

**RELAXANT EFFECT OF *MONDIA WHITEI*
EXTRACTS ON ISOLATED GUINEA PIG CORPUS
CAVERNOSUM**

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Summary

The present study aims to evaluate the response of *Mondia whitei* at the level of guinea pig corpus cavernosum. Penile erectile tissue was obtained from guinea pig. The corpus cavernosum (CC) was mounted in isolated tissue bath containing Krebs solution. Tissue strips were contracted with 10 μ M phenylephrine (Phe) and after obtaining a plateau of contraction, the aqueous, hexane and methylene chloride (CH₂Cl₂) extracts from *Mondia whitei* (0.5-5mg/mL) were used to relax the preparation in the absence and presence of indomethacin (5.6 μ M) or N-nitro-L arginine methylester (L ω -NAME, 500 μ M). Results of the work showed that *Mondia whitei* (0.5-5mg/mL) relaxed the smooth muscle of guinea pig corpus cavernosum pre-contracted with phenylephrine in a concentration-dependent manner. The CH₂Cl₂ extract was more effective in relaxing the preparation with an EC₅₀ of 1.31 compared to the hexane (EC₅₀, 1.85) and aqueous extracts (EC₅₀, 9.81). Neither L ω -NAME nor indomethacin affected the relaxant effect of the CH₂Cl₂ and hexane extracts. It could be concluded that *Mondia whitei* relaxes the corpus cavernosum through a direct inhibition of the alpha-adrenergic tone.

Keywords: *Mondia whitei*, corpus cavernosum, relaxation.

Introduction

Many aphrodisiac plants have been widely used since ancient time to modify impaired sexual functions of human beings (1,2,3,4). One of such plants is *Mondia whitei*, an aromatic plant of the Periplocaceae family. In Cameroon, it is referred to as "Limte", "Nkang bongo", "Yang" or "la racine". The roots are used either as spices, aphrodisiacs or for the treatment of urinary tract infection, jaundice and headache, while the whole plant is used to treat diarrhoea [5,6]. In our earlier studies, we demonstrated the androgenic and aphrodisiac properties of the aqueous and hexane

extracts from this plant in rats (7,8,9). The present study was undertaken to test the *in vitro* pro-erectile potential of *Mondia whitei* by recording the relaxant effect of the aqueous, hexane and methylene chloride (CH₂Cl₂) extracts from *Mondia whitei* on phenylephrine-induced contraction of the guinea pig corpus cavernosum.

Materials and Methods

Plant material

Fresh roots of *Mondia whitei* were collected in Bangangté, Cameroon. Botanical identification was done at the Cameroon National Herbarium (HNC) in Yaoundé 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 1.5-2cm, air-dried and powdered using an electric grinder (Moulinex).

Preparation of the aqueous extract

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

Preparation of organic solvent extracts

Seven hundred grams of the powdered roots were soaked in 6L of methylene chloride:methanol (CH₂Cl₂:MeOH) (1:1 v/v) mixture at room temperature for 72h and filtered. The solvent was removed by vacuum distillation and dried to obtain a black paste (76g) referred to as the CH₂Cl₂:MeOH extract; 66g of this mass were exhausted for 30 minutes in 500mL of hexane and filtered. The solvent was removed as previously to obtain 5g of hexane fraction. It was so proceeded to obtain the CH₂Cl₂ (10g) fraction of *Mondia whitei*. For each sample, the working solution (100mg/mL) was extemporarily prepared by dissolving 1g of paste in 2mL of 0.3%Tween 80 and 8mL of distilled water.

Animals

Adult healthy male guinea pigs (350-450g) were used in the study. The animals were kept under standard conditions (12h light/12h night; 26°C) with free access to water and food.

Isometric tension measurements

The guinea pigs were killed by a blow on the head and exsanguinated. The entire penis was surgically removed and rapidly placed in a Krebs solution of the following composition (mM/L): NaCl 115.0, NaHCO₃ 25.0, CaCl₂ 2.5, KCl 4.7, MgCl₂ 1.2, KH₂PO₄ 1.2, D-Glucose 10.0. The tunica albuginea was carefully opened, and the erectile tissue dissected, cleaned of surrounding adipose and muscular tissues. The corpus cavernosum (CC) was cut into two parts and mounted under a 0.71g resting tension in an isolated organ bath of 20mL capacity (Ugo Basile Cat. 4000 One Muscle Chamber, Via G. Borghi 43, 21025 COMERIO – VA, Italy) containing Krebs solution, maintained at 37±0.5°C and bubbled with air. The preparation was allowed to equilibrate for 45min during which the bath solution was changed every 15min.

Experimental procedure

The CC was contracted with 10µM of Phe. After having obtained a plateau of contraction, *Mondia whitei* extracts (0.5-5mg/mL) were used to relax the preparation in a cumulative manner. In some experiments, in order to study the involvement of nitric oxide (NO) and prostaglandins, the relaxant effects of the most active extracts (Hexane and CH₂Cl₂) on Phe-induced plateau of contraction were investigated after pretreatment for 15 min of the CC with a non specific NO synthase inhibitor [N-nitro-L arginine methylester (Lω-NAME), 500µM] or a prostanoid synthesis inhibitor (indomethacin, 5.6µM). The relaxant activities were expressed as the percentage of inhibition of the plateau of contraction to Phe. EC₅₀ values were calculated using Graph Pad Software version 3.00. Statistical analysis of the data were performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test. A probability of p < 0.05 was accepted as significant.

RESULTS

Relaxing activities of *Mondia whitei*

Through out the study, there was no spontaneous contractile activity of the guinea pig CC strips. The selective α_1 -adrenoceptor agonist, phenylephrine (10 μ M), induced contraction of guinea pig CC preparation. Table 1 displays the effects of *Mondia whitei* on Phe-induced contraction. Hexane and CH₂Cl₂ samples exhibited a concentration-dependent relaxing property. Comparatively, the CH₂Cl₂ extract produced more relaxation with an EC50 of 1.31 compared to that obtained with the hexane (EC50, 1.85) and aqueous extracts (EC50, 9.81).

Effect of L ω -NAME or indomethacin upon *Mondia whitei*-induced relaxations

In the presence of L ω -NAME (500 μ M) or indomethacin (5.6 μ M), the relaxant effect of the hexane and CH₂Cl₂ remained statistically unchanged when compared to their respective normal values (Table 2).

Table 1: Percent relaxant effect and EC₅₀ of *Mondia whitei* extracts on the plateau of contraction induced by phenylephrine (10μM) in guinea pig strips.

Plant extract	Concentration of <i>Mondia whitei</i> (mg/mL)					EC ₅₀
	0.5	1	2	4	5	
Aqueous	0.81±0.75 ^a	1.62±0.59 ^a	3.76±1.21 ^a	1.13±0.20 ^a	4.21±5.52 ^a	9.81
Hexane	15.32±3.87 ^b	38.56±9.54 ^b	83.31±11.27 ^c	115.93±8.36 ^c	118.01±7.80 ^c	1.85
CH ₂ Cl ₂	21.52±3.33 ^{bc}	60.82±8.12 ^c	120.87±9.68 ^{bc}	131.92±9.74 ^c	131.29±9.98 ^c	1.31

Data in Mean ± SEM of four experiments.

a, b, c: Within the same column, values with the same superscript letter(s) do not differ significantly (p>0.05) (Newman-Keuls multiple comparison test).

Table 2: Percent relaxant effect and EC₅₀ of the hexane and CH₂Cl₂ extracts of *Mondia whitei* on the plateau of contraction induced by 10μM phenylephrine in the presence of 15 min pre-treatment with Indomethacin (5.6μM) or Lω-NAME (500μM).

Treatment	Concentration of <i>Mondia whitei</i> (mg/mL)					EC ₅₀
	0.5	1	2	4	5	
Hexane	15.32±3.87	38.56±9.54	83.31±11.27	115.93±8.36	118.01±7.80	1.85
indomethacin + hexane	8.14±1.65	39.39±8.66	81.76±5.17	113.73±2.57	120.61±5.36	1.47
Lω-NAME + hexane	16.68±3.50	46.43±8.42	95.63±16.42	126.59±3.74	134.33±4.62	1.57
CH ₂ Cl ₂	21.52±3.33	60.82±8.12	120.87±9.68	131.92±9.74	131.29±9.98	1.31
indomethacin + CH ₂ Cl ₂	23.47±5.26	59.71±12.63	107.73±4.43	128.87±3.69	134.19±4.36	1.36
Lω-NAME + CH ₂ Cl ₂	17.27±3.36	53.14±6.20	106.25±5.10	118.20±8.38	118.20±8.38	1.30

Data in Mean ± SEM of three experiments.

For all comparisons, p>0.05 (Newman-Keuls multiple comparison test): 1- Hexane vs (indomethacin + hexane) or (Lω-NAME + hexane) and 2- CH₂Cl₂ vs (indomethacin + CH₂Cl₂) or (Lω-NAME + CH₂Cl₂).

DISCUSSION

Relaxation of cavernous smooth muscle is critical for inducing and maintaining penile erection (10,11). Our results demonstrated that *Mondia whitei* causes concentration-dependent relaxant responses in Phe-induced CC contraction. The pharmacological characteristic of phenylephrine, a specific α_1 -adrenoceptor, is the contraction of the trabecular smooth muscle of the CC (12). Among the three plant extracts, the CH_2Cl_2 and hexane extracts showed significant results. Bioactive molecules such as steroids revealed in these extracts may account for the relaxant activities. The steroid-like effect of *Mondia whitei*, may be supported by our earlier data showing the androgenic activity of the plant (7,8). Similarly, Sperling *et al* and, Hnatyzyn and co-workers also demonstrated the relaxant effects of *Aspidosperma quebracho blanco* and *Achyrocline satureioides* respectively on isolated CC (13,14). In this study, *Mondia whitei*-induced relaxations were unaffected by L ω -NAME, a non specific NO synthase inhibitor. This result implies that nitric oxide is not involved in the relaxing effects of *Mondia whitei* and also corroborates with our preliminary finding showing that pre-treatment of sexually inexperienced male rats with L ω -NAME (10mg/kg) completely failed to influence the sexual stimulant effect induced by the hexane extract of *Mondia whitei* (500mg/kg) (personal communication). Inhibition of prostanoid production did not also modify the relaxant effect of the plant suggesting the non involvement of prostaglandins (15,16,17).

The ability of *Mondia whitei* to reduce α -adrenergically stimulated contraction of the corpus cavernosum suggests that this plant might have a therapeutic potential in the treatment of erectile dysfunction (ED) due to decrease in levels of NO (ageing ED) and thus validate its popular use as sexual stimulant plant. However, further experiments are needed to clarify this hypothesis.

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