wilcoson test)

D28 designs rats treated for 14 days and allowed a wash-out period of 14 days

Values within [], (), « » and {} represent mount frequency [MF], intromission frequency (IF), penile erection «PE» and ejaculation frequency {EF} respectively.

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Groups			Period of treatment		
	D0	D1	D7	D14	D28
Control: distilled water	$[32.60\pm11.48]^{a}$ $(11.00\pm4.34)^{a}$	$[45.80\pm11.65]^{a}$ (23.60±8.84) ^a	$[38.20\pm4.99]^{a}$ $(18.60\pm1.96)^{a}$	$[31.20\pm12.24]^{a}$ $(9.20\pm2.35)^{a}$	$[7.80\pm3.37]^{\alpha}$ $(00.00\pm00.00)^{\alpha}$
10 mL/kg b.w/day, n=5	${\scriptstyle (14.40\pm6.18)^{a}} {\scriptstyle (0.20\pm0.20)^{a}}$	${\scriptstyle (30.00\pm9.76)^{a}} {\scriptstyle \{1.00\pm0.45\}^{a}}$	${\scriptstyle ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~$	${\scriptstyle (11.00\pm3.13)}^{a} \ {\scriptstyle (0.60\pm0.24)}^{a}$	$(1.40\pm1.40)^{\alpha} \{00.00\pm00.00\}$
Testosterone propionate	$[21.20\pm2.06]^{ab}$ $(14.00\pm3.50)^{a}$	$[41.80\pm13.43]^{ab}$ $(31.00\pm12.73)^{ab}$	$[70.80\pm7.81]^{a^*}$ (45.00±3.41) ^{b**}	$[69.40\pm11.15]^{b^*}$ (53.80±9.44) ^{b**}	[41.40±11.03] (29.40±7.85)*
20 mg/kg .w/day/3days	$(22.60\pm3.53)^{a} \{0.00\pm0.00\}^{a}$	${\scriptstyle (31.40 \pm 11.35)}^{ab} {\scriptstyle \{2.60 \pm 0.75\}}^{ab}$	${\scriptstyle { { { { { { { { { { { { { { { { { { $	$(66.40 \pm 9.13)^{b^{**}} \{3.00 \pm 0.55\}^{b^{**}}$	$(27.40\pm7.14)^{\alpha^*}$ $\{3.40\pm0.93\}^*$
Dracaena arborea:					
aqueous extract	$[14.20\pm1.77]^{a}$ $(4.00\pm1.10)^{a}$	$[21.80\pm1.50]^{ab}$ $(18.40\pm1.12)^{b}$	$[35.60\pm6.38]^{b}$ $(5.40\pm1.99)^{ab^{**}}$	$[56.40\pm12.75]^{b}$ $(22.80\pm5.07)^{b^{*}}$	$[7.40\pm3.19]^{\alpha}(0.60\pm0.60)^{\alpha}$
100 mg/kg b.w/day,	$(8.00 \pm 2.43)^{a} = \{0.20 \pm 0.20\}^{a}$	${\approx}24.80{\pm}1.16{\ast}^{b} {\{0.00{\pm}0.00\}}^{a^{*}}$	${\approx}21.80{\pm}6.01{>\!\!\!>}^b \{0.20{\pm}0.20\}^a$	${\scriptstyle (39.80\pm10.25)}^{\rm b*} \ {\scriptstyle \{1.20\pm0.58\}}^{\rm a}$	$(3.60\pm 2.23)^{\alpha}$ {0.00±0.00}
n=5					
500 mg/kg b.w/day,	$[17.40\pm2.27]^{a}$ $(2.40\pm1.60)^{a}$	$[23.00\pm6.11]^{ab}$ $(6.80\pm2.03)^{ab}$	$[26.00\pm1.34]^{b^*}$ $(5.00\pm2.10)^{ab^{**}}$	$[34.60\pm4.04]^{a}$ $(25.80\pm5.70)^{b^{*}}$	$[3.80\pm2.33]^{\alpha}$ $(2.60\pm1.60)^{\alpha}$
n=5	${\scriptstyle { { \langle 4.40 \pm 2.86 \rangle }^{a} }} { \{ 0.20 { \pm 0.20 \} }^{a} }$	${\scriptstyle (12.80\pm5.69)^{a}} {\scriptstyle (00.00\pm00.00)^{a^{*}}}$	${\scriptstyle \ll 14.20 \pm 5.44 }{\scriptstyle > a} {\scriptstyle \{ 0.20 \pm 0.20 \}}{\scriptstyle > a}$	$(27.40\pm7.97)^{a} \{0.20\pm0.20\}^{a}$	$(5.20\pm3.25)^{\alpha}$ {00.00±00.00}
Dracaena arborea:					
ethanolic extract	$[29.40\pm6.93]^{ab}$ $(17.20\pm5.27)^{a}$	$[50.40\pm10.95]^a$ $(29.00\pm7.82)^b$	$[70.20\pm22.58]^{ab}$ $(47.60\pm17.07)^{b^*}$	$[96.40\pm18.43]^{b^*}(57.40\pm14.15)^{ab^{**}}$	[53.40±14.44]* (30.00±10.02)**
100 mg/kg b.w/day,	$<\!\!<\!\!20.20{\pm}4.43 \\ >^{a} \{0.80{\pm}0.37\}^{a}$	${\scriptstyle (36.80 \pm 11.26)}^{\rm b} {\scriptstyle \{1.20 \pm 0.49\}}^{\rm a}$	${\ll}43.00{\pm}19.54{\ast}^{ab}~\{1.00{\pm}00.00\}^{a^*}$	$(69.80\pm15.89)^{b^{**}}$ $\{1.80\pm0.73\}^{a}$	$(47.00\pm13.62)^{**}$ $\{0.60\pm0.24\}^{*}$
n=5					
500 mg/kg b.w/day,	[48.00±11.31] ^a (17.00±7.29) ^a	[40.20±15.91] ^a (12.40±5.12) ^a	[37.40±19.26] ^a (21.80±12.56) ^a	[31.40±12.98] ^a (14.00±6.30) ^a	[26.60±8.49] (4.60±3.68)
n=5	«26.60±8.79» ^a {0.20±0.20} ^a	$(10.00\pm4.21)^{a}$ $\{00.00\pm00.00\}^{a^{*}}$	«31.00±17.59» ^a {00.00±00.00} ^a	$(22.00\pm9.74)^{a}$ $\{0.40\pm0.40\}^{a}$	«7.40±5.31» {00.00±00.00}

Table 4: Effects of aqueous and ethanolic extracts from the roots of Dracaena arborea on [MF], (IF), «PE » and {EF} of castrated sexually experienced male rats

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

a,b,c: For the period of treatment varying from D0 to D14, values with the same superscript letter in the same line (for each copulatory parameter) do not differ significantly (p>0.05) (ANOVA Repeated Measures, Wilcoson test)

*: p<0.05; **:p<0.01; ***:p<0.001 compared to respective control (Kruskal-Wallis, Mann Whitney test).

 α : p<0.05 compared to day 14 of treatment (ANOVA Repeated Measures, Wilcoson test)

D28 designs rats treated for 14 days and allowed a wash-out period of 14 days

Values within [], (), « » and {} represent mount frequency [MF], intromission frequency (IF), penile erection «PE» and ejaculation frequency {EF} respectively.







Figure 1: Effects of the ethanolic extract (100 mg/kg) of *Dracaena arborea* on the mount latency (A) and frequency (B), intromission latency (C) and frequency (D), penile erection (E), ejaculation latency (F) and frequency (G) of rat pre-treated with L ω -NAME (10mg/kg), atropine (10mg/kg) or haloperidol(10mg/kg).

Each bar represents Mean \pm SEM of 5 values.

C1: Control 1 (Distilled water, 10mL/kg); Da: Ethanolic extract (100mg/kg) of *Dracaena arborea*; C2: Control 2 (0.25% Tween 80 in NaCl 0.9%, 10mL/kg); LN: Lω-NAME+ plant extract; Atr: Atropine + plant extract; Hal: Haloperidol + plant extract.

Discussion

Mixture of the roots of Dracaena arborea with "palm wine" is used in cameroonian ethnomedicine for the treatment of male impotency. Results of the present study confirm the claims on the use of Dracaena arborea as sexual stimulating agent. Oral administration of the aqueous and ethanolic extracts of Dracaena arborea increased the erection, mount and intromission frequencies and the effect was more expressed after 7 days of treatment of gonado-intact sexually experienced male rats. Enhancement of sexual performance in sexually experienced animals has been proven (25). An interesting finding in the present work is the increase of the sexual performance of the treated animals with the increase of treatment, and the ethanolic extract being more potent than the aqueous extract. Flavonoids and sterols revealed in the extracts from Dracaena arborea may account for the pro-sexual effects of the plant extract. It could be suggested that these bioactive principles probably activate the central neurotransmitters involved in the regulation of male sexual behaviour or stimulate the corpus cavernosum for the release of nitric oxide (NO), the key element responsible for penile erection (26) or mediatee androgen biosynthesis (27). It is well established that androgens play a capital role in the control of erectile function (10, 28). In an attempt of assessing the influence of *Dracaena arborea* on androgen pathway in the modulation of male sexual behaviour, the effects of the aqueous and ethanolic extracts of this plant were investigated in castrated animals. Data from literature correlates castration with complete loss of sexual behaviour (29). On day 14 of treatment, Dracaena arborea significantly increased (p<0.01) the erection, mount and intromission frequencies and shortened (p>0.05) the mount, intromission and ejaculation latencies. These effects were more expressed at the dose of 100 mg/kg b.w of the ethanolic extract compared to control and all aqueous extract groups. This restoration of sexual activity in castrated animals by Dracaena arborea suggests an androgen-like effect of the plant. This hypothesis could be supported in the present study by the complete loss of sexual activity in orchidectomised rats treated with distilled water in the one hand, and by the enhancement of sexual activity in animals receiving a subcutaneous injection of testosterone proprionate in the other hand (30, 31). Arletti and

coworkers (32) reported similar effects after oral administration of *Turnera diffusa* and *Pfaffia paniculata* extracts in impotent male rats while other investigators demonstrated the androgenic properties of some aphrodisiac plants (33, 34). The ethanolic extract of *Dracaena arborea* appeared to be more potent than the aqueous extract in normal rats compared to castrated model lending some explanation and support to the traditional preparation of roots of *Dracaena arborea* in "palm wine", a traditional alcoholic solution for handling sexual weakness in males.

In order to determine the influence of Dracaena arborea on the pathway of some neurotransmitters involved in the regulation of male sexual behaviour, the effects of the ethanolic extract (the most active extract, 100 mg/kg b.w) was examined in the presence of inhibitors of NO synthase (L@-NAME), dopaminergic receptor (haloperidol) or metabotropic muscarinic receptor (atropine). The ability of the ethanolic extract of Dracaena arborea to induce libido in sexually experienced rats pre-treated with Lo-NAME indicates the non requirement of endogenous NO to induce penile erection (5, 35). Abolition of the sexual stimulant effects of Dracaena arborea after pre-treatment of rats with either haloperidol or atropine suggests a dopaminergic and/or a muscarinic-like effect of the plant. It is well established that dopamine and acetylcholine certainly among many neurotransmitters play an important role in the expression of male sexual behaviour (36, 37). The pro-erectile effect of acetylcholine results from the inhibition of the release of noradrenaline and/or some relaxant factors such as nitric oxide (5) whereas dopamine activates principally D₂ receptors leading to penile erection and seminal emission (36).

In conclusion, the present findings clearly show that the roots of *Dracaena arborea* possess potent sexual stimulant activity and provide some scientific evidence in favour of the claims regarding this action. However, further studies are required to investigate the effects of the bioactive compounds in order to assess their potential in the treatment of erectile dysfunction.

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References

1 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.

2 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.

3 Rampin O, Ferome N, Suaudeau C. Proerectile effects of apomorphine in mice. Life Sci 2003;72:2329-2336.

4 Lopez HH, Ettenberg A. Haloperidol challenge during copulation prevent subsequent increase in male sexual motivation. Pharmacol Biochem Behavior 2000;67:387-393.

5 Lundberg JM. Pharmacology of cotransmission in the autonomic nervous: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. Pharmacol Rev 1996;48:113-178.

6 Andersson KE. Pharmacology of penile erection. Pharmacol Rev 2001;53:417-450.

7 Caulfield MP, Birdsall NJ. International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol Rev 1998;50:279-290.

8 Burnett AL, Lowenstein CJ, Bredt DS, Chang TS, Snyder SH. Nitric oxide: a physiological mediator of penile erection. Science 1992;257:401-403.

9 Cartledge J, Suks M and Eardley I. the role of nitric oxide in penile erection. Expert Opin Pharmacother 2001;2:1-13.

10 Shabsigh R. Testosterone therapy in erectile dysfunction and hypogonadism. J Sex Med 2005;2:785-792.

11 Mills T, Stopper V, Wiedmeier V. Effects of castration and androgen replacement on the hemodynamics of penile erection in the rat. Biol Reprod 2004;54:234-238.

12 Neychev VK, Mitev VI. The aphrodisiac herb *Tribulus terrestris* does not influence the androgen production in young men. J Pharmacol 2005;101:319-323.

13 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.

14 Ang HH, Ngai TH, Tan TH. Effects of Eurycoma longifolia Jack on sexual qualities in middle aged male rats. Phytomedicine 2003;10:590-593.

15 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.

16 Watcho P, Zelefack F, Nguelefack TB, et al. Effects of the aqueous and hexane extracts of *Mondia whitei* on the sexual behaviour and some fertility parameters of sexually inexperienced male rats. Afr. J. Trad. CAM 2007;4:37-46.

17 Okunji CO, Iwu MM, Jackson JE, Tally JD. Biological activity of saponins from two Dracaena species. Adv Exp Med Biol1996;204:415-418.

18 Noumi E, Amvam Zollo PH, Lontsi D. Aphrodisiac plants used in Cameroon. Fitoterapia 1998;2:125-134.

19 Markham KR. Technique of flavonoid identification. Academic, New York 1982; pp1-113.

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

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

22 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, p 1991.

23 Meyerson BJ, Lindstrom L. Sexual motivation in the female rat. Acta Physiol Scand Suppl 1973;389:1-80.

24 Roubinian JR, Talal NJ, Greenspan S, Goodman JR, Sjjteri PK. Effects of castration and sex hormone treatment on survival, anti-nucleic antibodies, and glomerulonephritis in NZB/NZW Fl mice. J Exp. Med 1978;147:1568-1583.

25 Beach FA. Sexual attractivity, proceptivity and receptivity. Horm Behav 1976;7:105-138.

26 Silluvan ME, Thompson CS, Dashwood MR, et al. Nitric oxide and penile erection: Is erectile dysfunction another manifestation of vascular disease? Cardiovasc Res 1999;43:658-665.

27 Morelli A, Filippi S, Mancina R, et al. Androgens regulate phosphodiesterase type 5 expression and functional activity in corpora cavernosa. Endocrinology 2004;145:2253-2263.

28 Putnam SK, Du J, Sato S, Hull EM. Testosterone restoration of copulatory behaviour correlates with medial preoptic dopamine release in castrated male rats. Horm Behav 2005;39:216-224.

29 Baba K, Yajima M, Carrier S. Delayed testosterone replacement restores nitric oxide synthetase containing nerve fibres and the erectile response in rat penis. Br J Urol 2000;85:953-958.

30 Monga M, Kostelec M, Kamarei M. Patient satisfaction with testosterone supplementation for the treatment of erectile dysfunction. Arch Androl 2002;48:433-442.

31 Bialy M, Sachs BD. Androgen implants in medial amygdale briefly maintain non contact erection in castrated male rats. Horm Behav 2002;42:345-355.

32 Arletti R, Benelli A, Cavazzuti E, Scarpetta G, Bertolini A. Stimulating property of *Turna diffusa* and *Pfaffa paniculata* extracts on the sexual behaviour of male rats. Psychopharmacology 1999;143:15-19.

33 Ilarinov I. Androgenic and aphrodisiac action of the medicinal plant *Lithospermum arvense*. Eks med morfol 1989;28:28-33.

34 Gauthaman K, Adaikan PG, Prasad RN. Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in normal and castrated rats. Life Sci 2002;71:1385-1396.

35 Kalsi JS, Ralph DJ, Thomas F, et al. A nitric-releasing PDE5 inhibitor relaxes human corpus cavernosum in the absence of endogenous nitric oxide. J Sex Med 2005;2:53-57.

36 Velasco M, Lucchsinger A. Dopamine: Pharmacological and Therapeutic aspects. Am J Ther 1998;5:37-43.

37 Maeda N, Matsuoka N, Yamaguchi I. Role of the dopaminergic, serotonergic and cholinergic link in the expression of penile erection in rat. Jpn J Pharmacol 1994;66:59-66.