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EFFECTS OF THE HEXANE EXTRACT OF *MONDIA WHITEI* ON THE REPRODUCTIVE ORGANS OF MALE RAT.

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Abstract

The present study investigates the effects induced by the long term oral administration of the hexane extract of the dried roots of *Mondia whitei* at doses of 500 and 1000 mg/kg b.w. on body and organ weights, biochemical (cholesterol, total proteins), haematological (WBC, RBC, haematocrit) and physiological (mechanical response of vas deferens to Norepinephrine) parameters of rats treated for 30 days. Results showed that *Mondia whitei* did not affect the body and testes weights of rats. At high dose (1000 mg/kg b.w), there was a significant ($p<0.05$) increase of the relative weights of the caput epididymis, ventral prostate and seminal vesicle whereas at low dose (500 mg/kg b.w), there were growth of the ventral prostate and decrease in the relative weight of the proximal vas deferens as compared with control rats. At all doses, intratesticular cholesterol was decreased, serum and tissue total protein contents increased whilst the haematological variables were within the normal range. Norepinephrine (0.114-3.648 µM) produced concentration-dependent contraction of the vas deferens of rats treated with 500 mg/kg b.w and 1000 mg/kg b.w for 30 days. In rats allowed a washout period of 30 days, except the relative proximal vas deferens where a significant decrease ($P < 0.001$) was noticed, no change was recorded. These findings give evidence of the reversible androgenic effect of *Mondia whitei* and partially support its folk use as aphrodisiac.

Keywords: *Mondia whitei*, ethnomedical, cholesterol, aphrodisiac, accessory reproductive organs.

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Introduction

Medicinal plants have for long been used as a source of relief either in the form of traditionally prepared concoctions or in the form of pure active principles (Farnsworth et al., 1985). Adjanohoun et al (1996) and Noumi et al (1998) have listed a variety of plants used in cameroon traditional medicine for fertility regulation. *Mondia whitei L.* (Hook. F) Skeel (Periplocaceae), one of such plant, is a woody climber with a large tuberous root stock; It is widely distributed in tropical Africa, from Guinea through Cameroon to East Africa. In Cameroon, *Mondia whitei* is commonly known as "la racine" and principally found in the Southern and Eastern regions; the barks of the roots of this liana are usually eaten by men (400 mg/kg of body weight) for aphrodisiac purposes or for the treatment of male impotence. In our previous investigations, we reported the antispermatogenic and antifertility activities of the aqueous extract of the roots of *Mondia whitei* in rats (Watcho et al, 2001) in one study, and observed a relaxant effect of the hexane extract of this liana on the rat vas deferens precontracted with KCl and adrenaline on the other hand (personal communication, University of Dschang, Cameroon). The present study was then undertaken to determine the in vivo effect of the hexane extract of *Mondia whitei* on the intratesticular cholesterol concentration, haematological characteristics and on the sensitivity of isolated rat vas deferens to Norepinephrine in order to partially elucidate its folk use as a potent sexual stimulator.

Methodology

Plant extraction

Fresh roots of *Mondia whitei* were purchased in Dschang; Botanical identification was authenticated at the Cameroon National Herbarium (HNC) in Yaounde-Cameroon in comparison with the existing Voucher specimen N°42920/HNC collected by Westphal. The roots were cut into small pieces of around 1.5 cm long, dried in an oven (40° C) for 120 h, powdered and subjected to Soxhlet extraction (700 g) with methylene chloride/methanol mixture (1/1) for 72 h and filtered. The solvent was evaporated under reduced pressure to obtain a residue (66 g); ten grams of this residue were resuspended in hexane and filtered. The hexane was evaporated under reduced pressure to obtain the final residue (5 g) which was suspended in 0.3 %Tween 80 and distilled water at a final concentration of 100 mg/mL. The doses 500 mg/kg b.w and 1000 mg/kg b.w used in the study were 1/22 and 1/11 of the LD50 of *Mondia whitei* extract respectively.

Phytochemical screening test

The hexane extract of *Mondia whitei* was treated with several reagents and positive results were observed with steroids, triterpenes and aromatic compounds.

Animals and groups

Adult male Wistar rats weighing 165-220 g were selected from the inbred animal colony for experimental use. The animals were maintained under uniform

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husbandry conditions of light and temperature and were given laboratory diet and tap water *ad libitum*. A total of 28 rats were randomly divided into 5 groups: Group 1 served as control (n=6) and received 10 mL/kg of 0.3 %Tween 80, Group 2 (n=6) and Group 3 (n=6) treated daily with *Mondia whitei* hexane suspension at doses of 500 mg/kg of body weight and 1000 mg/kg of b.w respectively for 30 days. In order to study the reversibility of the effects induced by the plant suspension, Group 4 (n=5) and Group 5 (n=5) received *per os* 500 mg/kg of b.w (R500) and 1000 mg/kg of b.w (R1000) of plant suspension respectively and allowed a 30 day recovery period during which they received no treatment. Rats of all groups were weighed during the treatment and sacrificed 24 h after the last dose (day 31 or day 61). Blood was collected and the serum was separated, and the reproductive organs were dissected out and weighed.

Blood analysis

Whole blood was analysed for the red blood cells (RBC) and white blood cells (WBC) counts, and haematocrit (Benson et al, 1992; Theml, 2000).

Tissue biochemistry

Tissues from each rat were kept at –20° C until assayed for total protein (testes, epididymis) (Bradford, 1976) and cholesterol (testes) (using a commercial kit of Human Gesellschaft für Biochemica und Diagnostica mbh, Germany) estimations. Total proteins were also determined in the serum (Gornal et al, 1949).

Recording of the contractions of the isolated vas deferens

Immediately after the sacrifice, the right vas deferens of each rat was taken out and the proximal portion (nearer to the epididymis) isolated and mounted in an organ bath of 20 mL capacity containing fresh Krebs solution of the following composition (mM/L): NaCl 115.00, $NaHCO_3$ 25.00, $CaCl_2$ 2.50, KCl 4.70, $MgCl_2$ 1.20, KH_2PO_4 1.20, D-Glucose 10.00. The physiological salt solution (PSS) was maintained at 37 ± 0.5° C and continuously bubbled with air. Following equilibration for 60 min, contractile responses were elicited by adding Norepinephrine (NE) (Sigma Chemicals, USA) (0.114-3.648 µM) non-cumulatively to the Krebs solution. The contact time was 5 min and the per cent contractile response was calculated at each concentration of NE using the following formula: Per cent contractile response (%) = [Assay / Maximal Control value] x 100. The contractions were recorded by means of an isometric transducer (Ugo Basile, Italy) connected to a single channel recorder (Ugo Basile, Italy) which was calibrated to record change in the tension generated on g vs cm displacement basis.

Statistical analysis

Data are expressed in mean ± SEM. One way ANOVA with post hoc Dunnet's Multiple comparison test were performed using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The value of $p<0.05$ was considered to be statistically significant.

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Results

Body and relative organ weights

As shown in Table 1, there was a significant increase in the relative weights of ventral prostate ($p<0.001$), seminal vesicle ($p<0.05$) and the caput segment of the epididymis ($p<0.001$) of rats receiving 1000 mg/kg b.w for 30 days when compared to control. In rats treated with 500 mg/kg b.w, the ventral prostate was increased ($p<0.05$) whereas a significant decrease of the relative weight of the proximal vas deferens ($p<0.05$) was observed. At all treatments, the body weight and the relative weights of the testes, corpus epididymis and distal vas deferens remained unchanged when compared to control. In rats allowed a wash-out period of 30 days, except the relative proximal vas deferens where a significant decrease ($p<0.001$) was noticed, no change was recorded.

Blood analysis

Blood variables i.e. RBC, WBC and haematocrit were found within the normal ranges after *Mondia whitei* treatments (Table 2).

Biochemical analysis

Intratesticular cholesterol was significantly decreased in rats treated with 500 mg/kg b.w. ($P < 0.05$) and 1000 mg/kg b.w. ($P < 0.001$) of the hexane suspension of *Mondia whitei*. At all doses, there was a significant ($P < 0.05$) increase in the serum and total proteins contents of the treated animals. However, no change was recorded for the rats allowed a 30 day recovery period (Table 2).

Recording of the contractions of the vas deferens

The effects of NE (0.114-3.648 µM) on the vas deferens are outlined in Table 3. It's seen that NE stimulates the muscle in a dose-dependent manner and the dose of 1000 mg/kg b.w of *Mondia whitei* appeared to be more effective than the dose of 500 mg/kg b.w. However, there was no significant variation between control values and results obtained from rats allowed a 30 day recovery period (groups 4 and 5) after the normal treatment.

Discussion

Cholesterol is the major substrate responsible for the anabolic effect of testosterone in males (Carreau, 1996; Bhasin et al, 1998). A significant decrease in the intratesticular concentration of cholesterol was observed in rats treated for 30 days with the hexane suspension of *Mondia whitei* suggesting its conversion into androgens (mainly testosterone) and which is dependent on the availability of LH (Bargatel and Bremner, 1996, Kamtchouing et al, 2002; Vijaykumar et al, 2004). At high dose (1000 mg/kg b.w), a significant increase in the relative weights of the caput epididymis ($p<0.001$), ventral prostate ($p<0.001$) and seminal vesicles ($p<0.001$) associated to the increase in their total protein contents was observed and could then support the view of

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increase androgen levels. It is well established that sex differentiation, growth and maintenance of the epididymis, prostate and seminal vesicles are androgen-dependent processes (De Krester, 1987; Patil et al, 1998; Ang et al, 2000). In rats, any increase in serum testosterone or treatment with androgens are associated with increased secretory activity and increased weight of these organs (Dewan et al, 2000; Gonzales, 2001;).The accessory sex organs possess 5 alpha-reductase activity, which converts testosterone to dihydrotestosterone, the active hormone (Johnson and Everitt, 1988). It is suggested that the hexane suspension of *Mondia whitei* may exert its selective androgenic-like activity through similar mechanism. However, these findings contrast with our previous observations where we reported the reversible antispermatogenic and antifertility effects of the aqueous extract of *Mondia whitei* after 55 days of treatment of adult rats (Watcho et al, 2001). This disagreement could be attributed to the presence of non polar biologically active molecules namely steroids, triterpenes and aromatic compounds in the hexane extract of *Mondia whitei*, and which may interact with the male reproductive system. Further, the decrease observed in the relative weight of the proximal vas deferens is indicative of the selective action of *Mondia whitei* and, could be due to an unknown mechanism since it is also believed that the vas deferens is an androgen-sensitive organ (Dohle et al, 2003). In the present study, the plant material showed low systemic toxicity as indicated by the normal values of several haematological parameters, most importantly haematocrit (42-45%) (the percentage of red blood cells per standardised volume of blood). Generally, it is believed that normal values for heamatocrit range from 35% to 50% (Alexander and Griffiths, 1993). Similar observations have also been reported by Palmeiro et al (2003) and, Ashok and Meenaskshi (2004). The return to normal range of all measured parameters after the recovery period of 30 days also support the non-toxic and reversible effects of *Mondia whitei*.

In connection with the findings mentioned above, one of the objectives of the study was to examine the influence of the plant suspension on the contractile pattern of the vas deferens. *Mondia whitei* induced in a dose-dependent manner, supersensitivity to NE after 30 days of treatment. It is widely believed that NE is a specific alpha agonist whose receptors are abundant in the proximal part of the vas deferens compared to the distal section. It contracts the vas deferens by interacting either with alpha 1 or alpha 2 receptors (Bulman et al., 1993; Kato et al., 2000). This ability of *Mondia whitei* to potentiate NE action could be linked to the increase in sites of alpha receptors in the proximal region of the vas deferens, thus leading to an enhancement of the response. This statement is physiologically important since vas deferens plays a key role in sperm transport and seminal emission (Reddy and Prakash, 1999). Our results disagree with our recent *in vitro* findings (personal communication) where we observed a relaxant effect of the hexane, methanol and methylene chloride/methanol (1:1) extracts of *Mondia whitei* on KCl and adrenaline-induced rat vas deferens contractions.

This contradiction suggests that *in vivo, Mondia whitei*'s action may be mediated through its metabolite(s) and which could also be rapidly excreted as observed in the post-treatment study in which the treatment of rats with the plant suspension was followed by a wash-out period of 30 days.

Overall findings indicate that *Mondia whitei* extract may have reversible androgenic effect and potentiate the action of NE on rat vas deferens, and could then partially support its traditional use as an aphrodisiac.

Table1: Body weight and relative organ weights of rats after *Mondia whitei* treatment for 30 days.

Treatment	Body weight (g)		Relative organ weights (mg/100g)							
	Initial	Final	Testes	Epididymis			Vas deferens		Ventral prostate	Seminal vesicle
				Caput	Corpus	Cauda	Proximal	Distal		
Control, n=6	170	223	462.75 ± 29.20	71.75 ± 1.85	14.25 ± 0.39	68.42 ± 3.16	19.83 ± 0.87	25.33 ± 1.52	153.50 ± 2.45	294.83 ± 18.37
Hexane extract 500 mg/kg b.w, n = 6	180	234	451.08 ± 13.94	79.50 ± 3.36	12.08 ± 1.85	66.75 ± 2.12	13.83 ± 1.26[a]	21.33 ± 1.38	239.50 ± 28.50[b]	372.17 ± 38.72
Hexane extract 1000 mg/kg b.w, n = 6	200	264	503.50 ± 9.98	86.17 ± 1.88[b]	13.33 ± 1.46	71.58 ± 3.22	14.17 ± 2.65	25.25 ± 2.46	250.33 ± 22.45[b]	399.33 ± 16.42[a]
R500, n = 5	205	263	461.20 ± 18.00	70.50 ± 3.10	12.30 ± 0.84	68.10 ± 5.34	11.40 ± 0.96[b]	21.50 ± 0.72	152.80 ± 5.24	311.40 ± 10.08
R1000, n = 5	190	245	464.60 ± 14.37	71.00 ± 3.68	12.20 ± 0.91	70.80± 2.16	11.80 ± 0.59[b]	24.70 ± 1.18	154.40 ± 4.65	265.40 ± 33.13

All values: mean ± SEM (n=5-6).
n = Number of rats used.
R500 and R1000 : Rats treated with 500 mg/kg b.w and 1000 mg/kg b.w of *Mondia whitei* for 30 days respectively and allowed a 30 day wash-out period
a: $p<0.05$ b: $p<0.001$ when compared to control.

TAble 2: Effect of the hexane extract of *Mondia whitei* on cholesterol, total proteins and haematological parameters of rats.

Treatment	Cholesterol Testes (µg/mL)	Total proteins Serum (mg/mL)	Testes (µg/mg)	Epididymis (µg/mg) Caput	Corpus	Cauda	Globular counts RBC (10^6/mm^3)	WBC (10^3/mm^3)	Haematocrit (%)
Control, n = 6	2.13 ± 0.23	21.57 ± 0.41	10.67 ± 0.19	9.70 ± 0.16	7.89 ± 0.14	9.74 ± 0.33	7.16 ± 0.31	5.20 ± 0.46	42.67 ± 0.77
Hexane extract 500 mg/kg b.w, N = 6	1.62 ± 0.07[a]	24.17 ± 0.33[b]	17.96 ± 0.65[b]	11.78 ±0.26[b]	10.11 ±0.36[b]	11.53 ± 0.52[a]	7.23 ± 0.35	5.37 ± 0.38	43.32 ± 1.78
1000 mg/kg b.w, n = 6	1.30 ± 0.06[b]	26.11 ± 0.70[b]	20.15 ± 0.22[b]	13.53 ±0.29[b]	11.91 ±0.43[b]	14.79 ± 0.71[b]	7.58 ± 0.29	5.33 ± 0.59	45.46 ± 1.55
R500, n = 5	2.18 ± 0.04	21.56 ± 0.34	10.06 ± 0.11	9.48 ± 0.21	7.79 ± 0.27	9.70 ± 0.12	7.16 ± 0.77	5.02 ± 0.24	41.94 ± 1.54
R1000, n =5	2.18 ± 0.06	21.51 ± 0.34	10.55 ± 0.10	9.67 ± 0.17	7.82 ± 0.10	9.72 ± 0.12	7.14 ± 0.26	5.20 ± 0.16	42.70 ± 0.82

All values: mean ± SEM (n=5-6).
n: Number of rats used.
R500 and R1000 : Rats treated with 500 mg/kg b.w and 1000 mg/kg b.w of *Mondia whitei* for 30 days respectively and allowed a 30 day wash-out period.
WBC: White blood cells; RBC: Red blood cells.
a: $p<0.05$ b: $p< 0.001$ when compared to control.

Table 3: Per cent contractile responses (%) of NE on isolated vas deferens of rat treated with *Mondia whitei* for 30 days.

	Mondia whitei (mg/kg b.w)				
NE (x 10^{-3} µ M)	0	500	1000	R500	R1000
114	3.74 ± 0.32	4.70 ± 0.69	9.46 ± 3.27	3.55 ± 0.45	3.17 ± 0.52
228	16.15 ± 0.70	20.68 ± 4.27	27.81 ± 7.76	15.79 ± 2.18	4.45 ± 0.55
456	34.31 ± 2.27	46.92 ± 5.27	64.76 ± 5.31[b]	31.23 ± 3.00	14.75 ± 1.24[a]
648	46.58 ± 4.28	62.97 ± 10.76	96.72 ± 6.35[b]	42.02 ± 6.87	38.12 ± 1.13
824	69.79 ±2.00	87.32 ± 4.86[a]	124.82 ± 2.00[b]	70.30 ± 6.87	69.01 ± 4.01
912	100 ± 0.00	136.78 ± 2.42[b]	146.79 ± 2.49[b]	101.85 ± 10.31	100.12 ± 4.89

Each value represents mean ± SEM of 5-6 experiments.
R500 and R1000 : Rats treated with 500 mg/kg b.w and 1000 mg/kg b.w of *Mondia whitei* for 30 days respectively and allowed a 30 day wash-out period.
a: $P < 0.05$ b: $P < 0.001$ when compared to control.
NE: Norepinephrine.

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