Nigerian Journal of Physiological Sciences 22 (1-2): 129- 134 ©Physiological Society of Nigeria, 2007

Available online/abstracted at http://www.biolineinternational.org.br/njps; www.ajol.info/journals.njps; www.cas.org

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

S. O. ELIAS, C. O. LADIPO *, B. P. ODUWOLE **, P. M. EMEKA ***, P. D. OJOBOR * and O. A. SOFOLA *

Departments of Physiology, Lagos State University College of Medicine, P.M.B. 21266, Ikeja, Lagos.
** Department of Physiology, College of Medicine, University of Lagos, Idi-Araba, Lagos*
***Department Parasitology, Lagos State University Teaching Hospital, Ikeja, Lagos*
**** Department of Pharmacology, College of Medicine, University of Lagos, Idi-Araba, Lagos*
E-mail: soelias2@yahoo.co.uk, Tel: 234-803-7149383

Summary: The present work investigated the effect of *Morinda lucida* (*M. lucida)* extract on isolated uterine smooth muscle of pregnant and non-pregnant mice. Pregnant and non-pregnant mice were pretreated with oral stilboesterol (0.1mg/kg body weight) and killed by cervical dislocation. Thin strips of the uterus were cut and mounted in a 20-ml organ bath containing De Jalon solution bubbled with 95%O_2-5% CO_2 gas mixture. The strips were connected to a force transducer coupled to a Grass 7D Polygraph for the recording of isometric tension. Effects of graded concentrations of oxytocin (OXY; 10^{-5}–10^{-2} mol/L), acetylcholine (ACh; 10^{-9}-10^{-5} mol/L) and *M. lucida* extract (0.015–1.5mg/ml) were recorded. Fresh uterine strips were then incubated with *M. lucida* extract for 5mins and cumulative response to OXY was repeated. Another set of fresh strips was incubated in L-NAME for 15mins and the cumulative responses to *M.lucida* extract were repeated. OXY resulted in increased contractile responses in both pregnant and non-pregnant uterine muscles. *M. lucida* resulted in relaxation of the uterine smooth muscle in both pregnant and non-pregnant mice at all doses. However, at 1.500mg/ml, *M. lucida* completely blocked spontaneous uterine contractions. Following incubation with L-NAME, *M. lucida* extract led to a slightly greater relaxation of the uterine strips. In conclusion, *M. lucida* reduced contractility of uterine smooth muscle in both pregnant and non-pregnant mice as well as blocking contractile responses to OXY and Ach in uterine smooth muscle of pregnant and non-pregnant mice. There was no significant alteration of *M. lucida* activity by L-NAME suggesting that the action of the compound on uterine muscle is not associated with impaired nitric oxide synthase.

Key Words: *Morinda lucida, uterine contraction, nitric oxide, mice*

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 yoeli 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^{o}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^{o}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^{ω}-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.

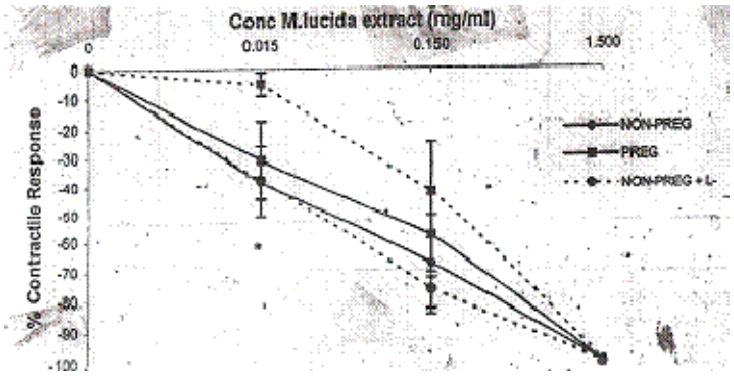

Figure 1: Typical tracing of the effect of administration of: (1)-M lucida alone, (2)-M lucida after incubation with L-NAME, (3)-Oxytocin after incubation with M lucida and (4)-Acetylcholine after incubation with M lucida, in uterine strips of (a)-non-pregnant and (b)-pregnant mice respectively.

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

	M. lucida alone		*M. lucida with L-NAME*	
	Non-Pregnant (n=5)	Pregnant (n=7)	Non-Pregnant (n=5)	Pregnant (n=7)
EC_{50} (mg/ml)	0.052±0.02	0.105±0.04	0.001±0.00	0.245±0.10
Maximum Response (mN)	8.28±1.70	6.74±0.73	7.19±1.13	5.47±0.54

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 I). 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)

	Non-Pregnant (n=5)		Pregnant (n=6)	
Dose (IU/ml)	OXY alone	OXY with *M lucida*	OXY alone	OXY with *M lucida*
10^{-5}	23.68±12.93 (0.78±0.34)	0.00±0.00 (0.00±0.00)	30.15±14.20 (0.49±0.29)	0.06±0.04 (0.08±0.05)
10^{-4}	40.69±17.44 0.92±0.59)	0.06±0.02 0.16±0.09)	47.33±17.53 (0.49±0.32)	0.30±0.05) (0.48±0.25)
10^{-3}	67.12±19.36 (0.86±0.34)	0.12±0.09 (0.12±0.08)	77.49±11.28 (0.97±0.58)	1.06±0.51 (1.60±0.72
10^{-2}	90.00±10.00 (0.98±0.55)	0.20±0.09 (0.08±0.05)	84.87±10.03 (1.09±0.50)	2.18±0.71 (2.32±0.69)

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

(b)

	Non-Pregnant (n=5)		Pregnant (n=6)	
Dose (M)	ACh alone	ACh with *M lucida*	ACh alone	ACh with *M lucida*
10^{-9}	5.49±2.64 (0.78±0.34)	0.00±0.00 (0.00±0.00)	-27.47±15.72 (0.78±0.34)	0.00±0.00 (0.00±0.00)
10^{-8}	21.46±7.30 0.92±0.59	0.00±0.00 (0.00±0.00)	-25.62±11.11 (0.92±0.59)	0.00±0.00 (0.00±0.00)
10^{-7}	22.07±9.14 (0.86±0.34)	0.00±0.00 (0.00±0.00)	1.08±0.49 (0.86±0.34)	0.00±0.00 (0.00±0.00)
10^{-6}	49.00±16.81 (0.98±0.55)	0.08±0.05 (0.02±0.01)	37.51±14.24 (0.98±0.55)	0.00±0.00 (0.00±0.00)
10^{-5}	100.00±0.00 (0.98±0.55)	0.08±0.05 (0.02±0.01)	100.00±0.00 (0.98±0.55)	1.12±0.46 (0.96±0.39)

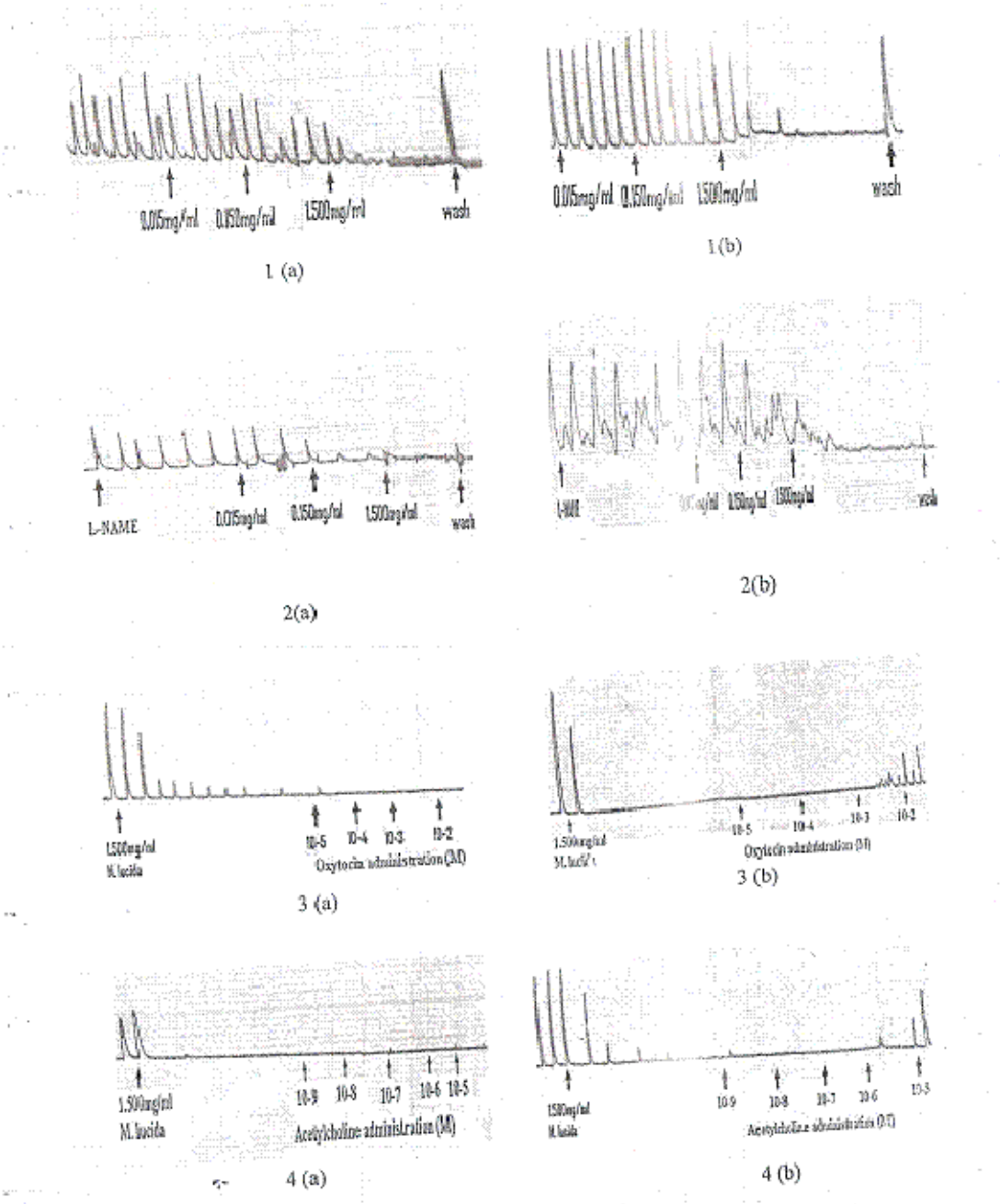

Figure 2: Line graph showing responses to M. lucida in uterine strips of (a) non-pregnant mice (Non-Preg, n=5), (b) pregnant mice (Preg, n=7), (c) non-pregnant mice (Non-P) after incubating in L-NAME; and (d) pregnant mice (Preg, n=7) after incubating in L-NAME. Each point represents a mean of the observation ± 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^{-4} 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^{-9}-10^{-5} 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). *M. lucida* is used for the treatment of malaria Sittie *et al (*1999), Lemmich *et al (*1999); Watt (1962), Beyer-Bradwijk (1962) and it is found to be cytotoxic, with LC_{50} values of 2.6 μg/ml (Ajaiyeoba *et al*, 2006). Earlier works have reported that *M. lucida* has a vasorelaxant effect on vascular smooth muscle, which is due to endothelium-dependent and –independent mechanisms, the former of which involves the nitric oxide-cGMP pathway (Ettarh and Emeka, 2004). Endothelial cells produce factors like nitric oxide (NO), prostacyclin and the endothelium-derived hyperpolarizing factor, which mediate the endothelium-dependent relaxation (Vapaatalo and Mervaala, 2001). The L-arginine-NO system has been demonstrated as possessing inhibitory effect on myometrial contractility (Kaya and Sarioglu, 1998). However, nitric oxide synthase inhibition appears to have no significant effect on *M. lucida* action on myometrial contractility in the present study.

In conclusion, this study demonstrates that *M. lucida* has a relaxant effect on isolated uterine smooth muscle of both non-pregnant and pregnant mice. This observation appears to be independent of bioavailability of nitric oxide in the uterus. However, further work investigating the effect of *M. lucida* on calcium mobilization from intracellular or extracellular stores will need to be explored.

References

Agomo, P. U., Idigo, J. C., Afolabi, B. M. (1992). 'Antimalarial' medicinal plants and their impact on cell populations in various organs of mice. *Afr. J. Med. Med. Sci.* 21: 3946.

Ajaiyeoba, E. O., Abiodun, O. O., Falade, M. O., Ogbole, N. O., Ashidi, J. S., Happi, C. T. and Akinboye, D. O. (2006). In vitro cytotoxicity studies of 20 plants used in Nigerian antimalarial ethnomedicine. *Phytomedicine.* 13:295-298.

Ettarh, R. R., Emeka, P. (2004). Morinda lucida extract induces endothelium-dependent and –independent relaxation of rat aorta. Fitoterapia 75: 332-336.

Gamaniel, K., Ojo, T., Wambebe, C., Awodogan. A., Samson, A., Shittu, A., Vangtau, H., Azuine, M. A. and Akah, P. A. (1995). New cytotoxic quinazoline derivatives that relax the isolated rat uterus and the rabbit ileum. *West Afr. J. Pharmacol. Drug Res.* 11: 80-86.

Kaya, T., Cetin, A. and Sarioglu, Y. (1998). Changes in the nitric oxide system of rat myometrium during midgestation and delivery at term. *Pharmacol Res*. 37: 403-408.

Koumaglo, K., Gbeassor, M., Nikabu, O., de Souza, C., Werner, W. (1992). Effect of three compounds extracted from morinda lucida on Plasmodium falciparum. *Planta. Med.* 58: 533-4.

Nwaigwe, C. I., Adegunloye, B. J. & Sofola, O. A. (1997). Effect of chloroquine on the contractility of the smooth muscle of the rat uterus, trachea and urinary bladder. *J. Basic. Clin. Physiol. Pharmacol.* 8: 279-285

Raddy, V. G. and Sinna. S. (2000). Chloroquine poisoning: report of two cases. *Acta Anaesthesiol Scand* 44: 1017-1020.

Silverthorn, D. U. (2004). Pregnancy and parturition in Human Physiology: An Integrated Approach (third edition) pp 821-828, Pearson Education, Inc.

Sittie, A. A., Lemmich, E. and Oslen, C. E. (1999). Structure-activity studies: in-vitro antileishmanial and antimalaria activity of anthraquinous from *Morinda lucida*. *Planta. Med.* 65: 259-61.

Vapaatalo, H. and Mervaala, E. (2001). Clinical important factors influencing endothelium function. *Med. Sci. Monit.* 7: 1075-1085.

Watt, J. M., Beyer-Bradwijk, M. G. (1962). The medicinal and poisonous plants of Southern and Eastern Africa. London: E.S. Livingstone Ltd.

Received: 18/9/2006
Accepted: 18/12/2007