



Pro-Sexual Effects of Aqueous and Methanol Extracts of *Phyllanthus muellerianus* (Kuntze) Exell (Phyllanthaceae) on a Model of Low Sexual Desire Disorder in Female Rats

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Abstract

Low sexual desire disorder is a recurrent deficiency in desire for sexual activity which impairs lifestyle. It is a frequent problem in women with limited therapeutic options. *Phyllanthus muellerianus* (Kuntze) Exell is a plant used by traditional healers to boost libido in women. This study aimed at evaluating the aphrodisiac properties of root barks extracts of this plant on a model of Low Sexual Desire Disorder (LSDD) in female rats. Low sexual desire was induced by ovariectomy plus low steroid hormones supplementations. Thirty ovariectomized rats were treated for 21 days with either distilled water, aqueous or methanol extracts (60 or 372 mg/kg) of *P. muellerianus*. All animals were supplemented with a low dose of 17 β -estradiol and progesterone prior to sexual behavior test. Sexual behavior test was performed each week by pairing each female rat with a sexually experienced male. Sexual motivation (approach, ear wiggling, hops and darts, anogenital presentation and aggressive behavior frequency) and sexual receptivity parameters (lordosis frequency and quotient) were recorded. At the end of treatments, animals were sacrificed, uteri and vagina collected, weighed and conserved for total uterine proteins assay and histology. LSDD was characterized by significant inhibition of sexual motivation parameters and lordosis frequency compared to a normal control. Moreover, poor reproductive tissues growth characterized by low total uterine proteins, uterine wet weight and uterine epithelia size was noted in LSDD group compared to normal control. Interestingly, plant extracts significantly improved sexual motivation parameters ($p < 0.05-0.001$) compared to LSDD group. In addition, moderate reproductive tissues growth was noted in plant treated groups as confirmed by amelioration of uterine cell integrity. Present results show that *P. muellerianus* exhibits pro-sexual effects through amelioration of sexual motivation and reproductive tissues growth on a model of LSDD.

Keywords: Female sexual dysfunctions; Estrogens; Sexual behavior; *Phyllanthus muellerianus*; Rats

Introduction

Female sexual response cycle is made up of desire, arousal, orgasm and resolution phases. Inhibition of

one of these phases leads to female sexual dysfunctions [1]. Female Sexual Dysfunctions (FSD) as described in the Diagnostic and Statistical Manual of

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Mental Disorders (DSM) include low sexual desire, orgasmic, sexual arousal and sexual pain disorders [2,3]. Available information shows that FSD is a common health problem, with a worldwide prevalence of 22%–43% [4]. A lack of sexual desire ranks as the most prevalent sexual concern, ranging from 7% to 14% of women between 18 and 80 years [5,6].

Low Sexual Desire Disorder (LSDD) is defined as a persistent or recurrent deficiency of sexual fantasies and desire for sexual activity that causes marked distress [2]. Several causes of LSDD have been reported among which psychological (stress), physiological (diabetes) and drug (antidepressants) problems [7,8]. Sexual desire also called sexual motivation is controlled by neural and hormonal pathways. The neural pathway involves sexual excitation (action of dopamine, oxytocin) or sexual inhibition (action of serotonin, opioids). Hormonal pathway involves ovarian hormones (estrogens and progesterone) which play an important role on sexual motivation by priming the excitatory sexual system thereby increasing libido [9]. In laboratory female rats, the synergic administration of estradiol within 48–54 hours and progesterone within 5–6 hours prior to sexual behavioral tests can absolutely induce reproductive behavior in ovariectomized animals [4,10]. Estrogens priming cause an increase in progesterone receptor (PR) levels in the hypothalamus and preoptic areas of the rat brain and progesterone amplifies the positive effect of estrogens on sexual behavior [11]. Moreover, estrogens action on sexual behavior is associated with modulation of tissues growth of some reproductive organs (uterus, vagina) favoring embryo implantation [12] suitable for reproduction. Hence, an experimental model of LSDD can be conceived through ovariectomy (suppression of the principal source of ovarian hormones) followed by sub-optimal (low) supplementation of 17β -estradiol and/or progesterone in female rats during sexual behavioral tests [4,13]. Female rats suffering from LSDD (sexually hypoactive female rats) are characterized by low sexual behavior [14] and impaired reproductive tissues growth due to estrogens deprivation or inaction. Some therapeutic approaches of LSDD have been elucidated such as antipsychotics and some dopamine agonists [15,16]. However, these management options are too expensive, with serious side effects (dizziness, anxiety) and contraindications [17]. Thus, a need for alternative and complementary options among which medicinal plants. Medicinal plants are widely used in African traditional medicine to ameliorate health conditions due to their wide variety of bioactive substances. A plant such as *Anthonotha macrophylla* P.Beauv. [18] has shown aphrodisiac properties by increasing sexual behavior in female rats. *Phyllanthus muellerianus* (Kuntze) Exell (Phyllanthaceae) commonly called “Mbolongo” in Eastern Cameroon, is a tropical

monoecious climbing shrub used for the management of libido and anovulation according to ethnobotanical survey [19]. Phytochemical studies have shown the presence of alkaloids (dopaminergic agonist), isoquercitrin (estrogens receptors agonist), phenol (cell proliferation), polyphenols, geraniin, sterols and glycosides [20]. Our research team showed that root barks of *P. muellerianus* restore ovarian functions in an animal model of polycystic ovarian syndrome through increase of estradiol content [21]. Hence, this plant possesses estrogenic (isoquercitrin) and dopaminergic compounds (alkaloids) necessary to boost libido as claimed by traditional use. Therefore, *P. muellerianus* may represent a favorable candidate for ameliorating low sexual desire in females. Considering the above-mentioned phytochemical and pharmacological properties, the aim of the present study was to assess the effects of aqueous and methanol extracts of *P. muellerianus* on the sexual behavior and reproductive tissues growth in a model of Low Sexual Desire Disorder in female rats.

Materials and Methods

Plant material and preparation of aqueous and methanol extracts

Fresh root barks of *Phyllanthus muellerianus* were harvested in Makenene, Center Region of Cameroon. Botanical authentication was done at the Cameroon National Herbarium in Yaounde (voucher specimen N.BWPV03). These roots were then cut, shade-dried for 7 days, powdered with an electric mixer and the powder used to prepare different extracts. The preparation of plant extracts was done according to traditional usage as follows:

Aqueous extract was obtained by infusing 250 g of *P. muellerianus* powder in 1 L of distilled water for one hour. After filtration, the filtrate was oven-dried (45°C) to obtain 21.15 g of residue (Extraction yield: 8.46%).

The methanol extract of *P. muellerianus* was obtained by macerating 250 g of powder in 1 L of methanol during 72 h at room temperature. After filtration and solvent evaporation under reduced pressure, 15.17 g of a brownish residue were obtained (Extraction yield: 6.07%).

Determination of doses

Plant extracts solutions were prepared in distilled water and orally administered at the doses of 60 and 372 mg/kg for each extract according to a previous study in our research team where the therapeutic dose to humans was found to be 60 mg/kg [21]. This therapeutic dose of humans was then multiplied by a factor of 6.2 so as to give the therapeutic dose in animals (372 mg/

kg) as reported by [22].

Animals

A total of 30 female Wistar rats aged 10-12 weeks (average weight 180-200 g) and 30 adult male Wistar rats aged 12-14 weeks (average weight 200-250 g) were obtained from the animal house of the Research Unit of Animal Physiology and Phytopharmacology, University of Dschang (Cameroon). Rats were housed 3 per cage (plastic cages) and kept at room temperature under natural day and night cycle. They had free access to a standard soy-free diet and water ad libitum. This study was approved by the scientific committee of the Department of Animal Biology, University of Dschang. Animal handling and in vivo experiments were carried out in conformity with the European Union on Animal Care (CEE Council 86/609) guidelines adopted by the Institutional Ethics Committee of the Cameroon Ministry of Scientific Research and Technology Innovation (Reg. no. FWA-IRD 0001954).

Drugs

17 β -estradiol (E) and progesterone (P) were purchased from Sigma (St. Louis, MO, USA). These hormones were dissolved in ethanol (90%) and administered in oil. Total uterine proteins assay kit was purchased from sigma diagnostics, Hungary.

Experimental design

The ability of aqueous and methanol extracts of *P. muellerianus* to improve female sexual behavior in sexually hypoactive female rats was investigated. Ovariectomy completely abolishes sexual behavior and impairs reproductive tissues growth, since the principal source of estrogens is removed. Firstly, female rats were ovariectomized under diazepam/ketamine anesthesia, following the technique of [23], with little modification as described by [24]. After one week of post-surgical recovery, ovariectomized rats exhibited sexual behavioral trainings after sequential subcutaneous injections of high doses of estradiol benzoate (30 μ g) and progesterone (600 μ g), 54 hours and 6 hours, respectively prior to mating test [25]. At the end, ovariectomized rats who exhibited lordosis posture during three consecutive training sessions were considered sexually active and selected for this study.

Induction of low sexual desire (sexually hypoactive female rats)

Low Sexual Desire Disorder was induced in ovariectomized rats through low supplementations with estradiol (10 μ g /rat) and progesterone (300 μ g) 54 h and 6 h respectively, prior to sexual behavior test only. These doses were selected after a screening carried out in our research team. This low supplementation

with estradiol and progesterone in ovariectomized rats permits sensitization of few estrogens receptors (low sexual desire) prior to action of unknown pharmacological substances which may bound on these receptors to produce their effects or activate more estrogens receptors. Without this hormonal supplementation in ovariectomized rats, sexual behavior cannot be possible due to absence of sex hormones.

Animals grouping and treatment

Thirty ovariectomized rats (OVX) were divided into 6 groups of 5 animals each and orally treated with the following substances. Group 1 served as the normal control (NC) constituted of OVX under maximal supplementation of estradiol (30 μ g/rat) and progesterone (600 μ g/rat) while group 2 was the negative control (DW) constituted of OVX receiving distilled water (10 mL/kg) under low supplementation with estradiol (10 μ g /rat) and progesterone (300 μ g /rat) prior to sexual behavior test. Group 3-4 and 5-6 constituted test groups made of OVX receiving aqueous or methanol extract at doses of 60 or 372 mg/kg respectively under low supplementation with estradiol (10 μ g /rat) and progesterone (300 μ g /rat). Hormones were administered subcutaneously.

In this study the NC functioned as a positive controlled since it represented ovariectomized rats receiving the highest dose of estradiol and progesterone which gives an excellent sexual desire and receptivity state. This normal control permitted to compare the sexual behavior of the negative control and see how sexual behavior was reduced (Low sexual desire disorder).

Sexual behavior analysis

Sexual behavior test was performed on days 0, 7, 14 and 21 in a dark and quiet room containing circular mating arenas of 50 cm diameter \times 40 cm high. All female rats were gently paired with sexually experienced males and sexual behavior recorded for 30 minutes using a night owl camera (model: P-85-462RT) connected to a Digital video recorder (model: P-DVR8-5GB). From the recordings the following female behavioral parameters were scored per 30 minutes trial by an operator blinded to the study:

Female full solicitation (approach frequency) consisted of an approach to the male rat, followed by abrupt run away [13].

Female partial solicitations consisted of rapid lateral shaking of the head causing the appearance of ear vibrations (ear wiggling frequency), short lip or jump with stiff legs followed by immobility or a run of several steps (hops and/or darts frequency) and finally presentation of the posterior end to the male (anogenital presentation frequency) [18].

Aggressive behaviors were referred to the number of fights and defense postures done to reject the male

partner. This parameter is quantified through defense and rejection frequency [15].

Lordosis parameters: lordosis was characterized by a crouching posture, with the tail flicked to the side to facilitate the male mounting. Lordosis parameters are mainly lordosis frequency and quotient. Lordosis frequency (LF) refers to the number of lordosis posture exhibited during each test period while lordosis quotient (LQ) is determined after dividing LF by total number of mounts multiplied by 100 [13].

Also, some male sexual behavior parameters were equally recorded or calculated to assess changes that occurred as a result of the modification in the female sexual behavior after different pharmacological treatments. This included mounting frequency (MF) and latency (ML), intromission frequency (IF) and latency (IL), ejaculation frequency (EF) and latency (EL) [25].

Blood collection, tissue preparation and biochemical analysis

Twenty-four hours after the last treatment, female rats were anaesthetized by intraperitoneal injection of diazepam/ketamine and blood sample collected through catheterization of the abdominal artery. Uteri and vagina were removed, trimmed of fat and weighed. The left uterus was homogenized in NaCl so as to make a 10% (g/mL) solution and centrifuged (3000 trs/min during 15 minutes). The supernatant was collected to quantify total proteins through colorimetric method [26]. The right uterus and vagina were fixed in 10% formalin for histological analysis. The estrogenic effect of the plant extracts perceived in ovariectomized rats by reproductive tissue growth was evaluated based on uterine wet weight, total uterine proteins level, uterine and vaginal histomorphological analysis.

Histomorphological studies

Following uterus and vagina fixation, tissue specimens

were dehydrated in a graded series of ethanol (70-100%), cleared in toluene, and finally embedded in paraffin. Thereafter, 5- μ m thin sections were prepared using a microtome (Leica RM2235) and stained with hematoxylin and eosin prior to microscopic examination. The microscopic features of uterine and vaginal tissues (necrosis, presence of cornified layer, cells integrity) were assessed together with their epithelial height measurements using ocular histomorphometer integrated to a light microscope (OLYMPUS, BX51, X400), which was connected to a computer.

Statistical analysis

Data are presented as mean \pm SEM (standard error of mean). For sexual behavior parameters, significance level was determined by two-way repeated measure followed by Bonferroni post-hoc test for multiple comparisons. Data on sex organs weights, total proteins assay, epithelial heights were analyzed using one-way analysis of variance followed by Turkey HSD post hoc test for comparison of mean. Data were considered statistically significant when $p < 0.05$. All tests were performed using Statistica software (Version 8.0, StatSoft, Inc., Tulsa, USA).

Results

Effects of different treatments on the sexual behavior of female rats

Effects on full solicitation frequency

Approach frequency (AF) increased along the treatment period in all the groups when compared to day 0 (Table 1). DW group showed significant reduction (day 14: $p < 0.001$) in AF compared to normal control. A significant increase of AF was observed in aqueous (372 mg/kg: $p < 0.05$) and methanol

Table 1. Effects of different treatments on approach frequency of female rats

Treatments	Doses	Approach frequency (AF)			
		Day 0	Day 7	Day 14	Day 21
Normal control	10 ml/kg	2.60 \pm 1.25	21.20 \pm 3.60	24.60 \pm 1.81	19.60 \pm 4.45
DW	10 ml/kg	1.40 \pm 0.60	7.20 \pm 2.24	2.40 \pm 0.60 ^{###}	6.80 \pm 3.15
Aqueous Extract	60 mg/kg	1.80 \pm 0.80	12.80 \pm 4.33	11.60 \pm 4.65	11.40 \pm 3.04
	372 mg/kg	2.80 \pm 1.24	9.40 \pm 4.55	20.00 \pm 4.14*	8.00 \pm 3.11
Methanol Extract	60 mg/kg	1.40 \pm 0.60	11.20 \pm 4.51	20.20 \pm 3.69*	18.60 \pm 3.47
	372 mg/kg	2.00 \pm 0.71	2.80 \pm 0.49#	16.20 \pm 3.18	16.20 \pm 5.17

Values are expressed as mean \pm SEM, n = 5. DW= Distilled water; # $p < 0.05$ in comparison to normal control; * $p < 0.05$; *** $p < 0.001$ in comparison to DW

(60 mg/kg: $p < 0.05$) extracts along the treatment as compared to DW. Methanol extract at 60 mg/kg (82.11%) showed the best effect after two weeks of treatment.

Effects on partial solicitations

Partial solicitations parameters increased all along the treatment period and in all groups in comparison to day 0. DW showed significant reduction ($p < 0.05-0.001$) in EW, H/D and AN compared to normal control. EW, H/D and AN significantly increased in methanol extract at 60 mg/kg ($p < 0.05-0.001$) compared to DW with highest effects after 14 days of treatment (Table 2).

Effects on aggressive behavior

In all groups, aggressive behavior parameters reduced all along the treatment period in comparison with initial values (day 0). A significant increase of these parameters ($p < 0.01$, defense frequency) was recorded in DW group as compared to normal control. Interestingly, plant extracts significantly increased aggressive behaviors (except for aqueous extract at 60 mg/kg) after two weeks of treatment compared to DW (Table 3).

Effects on lordosis parameters

Table 4 shows that lordosis parameter significantly reduced ($p < 0.05-0.01$) lordosis frequency along the treatment period as compared to normal control. However, administration of plant extracts increased LF and LQ as compared to DW (Table 4). Methanol extract at dose of 60 mg/kg was found to be most effective during the last week of treatment (76.03%, LF; 98.89%, LQ).

Effects of female treatments on male sexual behavior parameters

We found an increasing trend in MF, IF and EF all along the treatment period as compared to their initial values. Compared to normal control, male partners of DW group presented reduction in MF, IF and EF as compared to normal control. Male partners of female rats treated with plant extracts showed increase in MF, IF just like those of normal control ($p < 0.01$, MF and IF) (Table 5, 6 and 7 respectively). Moreover, a significant increase in ML ($p < 0.001$) was observed in DW group as compared to normal control and male partners from plant extracts groups could reverse this when compared to DW group (Table 5).

Table 2. Effects of different treatments on frequency of ear wiggling, hops and/or darts and anogenital presentation of female rats.

Treatments	Doses	Ear wiggling frequency (EW)				Hops and/or darts frequency (H/D)				Anogenital presentation frequency (AN)			
		D 0	D 7	D 14	D 21	D 0	D 7	D 14	D 21	D 0	D 7	D 14	D 21
Normal control	10 ml/kg	0	13.40±1.78	18.00±1.52	11.40±1.91	0	18.60±3.17	28.00±6.61	23.40±6.90	0	9.00±0.32	13.60±2.99	8.60±1.03
DW	10 g/kg	0	3.00±0.95	0.40±0.40 ^{##}	4.00±2.19	0	7.20±3.02	0.80±0.58 [#]	8.60±2.82	0	2.80±1.74	0 ^{###}	1.00±0.55

Aqueous Extract	60 mg/kg	0	9.40±3.28	5.40±2.73	6.80±2.58	0	17.20±6.50	7.20±4.87	8.20±3.68	0	6.40±2.23	2.60±1.44	2.20±0.97
	372 mg/kg	0	7.40±2.84	11.80±2.71	5.40±2.20	0	4.00±2.63	18.40±4.31	7.80±5.75	0	4.80±2.94	7.40±1.21	4.40±2.80
Methanol Extract	60 mg/kg	0	7.00±2.88	16.00±1.76***	11.60±1.75	0	11.60±4.95	17.60±2.36	25.20±3.99	0	3.80±1.74	10.00±1.79*	7.60±1.54
	372 mg/kg	0	1.40±0.75	13.20±3.58*	13.00±5.17	0	3.00±2.53	18.80±5.51	19.80±7.02	0	1.00±1.00	5.40±1.40	5.40±2.42

Values are expressed as mean ± SEM, n = 5. DW = Distilled water; *p < 0.05; ***p < 0.001 in comparison to DW; # p < 0.05 in comparison to Normal control.

Table 3. Effects of different treatments on defense and rejection frequency of female rats

Treatments	Doses	Defense frequency				Rejection frequency			
		Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
Normal control	10 ml/kg	11.00±3.83	0	0	0	3.20±1.20	0	0	0
DW	10 ml/kg	8.40±1.57	7.20±2.24	15.80±3.61##	3.60±2.20	6.60±2.44	2.00±1.10	9.00±4.02	0.80±0.80
Aqueous Extract	60 mg/kg	10.20±2.46	3.80±2.33	2.00±1.30	7.60±1.78	10.00±3.70	1.80±1.56	2.20±1.50	3.60±1.94
	372 mg/kg	6.60±1.03	5.80±3.04	0**	8.00±4.25	3.00±0.89	4.40±2.64	0	4.40±3.67

Methanol Extract	60 mg/kg	13.20±5.76	9.80±3.09	1.20±0.58*	0	2.60±0.40	5.40±2.77	0.40±0.24	0
	372 mg/kg	8.80±2.40	5.40±2.75	1.60±1.03*	3.20±2.33	4.60±1.69	3.80±1.74	0.60±0.40	1.20±1.20

Values are expressed as mean ± SEM, n = 5. DW = Distilled water. ## p < 0.01 in comparison to normal control; *p < 0.05 in comparison to DW.

Table 4. Effects of different treatments on lordosis frequency and lordosis quotient of female rats

Treatments	Doses	Lordosis frequency (LF)				Lordosis quotient (LQ)			
		Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
Normal control	10 ml/kg	0	18.20±2.84	22.80±3.31	24.20±3.84	0	96.41±1.58	99.05±0.95	100.00±0.00
DW	10 ml/kg	0	5.60±5.10	2.20±1.56##	8.80±3.93#	0	24.47±11.91	26.00±19.39	64.44±17.36
Aqueous Extract	60 mg/kg	0	17.00±6.86	7.00±4.17	8.40±3.53	0	63.47±20.81	45.78±20.04	63.69±19.42
	372 mg/kg	0	6.80±4.18	15.80±2.75	8.80±4.80	0	40.00±24.49	82.62±11.43	45.30±21.37
Methanol Extract	60 mg/kg	0	10.00±4.66	17.00±1.87	18.40±3.11	0	55.31±22.77	97.13±1.86	98.89±1.11
	372 mg/kg	0	3.20±3.20	12.40±3.36	12.80±4.16	0	20.00±20.00	58.00±23.75	71.26±18.83

Values are expressed as mean ± SEM, n = 5. DW = Distilled water; #p < 0.05; ##p < 0.01 in comparison to normal control.

Table 5. Effects of female rat treatments on mount latency and frequency of male partners

Treatments	Doses	Mount latency (s)				Mount frequency			
		Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
Normal control	10 ml/kg	ND	9.60±2.16	8.20±1.02	10.40±3.56	0	18.80±2.78	23.00±3.27 [#]	24.20±3.84
DW	10 ml/kg	ND	710.20±252.01 ^{###}	170.40±90.91	131.20±59.85	0	7.60±4.35	4.40±1.94	11.20±3.34
Aqueous Extract	60 mg/kg	ND	76.60±27.69 ^{***}	258.20±151.49	107.40±60.35	0	20.60±5.28	10.00±3.97	10.60±3.14
	372 mg/kg	ND	139.00±81.26 ^{***}	39.80±28.11	147.40±70.05	0	9.20±3.26	16.40±2.93	11.80±4.22
Methanol Extract	60 mg/kg	ND	90.20±45.68 ^{***}	28.40±5.40	81.60±62.60	0	12.20±3.92	17.40±1.72	18.60±3.09
	372 mg/kg	ND	273.00±75.74 ^{**}	39.80±9.06	166.20±86.86	0	6.20±2.50	13.80±2.80	15.20±4.27

Values are expressed as mean ± SEM, n = 5. DW = Distilled water. ^{###}p < 0.001 in comparison to normal control, ^{**}p < 0.01; ^{***}p < 0.001 in comparison to DW. ND = Not determined (>30 minutes).

Table 6. Effects of female rat treatments on intromission latency and frequency of male partners

Treatments	Doses	Intromission latency (s)				Intromission frequency			
		Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
Normal control	10 ml/kg	ND	9.60±2.16	8.20±1.02	10.40±3.56	0	18.00±2.92	22.8±3.31	24.00±3.99

DW	10 ml/kg	ND	ND	ND	136.00±113.06	0	3.60±3.11	2.2±1.56##	8.8±3.92
Aqueous extract	60 mg/kg	ND	89.25±31.63	160.00±154.54	219.75±177.57	0	16.80±6.79	7.00±4.17	8.40±3.53
	372 mg/kg	ND	ND	51.00±39.22	355.33±212.88	0	6.80±4.18	14.60±3.61	8.80±4.80
Methanol Extract	60 mg/kg	ND	40.67±24.66	28.40±5.40	81.60±62.60	0	10.00±4.66	17.00±1.87	18.40±3.11
	372 mg/kg	ND	ND	42.25±11.26	142.50±103.96	0	3.20±3.20	12.40±3.36	12.80±4.16

Values are expressed as mean ± SEM, n = 5. DW = Distilled water; ND: Not determined (>30 minutes). ##p < 0.01.

Table 7. Effects of female rat treatments on ejaculations latency and frequency of their male partners

Treatments	Doses	Ejaculation latency (s)				Ejaculation frequency			
		Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
Normal control	10 ml/kg	ND	235.20±64.73	240.00±24.21	522.60±253.52	0	2.40±0.68	2.60±0.25	2.60±0.60
DW	10 ml/kg	ND	ND	ND	ND	0	0.40±0.40	0	1.00±0.63

Aqueous extract	60 mg/kg	ND	233.67±41.19	ND	438.67±68.83	0	2.00±0.95	0.60±0.40	1.00±0.45
	372 mg/kg	ND	ND	497.00±119.05	ND	0	1.00±0.63	1.80±0.37	1.20±0.58
Methanol Extract	60 mg/kg	ND	219.67±55.57	417.4±174.60	157.75±41.42	0	1.80±0.80	2.40±0.51	2.80±0.73
	372 mg/kg	ND	ND	ND	256.00±88.33	0	0.20±0.20	1.20±0.58	1.40±0.60

Values are expressed as mean ± SEM, n=5. DW= Distilled water. ND= Not determined (>30 minutes)

Effects of different treatments on uterine and vaginal tissue growth of female rats

Effects on the uterine wet weight, total proteins level, epithelial height and architecture

Treatment with plant extracts produced an increase in uterine wet weight (Figure 1A), total uterine proteins level (Figure 1B) as well as uterine epithelial height (Figure 1C) just like NC group as compared to DW. The highest increase was obtained with aqueous extract at 60 mg/kg (33.64% for uterine wet weight) and 372 mg/kg (uterine total proteins level) while methanol extract at dose of 60 mg/kg showed the best effect on the uterine epithelial height. Figure 2 represents micrographs of the uterus after a 21-days treatment with different substances. An atrophic uterus with an increase stromal lamination, a flattened cuboidal endometrial epithelium in a disturbed pattern was obtained in DW group. Plant extracts treated groups just like NC group showed large cuboidal cells with an organized epithelium, reduction in stromal lamination and an increase in glandular cells number as compared to DW.

Effects on vaginal wet weight, epithelial height and architecture

Results obtained indicate that no change of vaginal wet weight and vaginal epithelial height was observed

in all experimental groups (Figure 3A and Figure 3B). However, DW group did not display cornified layer on vagina microphotograph when compared to NC (Figure 4). Interestingly, all plant treated groups presented this cornified layer when compared to DW (Figure 4).

Discussion

Sexual behavior in female rats is characterized by sexual motivation and receptivity controlled by estrogens and progestogens [27]. In rodents, ovariectomy is well known to induce a drop in ovarian hormones and subsequently impairs sexual behavior. Hence, exogenous supply of well-known doses of these hormones can stimulate sexual behavior. However, ovariectomy followed by low supplementations of 17 β -estradiol (E) and progesterone (P) in female rats during sexual behavioral tests is not able to stimulate sexual behavior. But can activate some estrogens receptors (ER), without attending the threshold quantity necessary for the exhibition of sexual behavior. This low hormonal supplementation permits to activate few ER in OVX rats and characterizes low sexual behavior. Therefore, ovariectomy plus low supplementation of estradiol and progesterone can be a predictive model of low sexual desire disorder (sexually sluggish) in rodents [13]. In the present study, acute administration (day 0) of low doses of E (10 μ g) and P (300 μ g) to OVX failed to induce female sexual behavior but repeated

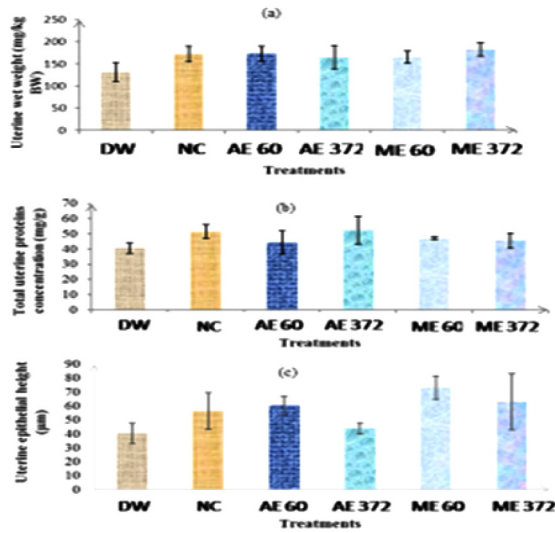


Figure 1. Effects of a 21 days treatment with *P. muellerianus* on uterine wet weight (A), uterine total proteins level (B) and uterine epithelial height (C). DW: Distilled water; NC: Normal control; AE: Aqueous extract; ME: Methanol extract. $P > 0.05$ in comparison to DW

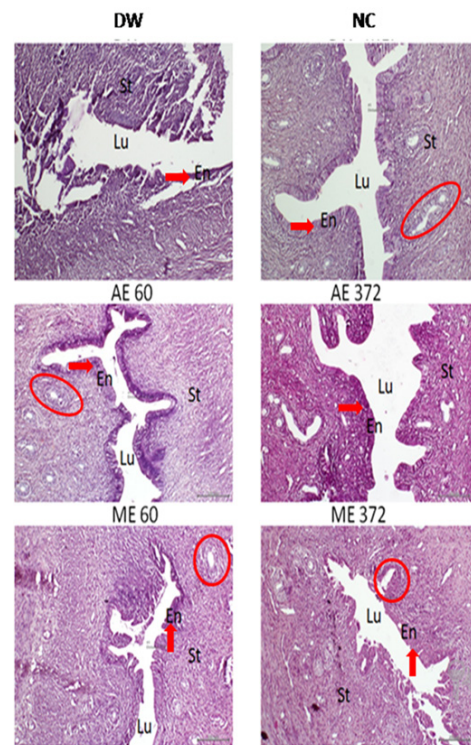


Figure 2. Effects of 21-days treatment with *P. muellerianus* on uterine microphotographs. DW= Distilled water; NC: Normal control; AE = Aqueous extract; ME = Methanol extract. Lu = Uterine lumen, En = Endometrium, St = Stroma

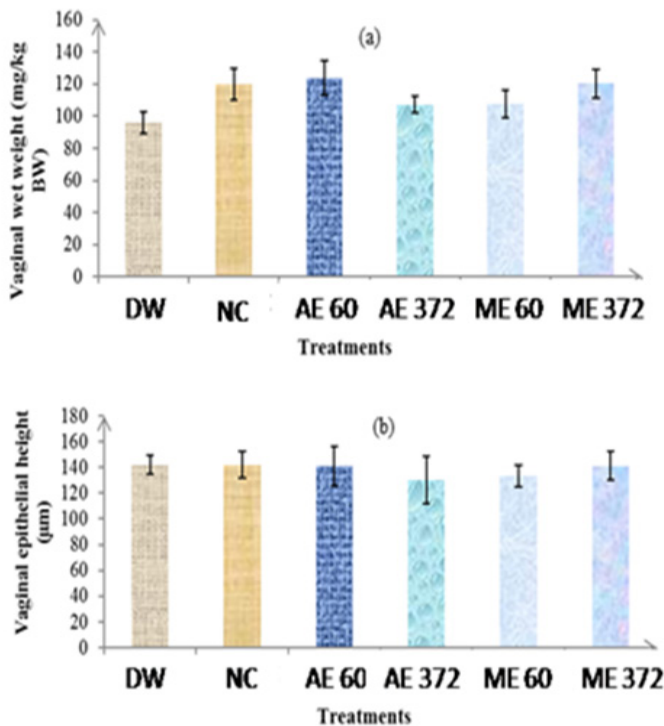


Figure 3. Effects of a 21-days treatment with *P. muellerianus* on vagina wet weight (A) and vaginal epithelial height (B). DW: Distilled water; NC: Normal control; AE: Aqueous extract; ME: Methanol extract. $p > 0.05$ compared to DW.

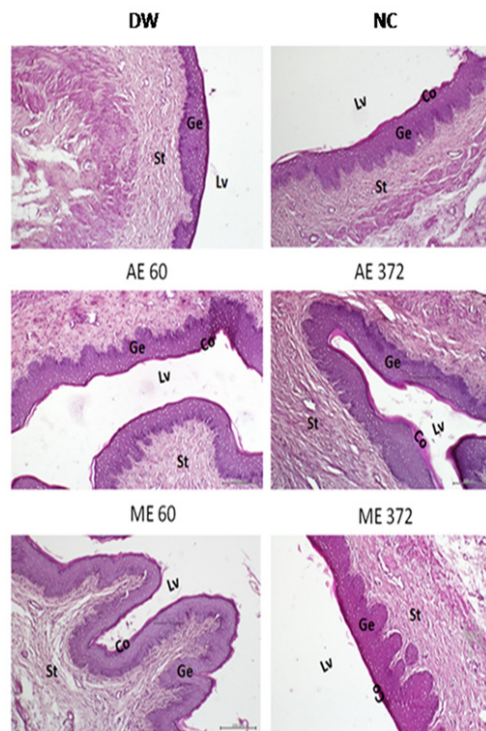


Figure 4. Effects of 21 days treatment with *P. muellerianus* on vagina microphotographs. DW = Distilled water; NC: Normal control; AE = Aqueous extract; ME = Methanol extract. Lv = Vaginal lumen, Co = Cornified layer, Ge = Stratum germinativum, St = Stroma

administration induced a low sensitization of some sexual behaviors parameters during three weeks of treatment. These results confirm the findings of [14]. This model is therefore characterized by low sexual motivation and receptivity which mimic low sexual desire disorder and the effects of natural or synthetic substances can be well evaluated.

Administration of plant extracts during three weeks to sexually sluggish female rats improved proceptive behaviors just like fully primed female rats. This was marked by a significant increase in full and partial solicitations (ear wiggling, anogenital presentation). Also, reduction in aggressive behavior was noted at all test periods. This increase was more pronounced after two weeks of treatment. Similar results were obtained by [13,15,28]. They showed that two weeks treatment of female rats with flibaserin or BP101 (synthetic substances) caused a significant increase in full and partial solicitations with reduction of defensive behavior. All together, these results reflect an increase in sexual desire after plant extracts treatment of sexually sluggish female rats. Proceptive behavior which is similar to women sexual desire are primarily promoted by E but amplified in a dose-dependent way by P administration [29]. It is also known that P acts on estrogen-induced progesterone receptors in the medial preoptic area (mPOA) to activate appetitive behaviors [14]. Moreover, ovarian hormones increase female sexual behavior by sensitization of central motive state. This central motive state stimulates excitatory pathway (dopamine) of sexual motivation over inhibitory pathway (serotonin), thereby promoting sexual desire and inhibiting female aggressiveness towards the male stimulus [7]. It therefore appears that these plant extracts may exert an estrogen-like action which sensitize excitatory neurochemical pathway of sexual desire. Our research team previously showed estrogen-like potentials of this plant in a model of PCOS rats [21]. However, further studies are necessary to determine the estrogenic potentials of this plant on the neurochemical pathways involved in sexual desire. Also the effect of these plant extracts on neurotransmitters such as dopamine or serotonin involved in the control of sexual behavior was not evaluated in this study. To considerably explore sexual behavior of female rats, we studied another component of sexual behavior that is sexual receptivity.

Three weeks treatment with plant extracts improved receptive behavior (LF and LQ) just like the normal control. Indeed, the increase in lordosis frequency correlates with an increase in mounts and intromissions frequencies. This might arise from high sexual activity of the stud male rats in contact with highly proceptive female rats. It is well documented that sexual receptivity expression requires the coordinated activity of forebrain, midbrain, brainstem and spinal

cord neurons which is strictly dependent on steroid hormones including estradiol and progesterone [27]. Estradiol plays a major role in the elicitation of receptive behavior by interacting with serotonergic system (activating serotonin receptor 1A) in the brain. Whereas progesterone is not able to induce lordosis response in OVX not pretreated with estrogens. Furthermore, steroid hormones are believed to regulate sexual receptivity by binding to intracellular receptors within certain hypothalamic and limbic structures, inducing protein synthetic changes that lead to neural excitability [30,31]. Therefore, drugs capable of inducing neural excitability in the brain are associated with increased sexual receptivity in animal studies and can reverse hypoactive sexual desire disorder state [32]. Our results bring forth more evidence of estrogen-like effects of these plant extracts as previously documented. Herbal treatment of female rats can also influence male sexual response. Evidence from literature review indicates that male rats are generally attracted by odors (pheromones) derived from clitoral gland [33]. In the present study, mating of plant treated female rat with stud male rat triggered high male sexual behavior which explains the increase in female sexual motivation. Beside their implication in sexual motivation and receptivity, estrogens are well known to stimulate the development of the genital tract (uterus and vagina) and also induce secretory changes [34]. Therefore, sex organs weights, total uterine proteins and histology of the uterus and vagina were targeted in the present study.

Available information showed that ovariectomy induced a decrease in uterine wet weight, uterine and vaginal epithelial heights whereas estrogen-like substances reverse this atrophy [12,34]. In the present study, treatment of female rats with *P. muellerianus* showed increase in uterine wet weight. In addition, an amelioration of total uterine proteins and uterine epithelial height was obtained after plant treatment. Uterine architecture was improved after plant treatment. The low effect of these extracts on uterine growth is reasonable due to the fact that adult female rats were used and not young ones which are more sensitive to pharmacological substances. Uterine growth is a biphasic event which implicates water imbibition as early phase and epithelial cell proliferation and differentiation as late responses. Phenolic compounds contained in plant extracts are reported to bind estrogen receptors and modulate the expression of many genes. This further induce an increase in total proteins level in the uterus, a marker of uterine cell proliferation which ameliorate uterine tissue layers [35]. These results suggest uterotrophic effects of these plant extracts, but further studies using immature female rat model where estradiol content can be directly measured is needed. The estrus phase is defined by the ap-

pearance of cellular necrosis in a tall columnar uterine epithelium while the vaginal epithelium is delimited by a shedding cornified layer [36]. Indeed, the effect of these extracts on the vagina microphotographs was evaluated. In the present study, we noticed the presence of shedding cornified layer in plant treated animals. This confirms that, plant extracts are capable to bound estrogen receptors and increase estrogenic content necessary to ameliorate sexual behavior in case of low sexual desire. Vaginal wet weight increase was also noticed in plant treated groups which shows tissue growth. These results demonstrate that *P. muellerianus* extracts improve female sexual behavior and may be through estrogen-like actions. Further mechanistic studies are needed to show the effect of this plant extracts on the neural pathway involved in the control of sexual behavior.

Conclusion

The present study provides the first evidence of the pro-sexual effect of *P. muellerianus* on low sexual desire disorder in female rats. These findings demonstrate that low sexual desire disorder is characterized by impaired sexual behavior with low reproductive tissues growth. *P. muellerianus* promotes female sexual behavior and reproductive tissues growth in these animals. Our study provides strong scientific evidence on the potential therapeutic solution of this plant in traditional medicine.

Conflict of Interests

The authors confirm that this article content has no conflicts of interest.

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