

Short Communication

Relaxant effect of *Jatropha gossypifolia* L. on uterine smooth muscle

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Abstract

Jatropha gossypifolia L. (Euphorbiaceae) is popularly known in Brazil as *pião roxo* and extensively used for hypotensive, spasmolytic and tocolytic purposes. In the present work, ethanolic extract and fractions from aerial parts of *J. gossypifolia* L. were assayed for their effects on calcium-evoked uterine smooth muscle contraction.

Rat uterus strips were incubated with ethanolic extract (0.1, 0.5 and 1.0 mg/ml), chloroformic fraction (0.25; 0.5 mg/ml) or aqueous fraction (0.25; 0.5 mg/ml). Ethanolic extract promoted a rightward displacement of calcium cumulative curves, as well as, reduced the maximal contractions in 27.3% and 80.3% (0.5 and 1.0 mg/ml, respectively). Chloroformic fraction inhibited the responsiveness of uterine muscle to calcium causing the maximal contractile response to be reduced in 27.4 % and 45.1 %, respectively. On the other hand, these parameters were only slightly reduced in the presence of aqueous fraction.

Taken together, our results suggest that ethanolic extract and fractions from *J. gossypifolia* reduce calcium-evoked contractile response of the uterine smooth muscle, corroborating its ethnopharmacological application as tocolytic remedy.

Keywords: *Jatropha gossypifolia* L., uterine smooth muscle, calcium influx and relaxation

Introduction

Herbal medicines have become the major source of bioactive agents and emerged as potential therapeutic tools. Use of *Jatropha gossypifolia* L. (Euphorbiaceae) as a traditional herbal medicine for treatment of diarrhea, hypertension and miscarriage is a common practice in Brazil and other tropical countries, where it is known as *pião-roxo* or bellyache bush [1-3].

In previous reports, we showed that ethanolic extract prepared from *J. gossypifolia* aerial parts (leaves and stems) has vasorelaxant [4] and antispasmodic [5] properties, which were mainly mediated through the impairment of calcium influx into vascular and intestinal smooth muscle cells. Such properties are also found in other *Jatropha* species, particularly *J. podagrica* [6, 7] and *J. elliptica* [8]. Regarding the uterine muscle, terpenoids isolated from *J. elliptica* [9] and *J. curcas* [10] have been shown to inhibit acetylcholine- and KCl-induced uterus contraction, whereas no data is available for *J. gossypifolia*.

Thus, in the present work we show that ethanolic extract, as well as, chloroformic and aqueous fractions from *J. gossypifolia* aerial parts are also able to impair calcium-evoked contraction of uterine muscle, in a way analogous to that observed in other smooth muscle territories.

Materials and Methods

Plant material

Jatropha gossypifolia L. aerial parts (stems and leaves) were collected from the urban area of São Luís (MA), Brazil, in August 2002 and identified by Dr. Rêgo T. from Ático Seabra Herbarium of the School of Pharmacy of Federal University of Maranhão, where a voucher specimen was deposited under #1006.

Extraction and partition

Dried and powdered stems and leaves (200 g) were extracted with 1.8 L of EtOH/H₂O (95:5) (3 x 600 ml). The combined ethanolic extracts (EE) were filtered and concentrated under vacuum giving a honey-like viscous residue (yield 7,04%). EE was submitted to a simple partition process using water and chloroform in a 2:1 volumetric ratio, from which was obtained the aqueous (AF) and chloroformic (CF) fractions, whose yields were 71 and 29%, respectively. For pharmacological tests, the EE and CF were dissolved in 0.9% NaCl plus 0.01% Tween 80 before use.

Animal and tissue preparation

Female Wistar rats weighing 180 – 200 g, were obtained from Animal House of the Federal University of Maranhão. They were

brought into oestrus by injecting oestradiol benzoate (1.0 mg/kg, sc, Sigma, St. Louis, MO, USA) 24 h before the experiments. Animals were decapitated with a small guillotine and uterine horns carefully removed out. After dissection of mesenteric fat, longitudinal uterine smooth muscle strips, with 15 mm in length, were suspended in a 5 ml organ bath chamber containing physiological salt solution (PSS) in the following chemical composition in mM: NaCl, 154; KCl, 5.6; NaHCO₃, 5.9; CaCl₂, 0.5 and glucose 5.5. Preparations were bubbled with air and kept at 30°C. The strips were initially equilibrated for 1 h under an optimal resting tension of 1.0g and PSS renewal every 15 min. Thereafter, preparations were exposed to a high-potassium calcium-free depolarizing solution, which was prepared by replacing 80 mM NaCl with equimolar KCl in the PSS, as previously described [15]. The amplitude of the contraction was considered the maximum responsiveness and taken as 100% value (Emax). Cumulative concentration – response curves for calcium (0.1 – 0.1 mM) were obtained at 60 min intervals either in absence or presence of EE (0.1, 0.5 and 1.0 mg/ml), CF (0.25 and 0.5 mg/ml) or AF (0.25 and 0.5 mg/ml), which were previously incubated for 5 min. The tension changes of the uterine smooth muscle preparations were measured with an isometric force-displacement transducer (F-60, Narco Bio-Systems, Houston, TX, USA) and recorded in a physiograph (Narcotrace 40, Narco Bio-Systems, Houston, TX, USA).

Statistical analysis

Mean EC₅₀ and its negative logarithm (pD₂) as well as the proportional maximal response (%Emax) for calcium were calculated for each of 5 – 6 preparations. All the data were expressed as mean ± SEM and analyzed by Student's t-test or ANOVA. Differences were considered significant at $p < 0.05$.

Results and discussion

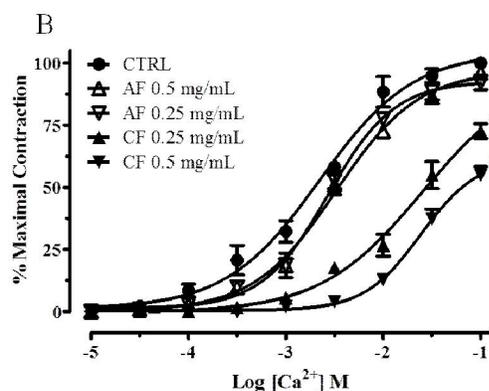
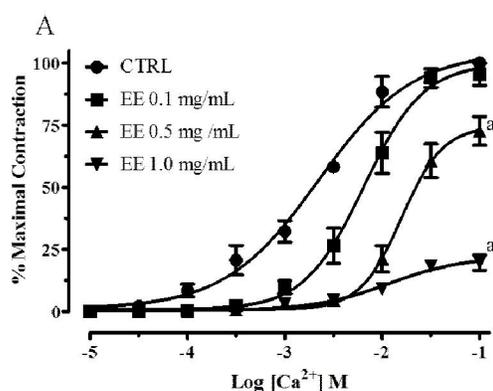


Figure 1: Effect of ethanolic extract (EE), chloroformic (CF) and aqueous (AF) fractions from aerial parts from *J. gossypifolia* on isolated rat uterine muscle contractile – response to CaCl₂ (Ca²⁺ in depolarizing PSS). Symbols and vertical lines indicate means ± SEM, respectively, $n=5-6$. ^a $p < 0.001$ vs control

In order to further characterize this effect, EE was submitted to chloroform/water partition, as described under material and

To directly investigate our hypothesis, EE was incubated with uterine strips suspended in a 5ml organ bath chamber, for 5 minutes, before the addition of cumulative concentrations of Ca²⁺ (0.1 μM – 0.1 M). EE promoted a rightward displacement of smooth muscle responsiveness to Ca²⁺, represented as pD₂ values, at all concentrations (Table 01). Emax was not inhibited by EE at 0.1 mg/mL (95.5 ± 4.5%). However, when the EE was present at the concentrations of 0.5 and 1.0 mg/mL, Emax was decreased to 72.7 ± 5.8% and 19.7 ± 3.0%, respectively (Figure 1A). Noteworthy, uterine responsiveness was completely recovered after washing of the preparations for maximum of 30 min. These data corroborate our previous findings where EE was shown to promote both inhibition of calcium-induced contraction and relaxation of previously calcium- or norepinephrine-contracted rat aortic rings [4].

Table 1: Parameters of concentration-response curves for the effects of ethanolic extract (EE), aqueous (AF) and chloroformic (CF) fractions from *J. gossypifolia* L. aerial parts on uterine smooth muscle contraction induced by Ca²⁺.

	E_{max} (%)	pD_2 (M)
Ca ²⁺ (control curve)	100	-2.65 ± 0.07
Ca ²⁺ + EE 0.1 mg/ml	95.5 ± 4.5	-2.19 ± 0.03 ^b
Ca ²⁺ + EE 0.5 mg/ml	72.7 ± 5.8 ^b	-1.80 ± 0.02 ^b
Ca ²⁺ + EE 1.0 mg/ml	19.7 ± 3.0 ^b	-1.93 ± 0.12 ^b
Ca ²⁺ + AF 0.25 mg/ml	96.1 ± 1.2 ^a	-2.48 ± 0.05
Ca ²⁺ + AF 0.5 mg/ml	92.2 ± 2.9 ^a	-2.57 ± 0.03
Ca ²⁺ + CF 0.25 mg/ml	72.6 ± 2.9 ^b	-1.60 ± 0.18 ^b
Ca ²⁺ + CF 0.5 mg/ml	54.9 ± 3.4 ^b	-1.61 ± 0.03 ^b

Values are mean ± SEM; $n = 5 - 6$. ^a $p < 0.01$ vs control, ^b $p < 0.001$ vs control.

methods, rendering CF and AF. Both fractions were assayed in the same system described above for EE. As shown in Figure 1B, CF also impaired the responsiveness of uterine muscle to calcium. At

lower concentrations, CF (0.25 and 0.5 mg/mL) promoted similar displacement of pD₂, from -2.65 ± 0.07 M to -1.60 ± 0.18 and -1.61 ± 0.03 M, respectively, as compared to EE (Table 1). In a way analogous to that observed on rat jejunum [5], CF decreased E_{max} to 72.6 ± 2.9 and $54.9 \pm 3.4\%$, respectively (Figure 1B). On the other hand, AF had no effect on calcium – evoked responsiveness, as assessed through pD₂ determination, besides it had slightly decreased the E_{max} (0.25 mg/ml, $96.1 \pm 1.2\%$; 0.5 mg/ml, $92.2 \pm 2.9\%$). The genus *Jatropha* comprises around 200 species from which a number of bioactive natural products, mainly diterpenes and triterpenes, have been reported [11]. Particularly, jatrophone and its derivatives 2 α - and 2 β -hydroxyjatrophone have been shown to promote smooth muscle relaxation through distinct mechanisms, such as impairment of Ach-receptor downstream pathways [9], K⁺-channels activation associated with Ca²⁺-channels blockage [8] and inhibition of protein kinase C-mediated pathways, as well [12]. Noteworthy, those terpenoids have been involved in a plethora of other biological activities with special emphasis on anticancer and antiproliferative properties [13, 14]. Nevertheless, in spite of the lower number of compounds isolated from *J. gossypifolia*, our data are consistent with the fact that EE and CF also harbor secondary metabolites which are responsible for their relaxative effect on uterine smooth muscle. Furthermore, our data still reinforce our previous documentation that the mechanisms by which they promote this effect possibly involve the impairment of Ca²⁺ influx through membrane voltage-operated channels [4, 5].

References

- [1]. Corrêa MP, de Azeredo Penna L. Dicionário das plantas úteis do Brasil e das exóticas cultivadas. Rio de Janeiro: Ministério da Agricultura - Instituto Brasileiro de Desenvolvimento Florestal; 1984, v.5, p.485.
- [2]. Lorenzi H, Matos FJA. Plantas medicinais no Brasil: nativas e exóticas. São Paulo: Instituto Plantarum de Estudos da Flora; 2002. p.213.
- [3]. Duke JA. Handbook of Medicinal Herbs. Boca Raton: CRC Press, 2002. p.406.
- [4]. Abreu IC, Marinho AS, Paes AM, Freire SM, Olea RS, Borges MO, Borges AC. Hypotensive and vasorelaxant effects of ethanolic extract from *Jatropha gossypifolia* L. in rats. *Fitoterapia* 2003; 74 (7-8): 650-657.
- [5]. Silva, SN, Abreu IC, Freire SMF, Cartágenes MSS, Ribeiro RM, Castro AS, Borges ACR, Borges MOR. Antispasmodic effect of *Jatropha gossypifolia* is mediated through dual blockade of muscarinic receptors and Ca²⁺ channels. *Rev Bras Farmacognosia* 2011, 21: 715-720.
- [6]. Ojewole JA, Odebiyi OO. Mechanism of the hypotensive effect of tetramethylpyrazine, an amide alkaloid from the stem of *Jatropha podagrica*. *Planta Med* 1981; 41(3): 281-287.
- [7]. Ojewole JA, Odebiyi OO. Neuromuscular and cardiovascular actions of tetramethylpyrazine from the stem of *Jatropha podagrica*. *Planta Med* 1980; 38(4): 332-338.
- [8]. Duarte DFP, Sant'Ana AEG, Calixto JB. Analysis of the vasorelaxant action of jatrophone in the isolated aorta of the rat: influence of potassium channel blockers. *Eur J Pharmacol* 1992; 215(1): 75-81.
- [9]. Calixto JB, Sant'ana, AEG. Evidence for the mechanism of the inhibitory action of jatrophone in the isolated rat uterine muscle. *Gen Pharmac* 1990; 21(1): 117-122.
- [10]. Falodun A, Nworgu ZM, Osayemwenre E. Smooth muscle relaxant evaluation of *Jatropha Curcas* Linn (Euphorbiaceae) and isolation of triterpenes. *Niger J Physiol Sci* 2011; 26(2): 133-137.
- [11]. Zhang XP, Zhang ML, Su XH, Huo CH, Gu YC, Shi QW. Chemical constituents of the plants from genus *Jatropha*. *Chem Biodivers* 2009; 6(12): 2166-2183.
- [12]. Silva AM, Brum RL, Calixto JB. The relaxant action of jatrophone in rat portal vein. A comparison with

Conclusions

In summary, our data showed for the first time that EE and CF from *J. gossypifolia* aerial parts caused a concentration-dependent and non-competitive inhibitory effect on Ca²⁺-induced contraction of isolated rat uterine muscle preparations. Moreover, contribute to the validation of this plant as a spasmolytic and tocolytic herbal medicine. Notwithstanding, additional studies are still needed, particularly focusing on the identification of the bioactive compounds.

Authors' contribution

AMAP has participated in the hypothesis' conception, carried out pharmacological protocols, analyzed results and drafted the manuscript. ALC has analyzed results and co-drafted the manuscript. SMFF has participated in the hypothesis conception and manuscript review. MORB has participated in the hypothesis' conception, results discussion and gave the final manuscript approval. All authors read and approved the final manuscript.

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- protein kinase C inhibitors. *Life Sci* 1995; 57(9): 863-871.
- [13]. Falodun A, Kragl U, Touem SM, Villinger A, Fahrenwaldt T, Langer P. A novel anticancer diterpenoid from *Jatropha gossypifolia*. *Nat Prod Commun* 2012; 7(2): 151-152.
- [14]. Theoduloz C, Rodriguez JA, Pertino M, Schmeda-Hirschmann G. Antiproliferative activity of the diterpenes jatrophone and jatropholone and their derivatives. *Planta Med* 2009; 75(14): 1520-1522.
- [15]. Calixto JB, Loch S. Ketamine-inhibition of calcium-induced contractions in depolarized rat uterus: a comparison with other calcium antagonists. *Br J Pharmacol* 1985; 85(1): 189-195..

