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EFFECTS OF THE AQUEOUS AND HEXANE EXTRACTS OF *MONDIA WHITEI* ON THE SEXUAL BEHAVIOUR AND SOME FERTILITY PARAMETERS OF SEXUALLY INEXPERIENCED MALE RATS.

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Abstract

The effects of *Mondia whitei* Hook (Skeels) were studied on the sexual behaviour and some fertility parameters of sexually inexperienced male rats. Animals were orally administered 100 mg/kg and 500 mg/kg of body weight (b.w) of either the aqueous or the hexane extracts of *Mondia whitei* whilst the control group received 10 mL/kg b.w of 0.3% Tween 80 once/day for 14 days. Their sexual behaviour was monitored on days 0, 1, 7 and 14 days of treatment and 14 days post-treatment. Some fertility parameters (index libido, quantal pregnancy, fertility index) of the treated rat were evaluated on day 13 of treatment by pairing it overnight with two proestrus females. Results showed that *Mondia whitei* extracts significantly ($p<0.001$) reduced the mount latency and the hexane extract was found to be more efficient than the aqueous extract. The treatment had no significant effect ($p>0.05$) on intromission, ejaculation and erection. The fertility of the animals remained unaffected. It's concluded that *Mondia whitei* had sexual enhancement of the sexually inexperienced male rats.

Key words: *Mondia whitei*, sexual behaviour, inexperienced male rat

Introduction

Male impotence also called Erectile dysfunction (ED) is a common medical condition that affects the sexual life of millions of men worldwide (Montorsi et al, 2003; Shabsigh and Anastasiadis, 2003;). The increasing number of men seeking help for impotence has expanded basic physiological and pharmacological research on sexual performance (Cicero et al, 2001). In the western societies, treatment options have progressed from psychosexual therapy and penile

prostheses (1970s), through revascularization, vacuum constriction devices, and intracavernous injection therapy (1980s), to transurethral and oral therapy (1990s) (Lue, 2000). In Developing countries on the contrary, the inability to afford modern medical healthcare has forced patients to seek traditional medical attention. In these countries, many plant extracts are traditionally used to improve sexual performances (Kamtchouing et al, 2002; Carro-Juarez et al, 2004). In Cameroon, several plants are claimed to possess aphrodisiac potential (Noumi et al, 1998). However, documented experiments or clinical data on many of these plants are lacking. *Mondia whitei* Hook (Skells) of the Periplocaceae family is one of such plants. In our previous findings, we reported the androgenic effect of the aqueous (Watcho et al, 2004) and hexane (Watcho et al, 2005) extracts of this plant in adult male rats. The aim of the present study was to determine the effect of an oral administration of the aqueous and hexane extracts from the dried roots of *Mondia whitei* on the sexual performance and fertility of sexually inexperienced male rats. This investigation will clarify the therapeutic efficacy of *Mondia whitei*.

Materials and Methods

Animals

A total of 200 adult Wistar rats (160-200 g; > 90 days) of either sex (25 males and 175 females) were obtained from our colony. The animals were raised at room temperature (23° C) with a natural light-dark cycle (12/12 h) and maintained at standard Laboratory rat diet and tap water given *ad libitum*.

Females were partitioned into ovariectomized and intact categories.

Ovariectomy was performed following the technique of Cariton (1986) 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 body weight/day/3days). One month later, they were brought into estrus by 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 (Meyerson and Lindstrom, 1973). 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.

Intact females were brought to heat artificially with a single subcutaneous dose of 10 µg estradiol benzoate and 600 µg progesterone, 48 h and 6 h before testing, respectively. These females were used in the fertility test.

Plant material

Fresh roots of *Mondia whitei* were collected in Dschang, Cameroon. Botanical identification was done at the Cameroon National Herbarium (HNC) in Yaounde in comparison with the Herbarium Voucher specimen N°42920/HNC collected by Westphal. The roots of

Mondia whitei were cut into small pieces of about 1.5-2 cm, air-dried and powdered using an electric grinder (Moulinex).

Preparation of the aqueous extract

200 g of the powdered roots were dissolved in 1.3 L of distilled water and kept for 72 h at 4° C, and occasionally stirred. After filtration, the solution obtained was evaporated in an oven (50°C) for 48-72 h to give 76.92 g of brown residue. The yield of extraction was 38.46%. The aqueous extract used in our study was prepared by dissolving 1 g of the brown residue in 10 mL of distilled water. The doses used in our study were 100 mg/kg b.w and 500 mg/kg b.w.

Preparation of the hexane extract

The powdered plant (700 g) was soaked in 6 L of CH_2Cl_2:MeOH (1:1) mixture at room temperature for 72 h and filtered. The solvent was removed by vacuum distillation and dried to obtain a black paste (66 g) which was exhausted for 30 min in 500 mL of hexane and filtered. The solvent was removed as previously to obtain 10 g of hexane extract. The working solution (100 mg/mL) was extemporary prepared by dissolving 1 g of paste in 2 mL of 0.3%Tween 80 and 8 mL of distilled water. The doses used were 100 mg/kg b.w and 500 mg/kg b.w.

Phytochemical tests

The hexane extract of *Mondia whitei* was treated with several reagents and, spectroscopy and physical analysis (RMN, 1H and 13C; mass spectrometry (SM) RMN etc) performed. Positive results were obtained with the following constituents: steroid (beta sitosterol), triterpens (amyrine alpha and beta acetate; lupeol) and aldehyde (vanillin or 3-hydroxy-4-methoxybenzaldehyde).

Treatments

Sexually inexperienced male rats were randomly assigned to one of the following groups (n=5, each): Group 1 received the vehicle orally (10mL/kg of 0.3% Tween 80) and served as control. Groups 2 and 3 received 100 mg/kg b.w and 500 mg/kg b.w of the aqueous extract of *Mondia whitei* whereas Groups 4 and 5 were treated with 100 mg/kg b.w and 500 mg/kg b.w of the hexane extract of *Mondia whitei* respectively. The different doses of *Mondia whitei* were administered orally to animals via a gastric tube 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 1 h of the application of each dose.

Sexual behaviour testing procedure

The sexual behaviour of male rat was tested during the period of darkness (08:00 p.m.) in a quiet room for a duration of 1 h. After a 10 min adaptation period 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 or calculated according to standard methods (Ageel et al, 1994; Carro-Juarez et al, 2004): 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; post-ejaculatory interval (PEI), the time from the first ejaculation to a new first intromission; penile erection (PE), the number of times the rat bent down to lick the penis. The test was ended when ML or PEI was more than 20 minutes.

Fertility test

On day 13 of treatment, each male was cohabited overnight with two receptive females (non-ovariectomized) and this was followed by the examination of the vaginal smear the next morning (7 am). The following reproductive parameters were then computed according to the method of Ratnasooriya and Dharmasiri (2000): index of libido = (mated/number paired) x 100; quantal pregnancy = (number pregnant/number mated) x 100 and fertility index = (number pregnant/number paired) x 100.

Statistical analysis

One-way analysis of variance (ANOVA) followed by post-hoc Newman-Keuls multiple comparison test was performed using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. A probability of $p < 0.05$ was accepted as significant.

Results

The effect of repeated doses of *Mondia whitei* (aqueous and hexane extracts) on the mounting behaviour of sexually inexperienced male rats is shown in Table 1. The mount latency dose-dependently decreased ($p<0.001$) with the increase in days of treatment whereas the number of mounts (MF) at all time points remained statistically unchanged when compared to both control and initial respective values (Day 0 and Day 1 of treatment). It was also observed that after 2 weeks of treatment, mounting behaviour activity was greater in hexane group than in aqueous group.

As shown in Table 2, neither aqueous (500mg/kg) nor hexane (100 and 500 mg/kg) extracts had no significant influence on the intromission latency and frequency. Daily administration of *Mondia whitei* extracts for 14 days did not bring any significant ($p>0.05$) change in the post-ejaculatory interval (PEI) of inexperienced rats compared to the corresponding control and Day 1 data. Except Day 7 data where a drop was observed, there was a time-dependent increase in the erectile frequency of animals receiving aqueous (500 mg/kg b.w) and hexane (100 and 500 mg/kg b.w) extracts of *Mondia whitei* (Table 3).

The libido index, quantal pregnancy and fertility index of inexperienced male rats treated with repeated doses of *Mondia whitei* extracts remained statistically unaffected (Table 4).

Discussion

Barks of the roots of *Mondia whitei* have been used since a pretty long time as an aphrodisiac agent alone or in combination with ingredients such as roots of *Albizia antunesiana* Harms (Mimosaceae) and stem bark of *Ozoroa insigni* Del (Anarcadiaceae) (Noumi et al, 1998; Carpentier et al, 2004).

Table 1: Effects of aqueous and hexane extracts of *Mondia whitei* on mount latency (s) and frequency of sexually inexperienced male rats.

Groups	Treatment				Post-treatment
	Do	D1	D7	D14	D14
Control (10 mL/kg of 0.3%Tween 80), n=5	71.2 ± 10.97^{a} [29.60 ± 8.05]	90.00 ± 16.97^{a} [36.00 ± 12.23]	15.8 ± 1.00^{b} [34.40 ± 2.88]	10.8 ± 0.52^{bc} [37.60 ± 1.71]	10.4 ± 0.61^{bc} [27.60 ± 3.01]
Mondia whitei Aqueous extract 100 mg/kg, n=5	82.80 ± 14.83^{a} [37.2 ± 8.59] **(125.68)**	56.00 ± 2.26^{*b} [54.4 ± 6.03] **(151.11)**	10.4 ± 0.83$^{***\ c}$ [40.80 ± 5.16] **(118.60)**	7.8 ± 0.82$^{***\ c}$ [40.20 ± 2.92] **(106.91)**	7.00 ± 0.80$^{***\ c}$ [35.60 ± 2.05] **(128.99)**
500 mg/kg, n=5	78.0 ± 9.30^{a} [32.20 ± 14.54] **(108,78)**	66.00 ±13.15^{b} [39.20 ± 3.43] **(108.89)**	8.40 ±0.83$^{***\ c}$ [29.20 ± 3.27] **(84.88)**	7.40 ± 0.45$^{***\ c}$ [40.00 ± 4.04] **(106.38)**	6.40 ± 0.46$^{***\ c}$ [27.80 ± 3.41] **(100.72)**
Hexane fraction 100 mg/kg, n=5	70.80 ± 12.40^{a} [31.20 ± 5.26] **(105.41)**	47.60 ± 2.24^{b} [35.80 ± 6.17] **(99.44)**	7.60 ± 0.50$^{***\ c}$ [30.40 ± 1.88] **(88.37)**	5.60 ± 0.22$^{***\ c}$ [43.60 ± 4.79] **(115.96)**	5.80 ± 0.33$^{***\ c}$ [38.80 ± 4.69] **(140.58)**
500 mg/kg, n=5	76.80 ± 6.21^{a} [30.80 ± 6.66] **(104.05)**	58.40 ± 2.34^{b} [38.40 ± 5.83] **(106.67)**	6.60 ± 0.45$^{***\ d}$ [32.80 ± 5.45] **(95.35)**	6.20 ± 0.18$^{***\ d}$ [49.20 ± 2.20] **(130.85)**	6.80 ± 0.66$^{***\ d}$ [38.60 ± 3.56] **(139.86)**

Values are Mean ± SEM; n= number of rats

Values within hooks represent mount frequencies.

Bold values within parenthesis represent percentage of control mount frequency.

Within the same column, * = $p < 0.05$; *** = $p < 0.001$ compared to corresponding control.

a,b,c: on the same line, values with the same superscript letters do not differ significantly ($p > 0.05$)

Table 2: Effects of aqueous and hexane extracts of *Mondia whitei* on intromission latency (s) and [frequency] of sexually inexperienced male rats.

Groups	Treatment				Post-treatment
	Do	D1	D7	D14	D14
Control (10 mL/kg of 0.3%Tween 80), n=5	4.00 ±1.10[a] [17.6 ± 1.15[a]]	3.00 ± 1.02 [a] [23.80 ± 5.74[a]]	9.60 ± 7.47 [a] [28.40 ± 6.99[a]]	2.20 ± 0.52 [a] [25.80 ± 2.61[a]]	2.20 ± 0.33 [a] [20.80 ± 2.90[a]]
Mondia whitei Aqueous extract 100 mg/kg, n=5	2.20 ± 0.33 [a] [18.20 ± 2.24[a]] **(103.41)**	2.20 ± 0.66 [a] [46.00 ± 7.18[* b]] **(193.28)**	2.20 ± 0.33 [a] [30.40 ± 5.41[ab]] **(107.04)**	2.40 ± 0.54 [a] [35.20 ± 4.27[ab]] **(136.43)**	1.40 ± 0.22 [a] [26.80 ± 2.29[ab]] **(128.85)**
500 mg/kg, n=5	3.00 ± 1.39 [a] [16.80 ± 6.82[a]] **(95.45)**	4.00 ± 1.33 [a] [23.60 ± 3.41[a]] **(99.16)**	9.20 ±3.18 [18.00 ± 3.37[a]] **(63.38)**	3.60 ± 0.92 [a] [29.60 ± 4.37[a]] **(114.73)**	4.00 ± 1.26 [a] [21.80 ± 3.22[a]] **(104.81)**
Hexanefraction 100 mg/kg, n=5	3.40 ± 2.39 [a] [17.40 ± 3.85[a]] **(98.86)**	5.40 ± 1.64 [a] [20.00 ± 2.04[a]] **(84.03)**	1.60 ± 0.22 [a] [23.60 ± 2.41[a]] **(83.10)**	2.80 ± 0.72 [a] [27.00 ± 3.33[a]] **(104.65)**	2.00 ± 0.40 [a] [18.0 ± 2.67[a]] **(86.54)**
500 mg/kg, n=5	3.00 ± 0.63 [a] [19.40 ± 4.64[a]] **(110.23)**	2.20 ± 0.59 [a] [25.40 ± 5.53[a]] **(106.72)**	2.80 ± 0.87 [a] [14.20 ± 1.63[a]] **(50.00)**	2.40 ± 0.36 [a] [32.80 ± 5.86[a]] **(127.13)**	2.20 ± 0.66 [a] [19.20 ± 2.81[a]] **(92.31)**

Values are Mean ± SEM; n= number of rats.
Values within hooks represent intromission frequencies.
Bold values within parenthesis represent percentage of control intromission frequency.
Within the same column, * = $p < 0.05$ compared to corresponding control.
a,b,c: on the same line, values with the same superscript letters do not differ significantly ($p > 0.05$).

Table 3: Effects of aqueous and hexane extracts of *Mondia whitei* on ejaculation latency (mn), [post-ejaculatory Interval (mn)] and (penile erection) of sexually inexperienced male rats.

Groups	Treatment				Post-treatment
	Do	D1	D7	D14	D14
Control (10mL/kg of 0.3%Tween 80), n=5	23.60 ± 5.30[a] [8.20 ± 0.33][a] (26.80 ± 7.62)[a]	22.20 ± 4.73[a] [8.40 ± 0.61][a] (31.20 ± 11.08)[a]	18.20 ± 4.36[a] [9.60 ± 0.36][a] (34.80 ± .,87)[a]	14.80 ± 2.34[a] [9.20 ± 1.11][a] (34.60 ± 2.89)[a]	25.40 ± 4.21[a] [10.80 ± 1.93][a] (28.40 ± 3.11)[a]
Mondia whitei Aqueous extract 100mg/kg, n=5	20.40 ± 3.60[a] [8.80 ± 0.66][a] (28.20 ± 7.61)[a]	21.40 ± 6.10[a] [11.20 ± 2.03][a] (46.40 ± 5.26)[a]	6.20 ± 1.07[b] [9.80 ± 0.33][a] (33.20 ± 5.16)[a]	5.40 ± 0.92[b] [8.00 ± 0.69][a] (39.40 ± 4.51)[a]	11.80 ± 1.14[a b] [9.20 ± 0.52][a] (31.60 ± 2.54)[a]
500mg/kg, n=5	19.20 ± 1.48[a] [7.80 ± 0.52][a] (23.20 ± 8.87)[a]	22.20 ± 6.20[a] [7.60 ± 0.61][a] (35.80 ± 4.84)[a]	15.40 ± 5.67[a] [9.20 ± 1.04][a] (23.40 ± 3.71)[a]	20.40 ± 7.11[a] [8.40 ± 0.73][a] (40.20 ± 4.30)[a]	25.60 ± 8.07[a] [11.00 ± 0.63][a] (18.00 ± 3.03)[a]
Hexanefraction 100mg/kg, n=5	19.80 ± 3.46[a] [8.80 ± 0.66][a] (26.20 ± 4.36)[a]	26.80 ± 6.41[a] [10.20 ± 0.52][a] (31.20 ± 4.39)[a]	13.40 ± 2.09[a] [9.60 ± 0.83][a] (22.00 ± 1.41)[a]	19.00 ± 2.53[a] [10.40 ± 0.92][a] (36.40 ± 3.24)[a]	35.40 ± 7.09[a] [11.80 ± 1.71][a] (31.80 ± 3.67)[a]
500mg/kg, n=5	24.20 ± 3.29[ab] [10.80 ± 1.48][a] (28.60 ± 5.83)[a]	11.20 ± 2.52[a] [11.40 ± 1.61][a] (34.60 ± 1.90)[a]	11.80 ± 1.45[a] [10.40 ± 1.22][a] (24.00 ± 3.81)[a]	17.00 ± 3.26[a] [10.00 ± 1.67][a] (43.80 ± 2.73)[a]	31.20 ± 4.05[b] [11.20 ± 0.95][a] (36.20 ± 5.21)[a]

Values are Mean ± SEM; n= number of rats.
Values within hooks represent post-ejaculatory interval.
Values within parenthesis represent erectile frequency (penile erection).
a,b,c: on the same line, values with the same superscript letters do not differ significantly ($p > 0.05$).

Table 4: Effects of aqueous and hexane extracts of *Mondia whitei* on fertility of sexually inexperienced male rats.

Groups	Total females mated	Total sperm positive females	Total pregnant females	Libido index (%)	Quantal gravidity (%)	Fertility index (%)
Control (10 mL/kg 0.3%Tween 80), n=5	10	8	8	80.00 ± 10.95	100.00 ± 0.00	80.00 ± 10.95
Mondia whitei Aqueous extract 100 mg/kg, n=5	10	9	8	90.00 ± 8.94	90.00 ± 8.94	80.00 ± 10.95
500mg/kg, n=5	10	8	8	80.00 ± 17.87	80,.0 ± 17.89	80.00 ± 10.95
Hexane extract 100mg/kg, n=5	10	10	9	100.00 ± 0.00	90.00 ± 8.94	90.00 ± 8.94
500mg/kg, n=5	10	10	10	100.00 ± 000	100.00 ± 0.00	100.00 ± 0.00

n= number of rats.

NB: Percentage represents mean of individual percentages in each group.

Results of the present study revealed the prosexual stimulatory property of *Mondia whitei* in sexually inexperienced male rats. The copulatory behaviour of normal male rat when tested with estrus female consists of repeated series of mounts and intromission culminating with ejaculation, and followed by a refractory period (PEI) (Slob and Van der Werff ten Bosch, 1997).

Administration of the aqueous and hexane extracts from *Mondia whitei* resulted in the reduction of the hesitation time of the sexually inexperienced males towards receptive females as indicated by the significant decrease ($p<0.001$) in the mount latency. Cicero et al (2001) and, Arletti and coworkers (1999) showed similar effects in rats treated with extracts from *Lepidium meyenii* and, *Turnera diffusa* and *Pfaffa paniculata* respectively. In our study, the hexane extract was more potent than the aqueous extract. Steroids and triterpens revealed in this extract may account for the proerectile effect of *Mondia whitei* (Drewes et al, 2003). These active principles may probably act by inducing changes in levels of neurotransmitters, modulating the action of these neurotransmitters on their target cells or by increasing androgen levels (Suresh Kumar et al, 2000). In this connection, we have already demonstrated the androgenic effect of the aqueous (Watcho et al, 2004) and hexane (Watcho et al, 2005) extracts of *Mondia whitei* in rats.

Although non significant, the increase in mount, intromission and erectile frequencies of animals treated for 14 days is of physiological interest since these sexual performance parameters constitute the real criteria for the determination of libido (Mbongue et al, 2003). This low enhancement could be attributed to the animal model used in this work. Indeed, it has been proved that isolation of young male from female generally leads to detrimental copulatory behaviour (Signoret and Balthazard, 1991).

In rats allowed a wash-out period of 14 days, it is noteworthy mentioning that most of the copulatory parameters remained non significantly elevated comparatively to data of Day 0 or Day 1 of normal treatment. This observation might be linked to the repeated contact of the treated males with receptive females in the one hand, and to the activation (with the increase of days of treatment) of a variety of mechanisms involved in the control of male sexual behaviour in the other hand (Arletti et al, 1999).

Results of the study showed that *Mondia whitei* extracts have low sexual enhancement of the sexually inexperienced male rats. Further experiment using experienced animal model is needed to better apprehend the prosexual effect of *Mondia whitei*.

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