MODULATION OF PENILE ERECTION IN RABBITS BY MONDIA WHITEI: POSSIBLE MECHANISM OF ACTION


Centre for Scientific Research into Plant Medicine, P. O. Box 73, Mampong- Akuapem, Ghana; Chemical Pathology Department, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana

Biochemistry Department, P.O. Box LG 54, University of Ghana, Legon, Ghana.

E-mail: kennie01951@yahoo.com

Abstract

Mondia whitei root was evaluated to validate its anecdotal use and determine its possible mode of action in the management of erectile dysfunction. Rabbits were administered with daily oral doses of 100-400 mg kg\(^{-1}\) crude ethanolic extract of \(M.\) whitei and sildenafil (50 mg kg\(^{-1}\)) as positive control for 6 weeks. Cavernosal tissue NOS activity and levels of NO and cGMP, and NOS and PDE protein expressions were investigated. The effect of the crude extract, chloroform and petroleum ether fractions \textit{in vitro} on cavernosal tissue NOS activity and levels of NO and cGMP were also investigated. Results indicate that the crude extract increased NOS activity by 7% at 200 mg kg\(^{-1}\) with corresponding increases in NO (88%) and cGMP (480%) levels. No significant changes in these measurements were observed with the 100 and 400 mg kg\(^{-1}\) doses whilst sildenafil slightly reduced them (15.9-37.5%). NOS and PDE protein expressions in test animals were not different from controls. Pre-incubation of cavernosal tissue \textit{in vitro} with the crude extract of \(M.\) whitei and its chloroform fraction markedly increased NOS activity (26-132%) and levels of NO (25%) and cGMP (400%) at 0.01 mg g\(^{-1}\) tissue but these were reduced to near control levels when their concentrations were increased to 0.10 mg g\(^{-1}\) tissue whilst the petroleum ether fraction had no effect. These findings suggest that \(M.\) whitei may influence erectile function through activation/stimulation of NOS with corresponding increases in tissue NO and cGMP levels and that certain chemical constituents present in the chloroform fraction may be responsible for biological activity.

Key words: Erectile dysfunction; nitric oxide; cGMP; sildenafil; organic fractions

Introduction

Erectile dysfunction (ED), also known as impotence, is defined as the inability to achieve or maintain an erection sufficient for satisfactory sexual intercourse. It is very common and occurs most frequently in middle age men of 40-50 years. However, it can affect men of all ages (NIH Consensus, 1993).

Erectile function is an integration of complex physiological processes involving the central nervous system (CNS), peripheral nervous system and hormonal and vascular systems. The degree of contraction of the corpus cavernosal smooth muscle determines the functional state of the penis. The balance between contraction and relaxation is controlled by central and peripheral factors that involve many transmitters and transmitter systems (Brosman and Leslie, 2006). ED occurs when there is an imbalance of or defects in any of the following: decreased nitric oxide (NO) generation, decreased cyclic guanosine monophosphate (cGMP) levels and increased phosphodiesterase (PDE) activity.

Cyclic GMP levels in the corpus cavernosum are determined by both the rate of synthesis by guanylate cyclase via NO action and the rate at which cGMP are broken down by PDE (Corbin et al., 2002). Phosphodiesterases (PDEs) are a diverse family of enzymes that hydrolyze cyclic nucleotides and thus play a key role in regulating intracellular levels of the second messengers cAMP and cGMP and hence cell function.
Thus increased PDE activity will promote detumescence (reversal of erection) while decreased PDE activity will enhance erection as a result of the longer half-life of cGMP. PDE5 is abundant in the corpus cavernosum and is the predominant PDE in this tissue (Corbin et al., 2002).

The NOSs are a group of enzymes responsible for the synthesis of NO and citrulline from the terminal nitrogen atom of L-arginine in the presence of oxygen and the co-factors, reduced nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme and tetrahydrobiopterin (BH4) (Furchgott and Zawadzki, 1980).

Many orthodox pharmaceuticals like sildenafil (Viagra) and vardenafil (Levitra) have exploited the above biochemical processes to promote penile erection. These drugs are quite expensive, $10-$15 per tablet, and beyond the reach of the ordinary worker. However, medicinal plants have been used for centuries as viable alternatives, with anecdotal evidence of their effectiveness for management of ED. Some of these include *Cortnanthe yohimbe*, *Xanthoparmelia scabrosa*, and *Mondia whitei*. *Cortnanthe yohimbe* is a tall evergreen forest tree native to Southwestern Nigeria, Cameroon, Gabon and the Congo. The bark of the tree contains approximately 0.8%-1.2% yohimbine as active constituent. *Xanthoparmelia scabrosa*, a lichen found in various parts of the world, contains epiditiopiperazinedione and 4-methylipiperazines as active constituents, which have been proven effective as PDE-5 inhibitors (Ernst-Russel et al. 1999).

*M. whitei* is from the Periplocaceae family has been used by many traditional medicine practitioners in Ghana for the management of ED. However, there are no empirical scientific data to support this ethnomedical use. This study was, therefore, conducted to evaluate the efficacy of *M. whitei* in the management of ED in rabbits with the view to determining its possible mode of action.

**Materials and Methods**

**Chemicals and Reagents**

Nitric Oxide Assay and cyclic GMP Enzyme Immunoassay kits were obtained from Assay Design Inc. (Ann Arbor, MI, USA). One-Step Western blotting kit for protein expression was from GenScript Corporation (Piscataway, NJ 09954, USA). Nitric Oxide Synthase Assay kit was purchased from Calbiochem (USA). Sildenafil manufactured by Pfizer (New York, USA) was purchased from Top-Up Pharmacy Ltd (Tema, Ghana). All other chemicals were obtained in the highest purity grade from British Drug Houses Ltd (Poole, UK).

**Experimental Animals**

Twenty-five sexually matured male rabbits weighing between 2.5-3.0 kg were obtained from the Noguchi Memorial Institute for Medical Research (NMIMR), housed individually in wooden cages at the Animal House of the Centre for Scientific Research into Plant Medicine (CSRPM) and subjected to 12 hrs of light and 12 hrs of darkness, fed standard rabbit feed and water. They were allowed to adapt for a period of one week before being used for the study. Studies were conducted in accordance with internationally acceptable principles for laboratory animal use and care. The animals were then divided into five groups of 5 animals each.

**Preparation of Plant Extract**

Roots of *M. whitei* (3 kg) (CSRPM voucher specimen number 210, and authenticated by Dr. Yaw Ameyaw, Plant Production Department, CSRPM) obtained from the production stores of the CSRPM, (Mampong-Akuapem, Ghana) were roughly milled and cold macerated with 70% ethanol for 3 days. The ethanolic extract was concentrated using the LABOROTIA 4000 rotary evaporator (Germany) at a temperature of 65°C. The concentrate obtained was reconstituted in water (500 mL) and re-concentrated to ensure complete removal of residual ethanol. Twenty five millilitres (25 mL) portions of the concentrated ethanolic extract were poured into flasks and freeze dried using a Heto Power Dry LL3000 freeze-dryer (Denmark) for 24 hrs. The dried powder was stored in air-tight containers and refrigerated until use.

**Phytochemical Screening**

The ethanolic extract of *M. whitei* was screened for the presence or absence of groups of phytochemicals such as saponins, reducing sugars, phenolics, cyanogenic glycosides, polyamides, phytosterols, triterpenes, anthracenosides, flavonoids and alkaloids (Sofowora 1982; Harborne 1983).

**Preparation of Organic Fractions of M. whitei**

Freeze-dried crude extract (30 g) was added to 100 mL water and left to dissolve. This was then poured...
into a separating funnel and equal volumes of petroleum ether, chloroform and ethyl acetate were added to the sample one at a time, shaken and allowed to separate. The various separated organic fractions were collected and organic solvents evaporated using the rotary evaporator to obtain the different fractions of *M. whitei*

**Animal Treatment**

The study design consisted of five (5) groups of rabbits with each group having five animals. Groups 1 and 5 served as control and positive (sildenafil) controls, respectively. Groups 2, 3, and 4 (Gp A, Gp B and Gp C, respectively) were the plant extract treatment groups, which received increasing doses (100-400 mg kg\(^{-1}\)) of *M. whitei*. The extracts or sildenafil were administered to the rabbits in 200 mL of drinking water to ensure that they had been drunk before further administration of drinking water to the animals *ad libitum*. This was done daily for a period of six (6) weeks.

**Preparation of Corpus Cavernosal Tissue**

The entire penis was removed from the animal and the corpus cavernosal tissue isolated from the enveloping tunica albuinea by careful dissection and immediately frozen in liquid nitrogen. The tissue was stored at -80°C until use. Also, fresh corpus cavernosal tissue of untreated rabbits was pre-incubated with *M. whitei* fractions dissolved in 1% DMSO at 0.1 mg g\(^{-1}\) tissue or 0.01 mg g\(^{-1}\) tissue for one hour, after which tissues were rinsed with PBS and stored at -80°C until use. The protein contents of tissue pellets and supernatants were determined by the method of Bradford (1976) using bovine serum albumin (BSA) as protein standard.

**Determination of Tissue Nitric Oxide (NO\(_2^-\)+NO\(_3^-\)) Levels**

Two millilitres (2 mL) of corpus cavernosal tissue homogenates were diluted two times with a reaction buffer provided in the assay kit. This was then ultrafiltered through a 10,000 molecular weight cut off (MWCO) filter using an Amicon ultrafiltration chamber. The filtrate was used for nitric oxide determination using a Nitric Oxide Assay kit (Assay Design Inc., Ann Arbor, MI, USA) which measures total nitrite and nitrate.

**Determination of cGMP Levels**

A weighed amount (0.2 g) of the frozen corpus cavernosal tissue of each sample was homogenized on ice in 10 volumes of cold 5% TCA (w/v) using a polytron-type homogenizer. The homogenate was centrifuged at 600 x g for 10 minutes and the supernatant was extracted with 3 volumes of water-saturated ether, after which the ether layer was discarded. The aqueous samples were assayed for cGMP using cGMP Enzyme Immunoassay Kit (Assay Designs Inc., Ann Arbor, MI, USA).

**Determination of NOS Activity**

Corpus cavernosal tissue (0.2 g) was homogenized on ice, in five-fold volume of phosphate buffered saline (PBS), pH 7.4 and centrifuged at 10,000 x g for 20 minutes. The supernatant was centrifuged at 100,000 x g for 15 minutes and then ultrafiltered using a 10 kDa MWCO Amicon filter. The NOS was measured in the supernatant using NOS Colorimetric Assay Kit (Calbiochem, USA) and performed according to the manufacturer’s instruction.

**NOS and PDE Expressions**

In NOS expression, frozen corpus cavernosal tissue (0.1 g) was homogenized in lysis buffer containing 10 mM Tris pH 7.4, 1% SDS, 1 mM sodium orthovanadate, 2 mM phenylmethylsulfonylfouride and 12.5 μg/mL aprotinin on ice, centrifuged at 10,000 x g for 20 minutes and the supernatant was used for SDS-PAGE. Fifty micrograms of protein from each sample was submitted to SDS-PAGE using the discontinuous buffer system. In phosphodiesterase expression, the cavernosal tissue (0.2 g) was homogenized on ice in buffer containing 50 mM Tris-Cl, 5 mM EDTA, 10 mM each of diithiothreitol and aprotinin, 10 μM pepstatin, 5 μM leupeptin and 1 mM each of sodium orthovanadate and sodium fluoride. The homogenate was then centrifuged at 16,000 x g for 20 minutes. Separation of proteins was done by SDS-PAGE as for NOS expression. Both NOS and PDE expressions were done by Western blotting (GenScript Corporation, Piscataway, NJ 09954, USA) using the SDS-PAGE separated protein bands in the gel.
Statistical Analysis

Data were reported as the mean and standard error of mean (SEM). Significance was calculated by one-way analysis of variance (ANOVA) and Student’s t-test for multiple comparisons. P values <0.05 were considered significant. The statistical tests were performed with SPSS 14.0 for Windows.

Results

Phytochemical Screening

The results of the phytochemical screening of the ethanolic extract of *M. whitei* are presented (Table 1). Results showed that *M. whitei* contained reducing sugars and triterpenes.

Table 1: Phytochemical screening of ethanolic extract of *Mondia whitei*

<table>
<thead>
<tr>
<th>Groups of Phytochemicals</th>
<th>Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Phenolics</td>
<td>-</td>
</tr>
<tr>
<td>Polyuronides</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Anthracenosides</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
</tbody>
</table>

(+: Detected; -: Not detected)

Yields of Crude Extract and Fractions of Crude Extract of *M. whitei*

The amount of freeze dried crude plant extract obtained was approximately 50,000 mg kg\(^{-1}\) of *M. whitei* roots. The yields of petroleum ether, chloroform and ethyl acetate fractions were 57,300, 153,000 and 9,330 mg kg\(^{-1}\) crude extract, respectively.

Tissue NOS Activity

Nitric oxide synthase activity in the corpus cavernosal tissue of the rabbits at termination of treatment with *M. whitei* extract and sildenafil is shown in Figure 1. The results indicate that there were non-significant differences between the control group and the other treatment groups. The NOS activity was decreased by 27% at the 100 mg kg\(^{-1}\) *M. whitei* dose whilst there appeared to be a slight increase (7%) with the 200 mg kg-1 dose but a 2% decrease from control with the 400 mg kg\(^{-1}\) dose. However, sildenafil reduced the NOS activity by 18% when compared to control.

Tissue Homogenate NO and cGMP Levels

The total nitric oxide levels, which are the sum of nitrite and nitrate levels, in corpus cavernosal tissue after treatment of rabbits with different doses of ethanolic extract of *M. whitei* and with sildenafil are shown in Figure 2. The results showed that there was no significant increase in cavernosal tissue NO levels of the 100 mg
kg$^{-1}$ $M$. whitei-treated rabbits compared to control. Although, there was marked increase (88%) in NO level at the 200 mg kg$^{-1}$ $M$. whitei dosage, at the dosage of 400 mg kg$^{-1}$ the NO levels sharply declined to 14% above control level. The tissue level of NO in the standard (sildenafil) treatment group was, however, slightly lower than that of the control. There were insignificant differences between all the treatment groups ($p>0.05$). Figure 3

![Figure 1: NOS activity in corpus cavernosal tissue of rabbits treated with vehicle, 100, 200 and 400 mg kg$^{-1}$ of $M$. whitei (Gp A, Gp B and Gp C, respectively), and 50 mg kg$^{-1}$ of sildenafil for 6 weeks. Data are expressed as the mean ± SEM of n=4.](image)

![Figure 2: NO levels in corpus cavernosal tissue of rabbits treated with vehicle, 100, 200 and 400 mg kg$^{-1}$ of $M$. whitei (Gp A, Gp B and Gp C, respectively), and 50 mg kg$^{-1}$ of sildenafil for 6 weeks. Data are expressed as the mean ± SEM of n=4.](image)
Figure 3: cGMP levels in corpus cavernosal tissues of rabbits treated with vehicle, 100, 200 and 400 mg kg\(^{-1}\) of *M. whitei* (Gp A, Gp B and Gp C, respectively), and 50 mg kg\(^{-1}\) of sildenafil for 6 weeks. Data are expressed as the mean ± SEM of n=4.

Figure 4: NOS activity in rabbit cavernosal tissue pre-incubated with vehicle (control), 0.01 or 0.10 mg g\(^{-1}\) tissue of petroleum ether fraction (PE), chloroform fraction (CH), and crude ethanolic extract (CE) of *M. whitei*, respectively. Results are means ± SEM of four determinations.
Figure 5: NO levels in rabbit cavernosal tissue treated with vehicle (control), 0.01 or 0.1 mg g⁻¹ tissue each of petroleum ether fraction (PE), chloroform fraction (CH), and crude ethanolic extract (CE) of M. whitei, respectively. Results are means ± SEM of four determinations.

Figure 6: cGMP levels in rabbit cavernosal tissue treated with vehicle (control), 0.010 or 0.10 mg g⁻¹ tissue each of petroleum ether fraction (PE), chloroform fraction (CH), and ethanolic extract (CE) of M. whitei, respectively. Results are means ± SEM of four determinations.
**Figure 7:** Effect of *M. whitei* on expression of eNOS (Gel B is a continuation of Gel A).

MW = Molecular weight marker; +C = Standard NOS; lanes 1 to 5 = Controls (Vehicle); lanes 6 to 10 = Group 2 (100 mg kg\(^{-1}\) of *M. whitei*); lanes 11 to 15 = Group 3 (200 mg kg\(^{-1}\) of *M. whitei*); lanes 16 to 20 = Group 3 (400 mg kg\(^{-1}\) of *M. whitei*); lanes 21 to 25 = Group 5 (positive control-50 mg kg\(^{-1}\) of sildenafil).

**Gel A**

<table>
<thead>
<tr>
<th>MW in kDa</th>
<th>MW</th>
<th>+C</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>200.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>116.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Gel B**

<table>
<thead>
<tr>
<th>MW in kDa</th>
<th>MW</th>
<th>+C</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>200.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>116.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

140.0 kDa
shows cGMP in the corpus cavernosal tissue of the rabbits at termination of treatment with *M. whitei* and sildenafil. There was no change in the tissue cGMP levels in the 100 mg kg$^{-1}$ *M. whitei*-treated animals compared to control. The cGMP level was insignificantly elevated by 480% at the 200 mg kg$^{-1}$ dose with a reduction to 230% of control at the 400 mg kg$^{-1}$ dose. Sildenafil, however, caused a 32% reduction in cGMP levels.

**NOS Activity of Tissue Pre-incubated with Organic Fractions of M. whitei**  

Figure 4 shows NOS activity in corpus cavernosal tissues pretreated with different organic fractions of *M. whitei in vitro* at 0.01 or 0.10 mg g$^{-1}$ tissue. The chloroform fraction caused a marked increase in NOS
activity with the 0.01 mg g\(^{-1}\) tissue fraction compared to control but this increase was less marked when the fraction concentration was increased to 0.10 mg g\(^{-1}\) tissue. There were insignificant increases (p>0.05) of NOS activity in tissues treated with 0.01 mg g\(^{-1}\) tissue of the petroleum ether fraction and the crude ethanolic extract. However, NOS activity was attenuated in tissues pre-incubated with 0.01 mg g\(^{-1}\) tissue of the petroleum ether fraction and the crude ethanolic extract.

**NO Levels of Tissue Pre-incubated with Organic Fractions of M. whitei**

The NO levels after incubation of corpus cavernosal tissue with various organic fractions of *M. whitei* at 0.01 or 0.10 mg g\(^{-1}\) tissue are shown in Figure 5. The chloroform fraction and the crude ethanolic extract at 0.01 mg g\(^{-1}\) tissue increased tissue NO levels by 25% and 18%, respectively while the effect of the same amount of the petroleum ether fraction was not different from control. Increase in amount of the fractions or crude extract to 0.10 mg g\(^{-1}\) tissue insignificantly reduced the NO levels to below control value.

**cGMP Levels of Tissue Pre-incubated with Organic Fractions of M. whitei**

Figure 6 shows the cGMP levels of rabbit corpus cavernosal tissue pre-incubated *in vitro* with 0.01 or 0.10 mg g\(^{-1}\) tissue of petroleum ether, chloroform fractions and the crude ethanolic extract of *M. whitei*. The chloroform fraction at both 0.01 and 0.1 mg g\(^{-1}\) tissue markedly increased the cGMP levels in the tissue above the control levels but to a slightly lesser extent with the 0.1 mg g\(^{-1}\) tissue fraction. The effect of the petroleum ether fraction or crude ethanolic extract on tissue cGMP levels at both 0.01 and 0.10 mg g\(^{-1}\) tissue were not markedly different from control levels.

**NOS and PDE Expressions**

The results of the protein expressions of NOS and PDE by Western blotting are shown in Figures 7 and 8, respectively. The gels showed bands of similar sizes for the SDS-PAGE proteins obtained from all the treatment groups. Bands of molecular weight 140 kDa and 102 kDa corresponded to the eNOS and PDE proteins, respectively. The colour intensity of the stained bands for either NOS or PDE appeared to be similar, but quantitative differences in expressions could not be determined between the various treatment groups.

**Discussion and Conclusions**

In recent times there has been the upsurge in herbal alcoholic beverage (bitters) consumption by both the young and old in Ghana. These bitters are advertised as having aphrodisiac properties, thus are highly patronized. The most popular herbal plant used in preparation of these bitters is *M. whitei*. The plant has been shown to increase levels of cGMP (Ofosuhene, 2005), which is known to cause vasodilation, thus allowing blood flow into the penile tissues resulting in erection. However, the activity and expression of the enzyme NOS which is responsible for synthesis of NO was not studied.

Varying doses (100-400 mg kg\(^{-1}\)) of crude ethanolic extract of *M. whitei* were administered to male rabbits for a period of 6 weeks. The activity of cavernosal tissue NOS and levels of NO and cGMP were measured and furthermore, NOS and PDE protein expressions in cavernosal tissue were investigated. Nitric oxide synthase (NOS) activity measurements may be taken as an indirect indicator of the potential NO synthesis (Shi et al., 2003). The results of this study indicated that NOS activity (Figure 1) was about 7% greater than that of the control at a dose of 200 mg kg\(^{-1}\) of the crude extract. However, increasing the dose to 400 mg kg\(^{-1}\) of the extract decreased the NOS activity by 2% as compared to the control. Thus the effect of the extract on NOS activity appears not to be dose-dependent. Sildenafil-treated animals also showed a slightly reduced NOS activity compared with the control. The extract, therefore, does not appear to induce NOS synthesis but possibly its activation. This is corroborated by the results obtained from the NOS expression (Figure 7); the colour intensity of NOS bands obtained appeared to be similar for all the animals used for the study at different dose levels. Differences in band intensity may have been detected if a densitometer was available for use. Also, Western blot did not show any reactivity for the NOS protein. However, the antibody cross-reacted with other enzymes (results not shown), suggesting that *M. whitei* did not cause induction of NOS synthesis.

Nitric oxide (NO) levels were also elevated (88%) in the animals administered with 200 mg kg\(^{-1}\) of extract, as compared with the control. NO levels were, however, elevated (14%) when the amount of extract administered was increased to 400 mg kg\(^{-1}\), indicating that increasing the dose of *M. whitei* did not cause an increase in NO. The NO levels at these two dosages of extract appeared to be closely tied to the expressed NOS activities at these dosages. A plausible explanation for this lack of dose-dependency in NOS activity and NO
levels with increasing dosage of extract may be that there is a threshold dose of 200 mg kg$^{-1}$ above or below which there is inhibition of NOS activity leading to the reduction in NO levels. The results therefore, showed that *M. whitei* has the ability to increase NO production at the right dosage. This may explain why in a pilot study in men between the ages of 25 and 50 years at the outpatients clinic of the CSRPM, it was observed that those who chewed below and above 3-4 chippings of *M. whitei* root per day showed reduced libido and loss of erectile function (unpublished results). There was also a decrease in the tissue NO levels of the sildenafil group, which is understandable since sildenafil is a PDE-5 inhibitor (Rotella, 2002) and would have caused cGMP to accumulate as a result of inhibition of cGMP hydrolysis. Accumulated cGMP may inhibit further synthesis of cGMP, probably through inhibition of soluble guanylate cyclase (sGC) or through indirect reduction in NO production. NO is known to activate sGC (Koesling, 1998; Ignarro et al., 1984).

In this study, a percentage increase (480%) of cGMP synthesis was observed in the 200 mg kg$^{-1}$ *M. whitei* animal treatment group as compared with the control. The synthesis of cGMP was, however, not dose-dependent because as the dose of *M. whitei* increased from 200 to 400 mg kg$^{-1}$, there was only a 110% corresponding increase in cGMP production, also suggesting once again that there is threshold dose beyond which *M. whitei* may have an inhibitory effect on cGMP production. Treatment with sildenafil, surprisingly, led to very low levels of cGMP in the cavernosal tissue, instead of high levels. It may be that the effect of sildenafil, at the 50 mg kg$^{-1}$ dose, on NO levels is more critical than its effect on PDE such that its reduction of NO levels may help in the development of *M. whitei* as a PDE-5 inhibitor. This could not be done above or below this dose (say at 100 or 400 mg kg$^{-1}$) it caused an opposite effect, suggesting a threshold dose below or above which *M. whitei* loses its aphrodisiac properties. The substances that activate/stimulate NOS, with the subsequent elevation of NO and cGMP levels, appeared to be contained in the chloroform fraction and to a lesser extent in the crude ethanolic extract since for the same weights of chloroform fraction and petroleum ether fraction, higher concentrations of active chemical constituents are likely to be found in the former than the latter.

Sildenafil, a known PDE inhibitor, showed opposite actions to that of *M. whitei* at these doses on sGC activity. This closely reflect the kinetic relationship between PDE and NOS with the consequent elevation of NO and cGMP levels. The two fractions showed similar corresponding effects on tissue NO and cGMP levels as for NOS. The petroleum ether fraction virtually had no effect on any of the parameters measured, namely NOS, NO and cGMP.

Phosphodiesterase protein expression in rabbit cavernosal tissue showed that the intensity of the enzyme protein bands (102 kDa) from the different dose groups were similar implying that PDE levels remained unchanged when extract was administered, and indicative of the fact that the *M. whitei* did not induce the synthesis of PDE. Measuring PDE activity by fluorimetric analysis would have given more information on the effect *M. whitei* has on PDE and whether *M. whitei* can be said to be a PDE-5 inhibitor. This could not be done because of technical problems. Also, Western blotting did not show any reactivity for PDE. However, the antibody used cross-reacted with other enzymes (results not shown) suggesting that there was induction of some enzyme synthesis other than PDE by *M. whitei*.

The effect of pre-incubation of rabbit cavernosal tissue with chloroform and petroleum ether fractions and the crude ethanolic extract, separately, at 0.01 or 0.10 mg g$^{-1}$ tissue on NOS activity and tissue levels of NO and cGMP indicated that the chloroform extracted constituents from the crude ethanolic extract were activators/stimulators of NOS in vitro, whereas those extracted by petroleum ether appeared to have no effect on NOS. The two fractions showed similar corresponding effects on tissue NO and cGMP levels as for NOS. The absence of NOS expression in vivo on pre-treatment of rabbits with varying doses of the crude extract lends support to the fact that the increase in NOS activity may be due to its stimulation/activation of NOS activity and not to the de novo synthesis of tissue NOS.

Furthermore, the results indicated that as the amount of organic fraction or crude extract was increased from 0.01 to 0.10 mg g$^{-1}$ tissue the NOS activity and levels of NO and cGMP were generally reduced to below control levels for the crude extract whereas there was reduction to near or markedly above control value for the chloroform fraction. The petroleum ether fraction virtually had no effect on any of the parameters measured. This closely reflect the in vivo situation in which as the dosage of crude extract increased from 100 to 400 mg kg$^{-1}$, the tissue NOS activity and levels of NO and cGMP were reduced to near or markedly below control levels. This further supports the initial suggestion that there is a threshold dose of *M. whitei*, which may produce certain tissue levels of chemical constituents, beyond or below which NOS activity, and NO and cGMP levels are reduced.

In conclusion, it may be said that *M. whitei* at a dose of 200 mg kg$^{-1}$ may help in the development of improved and sustained erection probably through activation/stimulation of NOS activity resulting in the elevation of levels of NO and cGMP, substances known to relax smooth muscle and mediate erectile function. It was observed that below or above this dose (say at 100 or 400 mg kg$^{-1}$) it caused an opposite effect, suggesting a threshold dose below or above which *M. whitei* loses its aphrodisiac properties. The substances that activate/stimulate NOS, with the subsequent elevation of NO and cGMP levels, appeared to be contained in the chloroform fraction and to a lesser extent in the crude ethanolic extract since for the same weights of chloroform fraction and crude ethanolic extract, higher concentrations of active chemical constituents are likely to be found in the former than the latter. Sildenafil, a known PDE inhibitor, showed opposite actions to that of *M. whitei*...
suggesting different modes of action. These findings appear to support the anecdotal evidence for the use of *M. whitei* in the management of erectile dysfunction.

**Acknowledgement**

The authors will like to express their sincerest gratitude to all the laboratory staff of the Phytochemistry and Animal Unit of CSRPM, Mampong, Akuapem, Ghana, staff of the Chemical Pathology and Parasitology Departments of the Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana.

**References**
