Effects of *Mondia whitei* extracts on the contractile responses of isolated rat vas deferens to potassium chloride and adrenaline

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ABSTRACT

Objective: To investigate the effects of the methylene chloride:methanol (CH_2Cl_2:MeOH, 1:1) extract of the dried roots of *Mondia whitei* Linn and its hexane and methanol fractions on potassium chloride (KCl) and adrenaline (Adr)-induced contractions of rat vas deferens.

Materials and Methods: Isolated strips of normal adult rat vas deferens were mounted in a Ugo Basile single-organ bath containing Krebs solution. Cumulative concentration-response curves of KCl (1–7 x 10^{-2} *M*) and adrenaline (1.21–8.45 x 10^{-7} *M*) were established in the absence and presence of *M. whitei* (50–400 µg/ml). In separate experiments, after obtaining a stable plateau of contractions with KCl (60 m*M*), *M. whitei* samples (50–400 µg/ml) were added cumulatively to relax the preparation. In KCl (60 m*M*), containing depolarizing medium, cumulative concentration-contraction curve to $CaCl_2$ (2–14 x 10^{-2} *M*) was elicited in the absence and presence of the hexane fraction of *M. whitei* (50–400 µg/ml).

Results: All the *M. whitei* samples produced rightward shift of the concentration-response curves to KCl and Adr. At high concentration of the plant extracts (400 µg/ml), a decrease of the maximal response to the contractile agents was observed compared with that obtained with the control. All the three extracts produced concentration-dependent relaxation of the plateau of contraction induced by KCl and the hexane fraction appeared to be the more potent. In calcium-free physiological salt solution, the hexane fraction of *M. whitei* produced rightward shift to the concentration-response curve to $CaCl_2$ and completely abolished the contractile effect of calcium at high concentration (400 µg/ml).

Conclusion: It is concluded that *M. whitei* extracts antagonized the contractile responses to KCl and Adr in isolated rat vas deferens, which could be due to the blockade of voltage-operated calcium channels.

Key Words: Epinephrine, *in vitro* preparation, KCl.

Introduction

Mondia whitei is an aromatic plant of the Periplocaceae family. In Cameroon traditional medicine, it is referred to as *Limte*, *Nkang Bongo*, *Yang*, or *La racine*. The roots are used as spices or to treat urinary tract infection, jaundice, headache, diarrhea,[1] and male sexual asthenia (impotence).[2] We have observed a short-term androgenic[3] (8 days of treatment) and a long-term reversible antispermatogenic and antifertility effects of the aqueous extract of *M. whitei* (400 mg/kg) in adult male rat.[4] To the best of our knowledge, no work has been done with this plant on the physiology of the vas deferens, a structure of male reproductive system, which plays a principal role in male fertility through its contractile properties.[5] The present work was carried out to investigate the *in vitro* effects of the CH_2Cl_2 : MeOH extract of the dried roots of *M. whitei* and, its hexane and methanol fractions on KCl and adrenaline-induced contractions of the rat vas deferens, a study which could permit us to partially postulate the implication of the plant in the handling of some male reproductive disturbances such as infertility.

Materials and Methods

Animals

Healthy male albino rats (150–250 g, >90 days) of the Wistar strain were used in the present study. The animals were raised at room temperature with a natural light-dark cycle and maintained at standard rat diet and tap water given *ad libitum*.

Plant collection and extraction

Fresh roots of *M. whitei* were obtained from the local market and authenticated by Dr. Pinta Jonas of the Botany

Department, Faculty of Science, University of Dschang, Cameroon. The roots were air-dried and ground using a mixer (Moulinex). The powdered roots (700 g) were soaked in 6 litres 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 (76 g), referred to as the CH_2Cl_2 : MeOH extract; 66 g of this mass were exhausted for 30 min in 2 l of hexane and filtered. The solvent was removed as previously to obtain 10 g of hexane fraction. It was so proceeded to obtain the methanol (10 g) fraction of *M. whitei*. For each sample, the working solution (100 mg/ml) was prepared extemporarily by dissolving 1 g of paste in 2 ml of 0.3% Tween 20 and 8 ml of distilled water.

Phytochemical tests

The hexane and methanol fractions of *M. whitei* were treated with several reagents and spectroscopy and physical analysis (RMN, ^1H and ^{13}C; mass spectrometry (SM) RMN, etc.) were performed. Positive results were obtained with the following constituents: steroids, triterpenes (mixture of amyrine α- and ß-acetate, lupeol, ß-sitosterol, and ß-sitosterol glucoside) and aromatic (2-hydroxy-4-methoxybenzaldehyde, 3-hydroxy-4-methoxy-benzaldehyde (vanillin), and 4-hydroxy-3-methoxy-benzaldehyde) compounds in the hexane fraction, sugar (glucose), and polyholosides [α-D-glucopyranosyl (6-1)-ß-D-glucopyranose and 1-methoxy-ß-D-glucopyranosyl (6-1)-ß-D-glucopyranose] in the methanol fraction.

Contractions of the isolated vas deferens

Effects on agonist-induced contractile responses

The rats were sacrificed by a blow on the head and bled to death by cutting the neck vessels. The vas deferens were promptly removed, cleansed of the connective tissue, and cut into two parts as to obtain a proximal portion nearer to the epididymis and a distal portion nearer to the sex accessory complex. The proximal part, which is more sensitive than the distal section,[6] was used in the study and mounted in an organ bath of 20 ml capacity containing fresh Krebs solution of the following composition (mM/l): NaCl 115, $NaHCO_3$ 25, $CaCl_2$ 2.5, KCl 4.7, $MgCl_2$ 1.2, KH_2PO_4 1.2, and D-glucose, 10. The PSS was maintained at 37 ± 0.5°C and continuously bubbled with air. The preparation was allowed to equilibrate for 45 min during which the bathing solution was changed every 15 min. Cumulative concentration-response curves to agonists such as KCl (1.0–7.0 x 10^{-2} M) and adrenaline (1.21–8.45 x 10^{-7} M) were recorded in the absence and presence of *M. whitei* samples (50–400 μg/ml). The contractile responses were expressed as a percentage of the maximal contractile response to KCl or Adr.

Effects on KCl-induced maximal contraction

In separate experiment, after having obtained a stable plateau of contractions [usually 15–20 min after challenging the preparation with supramaximal concentration of KCl (60 mM)], *M. whitei* extract and its fractions (50–400 μg/ml) were used to relax the preparation in a cumulative manner. The relaxant effects were expressed as the percentage of inhibition of the plateau of contraction to KCl.

Effects of the hexane fraction on calcium activity

In order to determine the effect of *M. whitei* extracts on calcium activity, the vas deferens was contracted with KCl and relaxed with PSS, and the bath solution replaced with a high K^+(70 mM), Ca^{2+}-free Krebs solution containing EGTA (1 mM). Among the three *M. whitei* solutions, the hexane fraction showed maximum changes in the two previous experiments and was thus used in this investigation. Cumulative concentration-contraction curve was obtained by a stepwise increase in $CaCl_2$ (2–14 x 10^{-2} M) in the absence and presence of the hexane fraction of *M. whitei* (50–400 μg/ml). The contractile response was expressed as a percentage of the maximal contractile response to $CaCl_2$.

In the above studies, contraction and inhibition (relaxation) of the vas deferens were recorded by means of an isometric transducer 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. The tension applied on the preparation was 0.8 g.

One-way ANOVA with post hoc Dunnett's or Newman-Keuls multiple comparison test were performed. The value of $P < 0.05$ was considered to be statistically significant.

Results

Effects on agonist-induced contractile responses

In rat vas deferens, KCl (1–7 x 10^{-2}*M*) and adrenaline (1.21–8.45 x 10^{-7}*M*) produced concentration-dependent contractions. In the presence of *M. whitei* (50–400 μg/ml), there were rightward shifts of the concentration-response curves to KCl and adrenaline with significant decrease in the maximal response. The hexane fraction of *M. whitei* produced comparatively more rightward shift and significant inhibition of the maximal response to KCl and Adr at highest concentration (400 μg/ml) as compared with the other fractions. [Figures 1 and 2, Table 1]

Effects on the plateau of contraction induced by KCl

On the plateau of contraction induced by KCl (60 m*M*), *M. whitei* extract and its fractions induced a concentration-dependent relaxant effects with the hexane fraction producing more relaxation with equivalent concentration (Table 2).

Effects of the hexane fraction on calcium activity

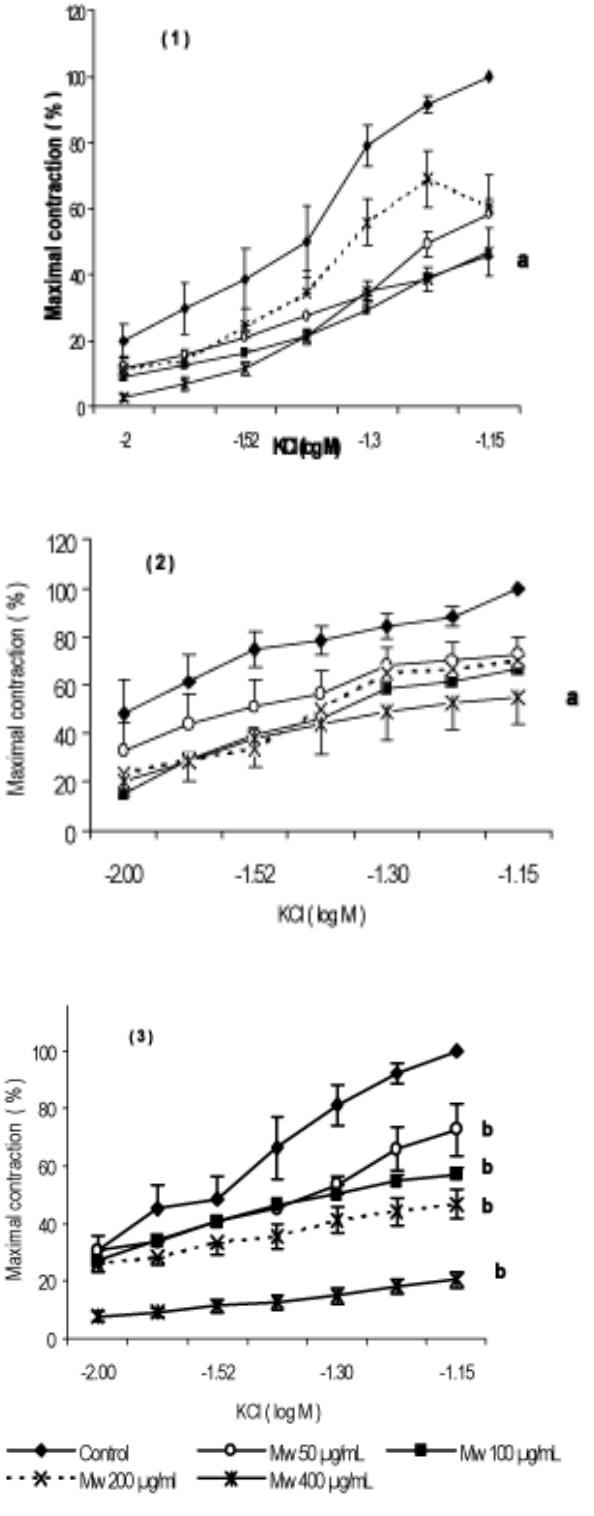
In calcium-free medium, the hexane fraction of *M. whitei* produced concentration-dependent rightward shift of the concentration-response curve of $CaCl_2$ (2–14 x 10^{-2} *M*) with significant decrease in the maximal response. [Figure 3 and Table 1]

Discussion

Smooth muscle contraction is dependent on the concentration of the cytosolic-free calcium, which activates the contractile elements.[7,8] In the present study, KCl and Adr induced contractions of isolated vas deferens of rat. The contractile effect of KCl is owing to a mechanism related to membrane depolarization and subsequent influx of external calcium through voltage-operated channels.[9] Receptors for adrenaline are abundant in the proximal part of the vas deferens compared with the distal section. It contracts the vas deferens by interacting with either α_1 or α_2 receptors, whereas relaxation is generally associated with ß-receptors.[10] Stimulation of α_1 receptors leads to the hydrolysis of phosphatidyl inositol into inositol triphosphate (IP_3) and

Figure 1. Effects of various concentrations of the CH_2Cl_2:MeOH (1) extract of *Mondia whitei (Mw)* and its methanol (2) and hexane (3) fractions on cumulative concentration-response curves to KCl. Each point represents mean of four experiments and the bars indicate SEM.
[a]P<0.05 and [b]P<0.001 significantly different from the corresponding control values.

Figure 2. Effects of various concentrations of the CH_2Cl_2:MeOH (1) extract of *Mondia whitei (Mw)* and its methanol (2) and hexane (3) fractions on cumulative concentration-response curves to Adrenaline. Each point represents mean of four experiments and the bars indicate SEM.
[a]P<0.05 and [b]P<0.001 significantly different from the corresponding control values.



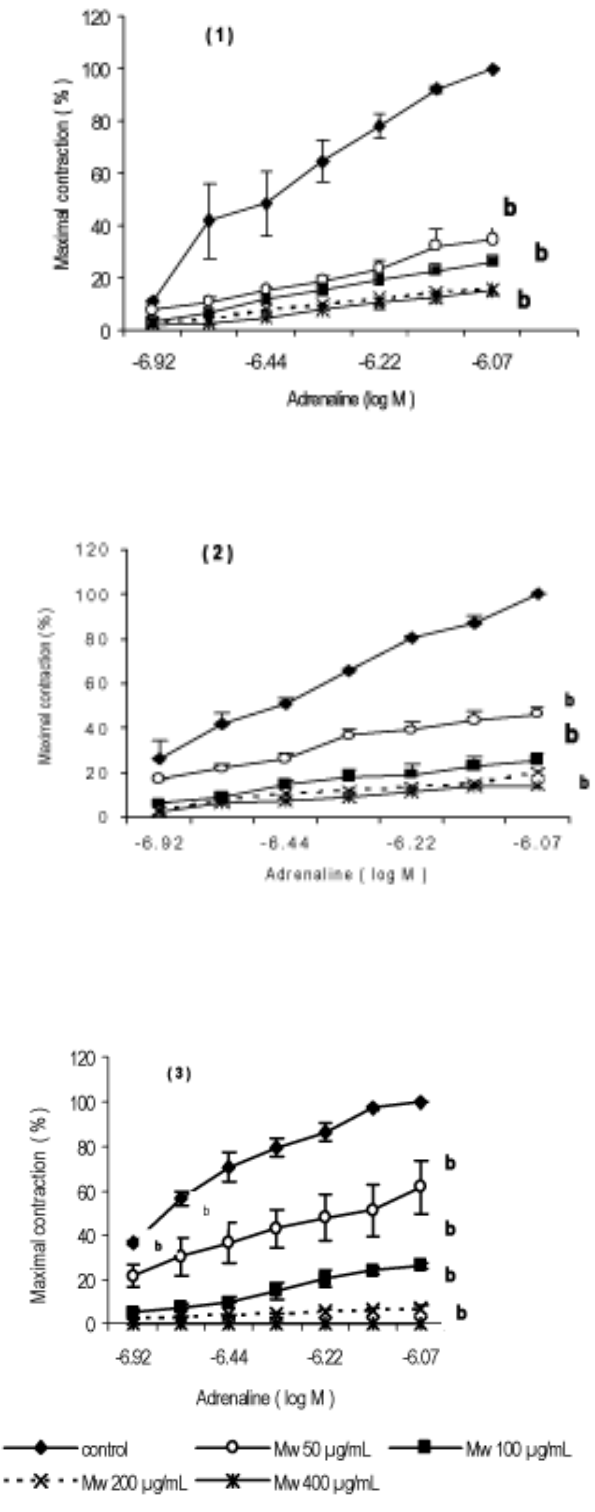


Table 1

Maximal contractile responses of various agonists in the absence and presence of *M. whitei* extract and its fractions (50 - 400 µg/ml) isolated in rat vas deferens

Agonist	*M. whitei extract*	*In the absence of M. whitei (Control)*	*In the presence of M. whitei (µg/ml)*			
			50	*100*	*200*	*400*
KCl	CH_2Cl_2:MeOH	100.00±0.00	52.24 ±4.83**	45.75±6.95*	71.19±8.08*	47.39±7.37**
KCl	MeOH	100.00±0.00	72.50 ±7.27	66.34±8.82	70.08±8.19	57.47±10.56*
KCl	Hexane	100.00±0.00	72.60 ±9.02*	57.14±2.34**	46.65±4.92**	20.45±2.78**
Adr	CH_2Cl_2 : MeOH	100.00±0.00	34.67 ±7.52**	25.97±2.50**	15.51±1.51**	15.16±2.05**
Adr	MeOH	100.00±0.00	46.38 ±3.28**	25.18±3.83**	20.19±2.93**	14.18±1.06**
Adr	Hexane	100.00±0.00	61.50 ±11.95**	26.32±1.44**	6.12±1.08**	3.59±0.41**
$CaCl_2$	Hexane	100.00±0.00	43.61 ±4.53**	27.66±3.72**	9.11±2.78**	0.00±0.00**

Values are **%**maximal response (mean ± SEM). *P<0.05. **P<0.001 compared to control. Adr - adrenaline.

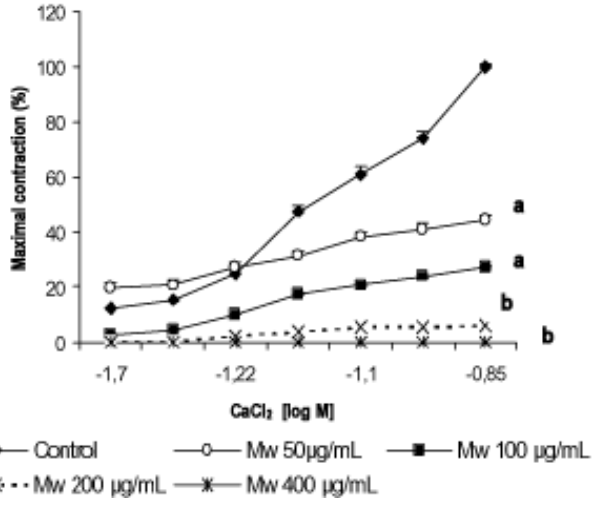
Table 2

Percent relaxant activity of *M. whitei* extract and its fractions against 60 m*M* KCl-induced plateau of contraction in isolated rat vas deferens

Agonist	*M. whitei extract*	*M. whitei (µg/ml)*				
		50	*100*	*200*	*300*	*400*
KCl	Hexane	13.67±5.12	22.52 ±4.78	44.22±4.84	64.37±4.64	75.76±4.97
KCl	CH_2Cl_2 : MeOH	8.76±3.27	18.55 ±4.59	40.85±1.94	52.57±1.47	57.82±3.35
KCl	MeOH	8.15±1.90	15.89 ±0.90	34.52±2.99	46.23±5.02	51.21±6.29*

Values are %relaxant activity (mean±SEM). *P<0.05 compared with the hexane fraction of *M. whitei.*

Figure 3. Effects of various concentrations of the hexane fraction of *Mondia whitei* (Mw) on cumulative concentration-response curves to $CaCl_2$. Each point represents mean of four experiments and the bars indicate SEM.
[a]P< 0.05 and [b]P< 0.001significantly different from the corresponding control.



diacylglycerol (DAG). IP_3 causes the liberation of the intracellular calcium ion stockage, whereas DAG depolarizes the cell membrane inducing the calcium ions reflux.[11] The stimulation of a_2 adrenoceptors activates the adenylcyclase responsible of the increase of cAMP that induces the influx of calcium ions which cause the contraction.[12–15] The rightward shift curves produced by all *M. whitei* extracts against KCl and adrenaline-induced contraction of the isolated vas deferens denotes noncompetitive antagonism and indicates reduced entry of Ca^{2+} through membrane Ca^{2+} channels.[16] This inhibitory effect was prominent at high concentration of hexane fraction (400 μg/ml) of the plant, as compared with that obtained with the CH_2Cl_2 : MeOH extract and its methanol fraction. This could be a contributing factor in reducing the continuous transport of sperms at rest and during ejaculation by the vas deferens. Alterations in contractility of rat-isolated vas deferens by many drugs have been reported.[17,18] Our findings also revealed a concentration-dependent relaxing effect of different extracts on the plateau of contraction induced by a supramaximal dose of KCl, and the hexane fraction was found to be more potent than the other two extracts in relaxing the preparation. This ability of *M. whitei* to relax KCl-induced sustained contractions suggests the involvement of voltage-operated calcium channels.

From these observations, the hexane fraction of *M. whitei* appeared to be the most active. Steroids and aromatic compounds revealed in this extract may be responsible for its high activity,[19,20] compared with the other samples in which these substances are either in less quantity (CH_2Cl_2 : MeOH extract) or absent (methanol fraction). In order to study the action of *M. whitei* on the movement of external Ca^{2+}, the effect of hexane fraction was examined on the $CaCl_2$-induced contractions in potassium-depolarized preparation. Since this extract was able to abolish the contractions induced by $CaCl_2$ in K^+-depolarized preparation in a concentration-dependent manner, it is likely that the hexane fraction of *M. whitei* inhibits the calcium influx through voltage-operated channels.

In conclusion, the results of the present investigation suggest that the hexane fraction of *M. whitei* extracts contains bioactive molecules that block both the receptor-operated and voltage-operated calcium channels, thus preventing the entry of calcium during depolarization of vas deferens by KCl and adrenaline. However, *in vivo* studies using the hexane fraction of *M. whitei* are needed to justify and confirm the above assertion.

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