MORINDA LUCIDA REDUCES CONTRACTILITY OF ISOLATED UTERINE SMOOTH MUSCLE OF PREGNANT AND NON-PREGNANT MICE

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Introduction
Morinda lucida (L.) Benth. (Rubiacae) is used in herbal decoctions in the treatment of “fevers” Sittie et al (1999), Lemmich et al (1999), malaria Watt (1962); Beyer-Bradwijk (1962) and diabetes Ettarh and Emeka (2004) in Africa. It has been reported that the main compounds that have useful antimalarial activities, which can be extracted from the stem bark and root of M lucida Benth are anthraquinones, digitolutein, rubiadin 1-methyl ether and damnacanthal Sittie et al (1999); Lemmich et al (1999); Koumaglo et al (1992). It has been shown that anthraquinones also have antibacterial properties (Koumaglo et al, 1992).

Studies have shown that administration of chloroquine to mice infected with Plasmodium yoelli nigeriensis parasites led to the survival of all the mice, while those treated with medicinal plants such as M. lucida exhibited various degrees of chemosuppression, which did not however lead to their survival (Agomo et al, 1992). Also, M. lucida has been reported to induce relaxation of vascular smooth muscle via endothelium-dependent and –independent mechanisms, the former of which involves the nitric oxide-cGMP pathway (Ettarh and Emeka, 2004).

Whereas these herbal preparations are widely used in pregnancy, not many reports have taken into consideration the effect of M. lucida on the uterus, pregnant or non-pregnant. We therefore investigated the effect of M. lucida on isolated uterine smooth muscle from non-pregnant and pregnant mice and the...
possible role of nitric oxide in its mechanism of action.

Materials and Methods

Plant

*M. lucida* fresh leaves were collected in Lagos, Nigeria in March 2002 and authenticated by Prof. D. Olowokudejo of Department of Botany and Microbiology, University of Lagos, Akoka, Lagos, Nigeria.

Preparation of Extract

Leaves of ground *M. lucida* that have been previously dried, weighing 250g were Soxhlet-extracted with water. The extract was concentrated under vacuum at 40°C, and appropriate stock solutions prepared on the day of the experiment.

Animal Preparation

The experiments were performed on 10 pregnant and 10 non-pregnant mice, which were obtained from the Laboratory Animal House Department of the Lagos State University College of Medicine. They were provided with mice pellets and water ad libitum. The mice were pretreated with 0.1 mg/kg-body weight of stilboesterol given orally 24 hours before the experiment.

Preparation of Mice Uterine Strips

The mice were anaesthetized with urethane and sacrificed by cervical dislocation. The uterine horn was exposed by means of blunt dissection, freed of connective tissue and cut into strips of 2mm long. The strip was suspended on fine stainless steel stirrups in a 20 ml organ bath and connected to a force transducer (Grass Model FT03), which was coupled to a 4-channel Grass Model 7D Polygraph for the recording of the isometric tension. The transducer was calibrated with 1g weight to give a 2cm paper deflection. The bath contained De Jalon solution Gamaniel *et al* (1995) of the following composition (1 L): NaCl-9g, NaHCO$_3$-0.5g, Glucose-0.5g 10% KCl-4.2ml, 1M CaCl$_2$-0.27ml). The solution was bubbled with a 95% O$_2$-5% CO$_2$ gas mixture. The temperature and pH were maintained at 37°C and 7.4 ± 0.2 respectively.

Experimental Protocols

Each tissue preparation was allowed to equilibrate for 60-90 min under a resting tension of 9.5mN. All investigations were carried out in spontaneously contracting uterine strips. Graded concentrations of oxytocin (OXY) $10^{-5}$-$10^{-2}$ mol/L were added into the organ bath and their effects noted. In addition, responses to graded concentrations of the following were investigated in fresh uterine strips from different mice after the equilibration period: acetylcholine (ACh; $10^{-9}$-$10^{-5}$ mol/L) and *M. lucida* (0.015 – 1.500 mg/ml). The tissues were then incubated in 1.500 mg/ml of *M. lucida* for 5 minutes after which the cumulative dose responses to OXY and ACh were repeated. Finally, graded dose response to *M. lucida* was investigated in different sets of tissues from different animals after incubation in $10^{-4}$M N$^\omega$-nitro L-arginine methyl ester (L-NAME) for 15 minutes.

Statistics

For each cumulative dose response study, the EC$_{50}$ values were calculated. The data are presented as means ± SEM and Student’s unpaired t-test was used to test for statistical differences among groups. P value less than 0.05 was considered significant.

Figure 1: Typical tracing of the effect of administration of: (1)-M lucida alone, (2)-M lucida after incubation with L-NAME, (3)-Oxytocin after incubation with M lucida and (4)-Acetylcholine after incubation with M lucida, in uterine strips of (a)-non-pregnant and (b)-pregnant mice respectively.
Table 1: EC$_{50}$ and Maximum Tension Response (mN) due to administration of *M. lucida* before and after incubating in L-NAME in non-pregnant and pregnant mice

<table>
<thead>
<tr>
<th></th>
<th>Non-Pregnant (n=5)</th>
<th>Pregnant (n=7)</th>
<th>Non-Pregnant (n=5)</th>
<th>Pregnant (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EC$_{50}$ (mg/ml)</strong></td>
<td>0.052±0.02</td>
<td>0.105±0.04</td>
<td>0.001±0.00</td>
<td>0.245±0.10</td>
</tr>
<tr>
<td><strong>Maximum Response</strong></td>
<td>8.28±1.70</td>
<td>6.74±0.73</td>
<td>7.19±1.13</td>
<td>5.47±0.54</td>
</tr>
</tbody>
</table>

Results

Effect of *M. lucida* on Mice Uteri

All doses of *M. lucida* led to relaxation of spontaneous uterine contraction in non-pregnant and pregnant mice. However, there was no difference in the EC$_{50}$ and maximum response of uterine smooth muscle of non-pregnant and pregnant mice to *M. lucida* (Table 1). Also, the frequency of contraction to *M. lucida* in the uterine strips of the experimental mice was similar (Figure 1).

Table 2: Effect of *M. lucida* incubation on uterine spontaneous contractile response (%) to (a) oxytocin (OXY) and (b) acetylcholine (ACh) in non-pregnant and pregnant mice [frequency of spontaneous uterine contraction (contraction per min) in parenthesis]

(a)

<table>
<thead>
<tr>
<th>Dose (IU/ml)</th>
<th>OXY alone (Non-Pregnant (n=5))</th>
<th>OXY with <em>M. lucida</em></th>
<th>OXY alone (Pregnant (n=6))</th>
<th>OXY with <em>M. lucida</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>10$^{-5}$</td>
<td>23.68±12.93 (0.78±0.34)</td>
<td>0.00±0.00 (0.00±0.00)</td>
<td>30.15±14.20 (0.49±0.29)</td>
<td>0.00±0.00 (0.00±0.00)</td>
</tr>
<tr>
<td>10$^{-4}$</td>
<td>40.69±17.44 (0.92±0.59)</td>
<td>0.06±0.02 (0.16±0.09)</td>
<td>47.33±17.53 (0.49±0.32)</td>
<td>0.30±0.05 (0.48±0.25)</td>
</tr>
<tr>
<td>10$^{-3}$</td>
<td>67.12±19.36 (0.86±0.34)</td>
<td>0.12±0.09 (0.12±0.08)</td>
<td>77.49±11.28 (0.97±0.58)</td>
<td>1.06±0.51 (1.60±0.72)</td>
</tr>
<tr>
<td>10$^{-2}$</td>
<td>90.00±10.00 (0.98±0.55)</td>
<td>0.20±0.09 (0.08±0.05)</td>
<td>84.87±10.03 (1.09±0.50)</td>
<td>2.18±0.71 (2.32±0.69)</td>
</tr>
</tbody>
</table>

*P<0.05 vs. non-pregnant uterine treated with *M. lucida*

(b)

<table>
<thead>
<tr>
<th>Dose (M)</th>
<th>ACh alone (Non-Pregnant (n=5))</th>
<th>ACh with <em>M. lucida</em> (Non-Pregnant (n=5))</th>
<th>ACh alone (Pregnant (n=6))</th>
<th>ACh with <em>M. lucida</em> (Pregnant (n=6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>10$^{-9}$</td>
<td>5.49±2.64 (0.78±0.34)</td>
<td>0.00±0.00 (0.00±0.00)</td>
<td>-27.47±15.72 (0.78±0.34)</td>
<td>0.00±0.00 (0.00±0.00)</td>
</tr>
<tr>
<td>10$^{-8}$</td>
<td>21.46±7.30 (0.86±0.34)</td>
<td>0.00±0.00 (0.00±0.00)</td>
<td>-25.62±11.11 (0.86±0.34)</td>
<td>0.00±0.00 (0.00±0.00)</td>
</tr>
<tr>
<td>10$^{-7}$</td>
<td>22.07±9.14 (0.98±0.55)</td>
<td>0.00±0.00 (0.02±0.01)</td>
<td>1.08±0.49 (0.98±0.55)</td>
<td>0.00±0.00 (0.96±0.39)</td>
</tr>
<tr>
<td>10$^{-6}$</td>
<td>49.00±16.81 (0.98±0.55)</td>
<td>0.08±0.05 (0.02±0.01)</td>
<td>37.51±14.24 (0.98±0.55)</td>
<td>0.00±0.00 (0.96±0.39)</td>
</tr>
<tr>
<td>10$^{-5}$</td>
<td>100.00±4.00 (0.98±0.55)</td>
<td>0.08±0.05 (0.02±0.01)</td>
<td>100.00±4.00 (0.98±0.55)</td>
<td>1.12±0.46 (0.96±0.39)</td>
</tr>
</tbody>
</table>
Figure 2: Line graph showing responses to M. lucida in uterine strips of (a) non-pregnant mice (Non-Preg, n=5), (b) pregnant mice (Preg, n=7), (c) non-pregnant mice (Non-P) after incubating in L-NAME; and (d) pregnant mice (Preg, n=7) after incubating in L-NAME. Each point represents a mean of the observation ± S.E.M.
Effect of M. lucida on Responses of Uterine Strips to OXY and ACh

Incubation in M. lucida (1.50 mg/ml) led to total blockade of contractile response to OXY in non-pregnant uterine strips of non-pregnant mice while uterine strips of pregnant mice produced slight contractile responses to $10^{-4} - 10^{-2}$ IU/ml of OXY (Table IIa). There was a significantly higher (P<0.03) degree and frequency of contractile response to $10^{-2}$ IU/ml of OXY in pregnant uterine strips of pregnant mice compared with uterine strips of non-pregnant mice (Figure 1). However, incubation with M. lucida led to complete inhibition of contractile response to ACh in experimental mice (Figure 1).

Effect of L-NAME on Responses of Isolated Uterine Smooth Muscles to M. lucida in Experimental Mice

There was a shift to the right in the dose response curve to M. lucida in pregnant mice (Figure 1) after incubation of the uterine strips in L-NAME (10^{-5} M) for 15 minutes. Also, relaxant effect of M. lucida on the strips at low dose (0.015 mg/ml) in the presence of L-NAME was significantly (P<0.05) attenuated. However, there was no significant difference in EC_{50} and maximum response to M. lucida alone or M. lucida with L-NAME in both groups (Table I).

Discussion

The present experiments have shown that M. lucida has a relaxant effect on uterine smooth muscle of both non-pregnant and pregnant mice; inhibiting both the degree and frequency of contractile responses. There was no significant difference in the sensitivity of the uterine strips to M. lucida in non-pregnant or pregnant mice.

M. lucida at a concentration of 1.500 mg/ml completely blocked the cumulative responses of the uterine strips to OXY ($10^{-5}-10^{-2}$ IU/L) and ACh ($10^{-5}-10^{-3}$ mol/L) in both pregnant and non-pregnant mice. However, there was a slight contractile response in uterine smooth muscle of pregnant mice to high dose of OXY ($10^{-2}$ IU/L) and ACh ($10^{-5}$ M), which was significantly greater than those observed in uterine smooth muscles of non-pregnant mice. This might probably be due to increase in OXY receptors associated with pregnancy Silverthorn (2004), which may have been responsible for this observation in pregnant mice.

Anti-malarial drugs like chloroquine have been reported to possess inhibitory action on the uterus (Nwaigwe et al, 1997). In many cases, chloroquine has been abused with its local use as an abortifacient effect without regards for its associated toxic effect (Raddy and Sinna, 2000).


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