Aqueous leaf extract of *Averrhoa carambola* L. (Oxalidaceae) reduces both the inotropic effect of BAY K 8644 on the guinea pig atrium and the calcium current on GH₃ cells

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ABSTRACT: It was previously showed that aqueous leaf extract (AqEx) of *Averrhoa carambola* depresses the guinea pig atrial inotropism. Therefore, experiments were carried out on guinea pig left atrium and on pituitary GH₃ cells in order to evaluate the effect of AqEx on the cellular calcium influx. The atrium was mounted in an organ chamber (5 mL, Tyrode, 27 ± 0.1 °C, 95 % O₂, 5 % CO₂), stretched to 10 mN, and paced at 2 Hz (0.5 ms, 400 V) and GH₃ cells were submitted to a whole cell voltage clamp configuration. In the atrium, the AqEx (1500 µg/mL) shifted to the right the concentration-effect curve of the positive inotropic effect produced by (+) BAY K 8644, an L-type calcium channel agonist. The AqEx increased EC₅₀ (concentration required to promote 50% of the maximum effect) of the inotropic effect of BAY K 8644 from 7.8 ± 0.38 to 115.1 ± 0.44 nM (N = 3; p < 0.05). In GH₃ cells assayed with 500 µg/mL of AqEx, the L-type calcium inward current declined 30 % (from 282 to 190 pA) at a constant voltage of 0 mV. These data suggest that, at least in part, the negative inotropic effect of AqEx on the guinea pig atrium is due to a reduction of the L-type calcium current.

Keywords: *Averrhoa carambola*, aqueous extract, L-type calcium current, guinea pig atrium, GH3 cells

INTRODUCTION

*Averrhoa carambola* L. (Oxalidaceae) is a tree originated from Asia but widely found in tropical countries. Brazilian folk medicine uses its fruit, juice or even hot tea prepared from leaves for treating headache,
vomiting, cough, and insomnia (Agra et al., 2008; Oliveira et al., 1989; Pio-Correa, 1931). Nevertheless, chronic nephropathic patients can develop severe and acute intoxication after eating the star fruit or drinking its juice. The main signals and symptoms were: intractable hiccups, sudden onset of limb numbness, muscle weakness, consciousness disturbance, and seizure (Muir & Lam, 1980; Martin et al., 1993; Chang et al., 2000; Neto et al., 2003; Tse et al., 2003; Chang & Yeh, 2004; Tsai et al., 2005). These clinical manifestations have only disappeared after administrating propofol or when the patients were submitted to hemodialysis (Tsai et al., 2005).

Vasconcelos et al. (2006) showed that it decreased the conduction velocity of the atrial impulse and increased the elapsed time to conduct the myocardial impulse from the right atrium to the His bundle. The authors reported that the AqEx induced different degrees of atrioventricular block, prolonged the QT interval, and increased the QRS complex duration.

The present study aimed to investigate if the AqEx of *A. carambola* leaves exerts a negative inotropic and chronotropic effects on the guinea pig atria. Furthermore, Vasconcelos et al. (2006) showed that it decreased the conduction velocity of the atrial impulse and increased the elapsed time to conduct the myocardial impulse from the right atrium to the His bundle. The authors reported that the AqEx induced different degrees of atrioventricular block, prolonged the QT interval, and increased the QRS complex duration.

The present study aimed to investigate if the AqEx of *A. carambola* leaf can change the cellular calcium influx offering thus a mechanism to explain the mechanical and electrophysiological effects promoted by the leaf extract of *A. carambola* on the mammalian myocardium.

**MATERIAL AND METHODS**

**Preparation of the aqueous leaf extract of Averrhoa carambola**

*A. carambola* L. leaves were collected near to the campus of the Universidade Federal de Sergipe (10° 53’ 57.55”S, 37° 07’ 14.30”W, altitude 10 m) from a healthy and agrotoxic-free plant. Botanical identification was performed by the specialized staff of the Herbarium of the Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil (Voucher #24,720). The water extract was prepared in a Soxhlet apparatus by submitting dry *A. carambola* leaves to the following sequence of solvents: hexane, chloroform, acetone, ethanol, and methanol. After this procedure the leaves were extracted with deionized water. This aqueous extract was concentrated in a rotative evaporator (Tecnal TE 210, Piracicaba, São Paulo, Brazil) and then stored at a room temperature (27 ± 3 ºC). No care was taken to protect it from the environmental light. To adjust the extract concentration in the organ bath, its water-insoluble residue was determined.

**Animals**

The experiments were carried out on the guinea pig (*Cavia porcellus*) atria obtained from adult animals (300 to 500 g) of both genders. The animals, supplied by the Bioteiy of the Universidade Federal de Sergipe, have had free access to food and water and were kept at room temperature (27 ± 3 ºC) with 12 h of light-dark cycles. Animal handling followed the rules of the Colégio Brasileiro de Experimentação Animal (COBEA).

**Drugs**

The following drugs and salts were used: reserpine, 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]pyridine-3-carboxylic acid methyl ester (± BAY K 8644), tetrodotoxin (TTX), tetrathyammonium chloride (TEA), ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), (N-[2-Hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) (HEPES), HEPES-buffered Dulbecco modified Eagle's medium (DMEM-HEPES), NaCl, KCl, MgCl₂, H₂O, BaCl₂, Glucose, CsCl, CsOH, CaCl₂, H₂O, NaH₂PO₄·H₂O, NaHCO₃, fetal bovine serum, penicillin, and streptomycin. Drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA) except reserpine that was gently supplied by Laboratório Gross S.A. (Rio de Janeiro, Brazil), and fetal bovine serum that were both purchased from Cultilab (Campinas, SP, Brazil).

**Solutions**

Tyrode solution was prepared according to Dorigo et al (1990), in mM: NaCl 120, KCl 2.7, MgCl₂, H₂O 0.9, NaHCO₃ 11.9, CaCl₂·2H₂O 1.37, Glucose 5.5, NaH₂PO₄·H₂O 0.4. External solution, in mM: NaCl 126; BaCl₂ 10; CsCl 5.4; HEPES 10; Glucose 10; TTX 3 x 10⁻⁴ (pH adjusted to 7.4 with CsOH). Internal solution, in mM: MgCl₂ 4; CsCl 130; EGTA 10; HEPES 10; TEA 10 (pH adjusted to 7.2 with CsOH).

**Experimental assemblage to evaluate the effect of the aqueous leaf extract of Averrhoa carambola on the guinea pig atrium contractility**

Reserpine-treated animals (5 mg/kg, i.p., 24 h before the experiment) were sacrificed by a blow applied to the skull base. The left atrium was rapidly removed and mounted in an organ bath (5 mL, Tyrode solution, 27 ± 0.1 ºC, 95 % CO₂ + 5 % O₂). Each preparation was stretched to a resting tension of 10 mN and remained under electrical stimulation (2 Hz, 400 V, 0.5 ms, DIGITIMER 3072, DIGITIMER D4030, Welwyn, Garden City, Hertfordshire, England). The atrial force was recorded isometrically (Transducer HP FTA 10-1 Sunborn, HP 8805B, Chicago, IL, USA) in a thermal paper polygraph (HP8805B, HP7754A, HP7754B, Chicago, IL, USA) and stored in a computer (DATAQ DI400, DI 205, WINDAQ PRO Acquisition, WINDAQ EX Calculate, Akron, OH, USA).
Protocol to evaluate the effect of the aqueous leaf extract of *Averrhoa carambola* on the contractile action of BAY K 8644

Effect-concentration curves were obtained by adding cumulatively BAY K 8644 - an agonist of the L-type calcium channels - to the organ bath. The contractile force amplitude was determined at each concentration (5, 10, 30, 50, 100, 300 and 500 nM) and plotted against the logarithm of the related concentration. This procedure was carried out before and after adding AqEx (1500 μg/mL) to the bath and EC$_{50}$ (concentration to produce 50 % of the maximal effect) was determined. For BAY K 8644 in the absence and in the presence of AqEx (500 μg/mL) on the membrane calcium current was measured by recording both the time-course and current-voltage sequences during superfusion with AqEx. No correction was made for the liquid junction potential.

Statistical analysis

The univariate analysis of variance ANOVA (General Linear Model) followed by the Tukey’s multiple comparisons test was used to determine differences between means. The results were considered statistically different when $p < 0.05$. Data in this paper are shown as mean ± SD.

RESULTS

Figure 1 (upper panel) shows the inotropic effect of (±) BAY K 8644 (5-100 nM) added cumulatively to the organ bath. Contraction force start increasing when BAY K 8644 concentration was 5 nM. After adding AqEx (1500 μg/mL) to the bath, the atrial force declined progressively until reach a near zero amplitude. In such situation, BAY K 8644 only produced a significant increase in force amplitude when its concentration was greater than 300 nM (lower panel).

Figure 2 shows concentration-response curves for BAY K 8644 in the absence and in the presence of AqEx (1500 μg/mL). The extract shifted to the right the Hill-Langmuir curve and reduced the maximum efficacy from 100 to 10 %. The EC$_{50}$ of BAY K 8644 increased from 7.8 ± 0.38 to 115.1 ± 0.44 nM in the presence of AqEx (N = 3 atra; $p < 0.0001$).

The effect of AqEx on the membrane calcium inward current was assayed on GH$_{1}$ cells submitted to a patch clamp in a whole-cell configuration. Suppression of inward Na$^{+}$ and outward K$^{+}$ was achieved, respectively, by adding TTX to the bathing medium and by replacing intracellular K$^{+}$ by Cs$^{+}$. Moreover, it was used TEA in the pipette solution to block potassium channels. Figure 3
depicts the effect produced by AqEx (500 μg/mL) on the L-type calcium inward current in GH3 cells. The extract reduced the calcium current from 282 to 190 pA.

The effect of AqEx on the current-voltage plot concerned to the calcium inward current through the L-type calcium channels can be seen in Figure 4. The AqEx (500 μg/mL) reduced in 32 % the calcium current but did not change either the voltage related to the maximum current (-10 mV) or the inactivation kinetics of the L-type calcium channel.

**DISCUSSION**

Aqueous extract of *A. carambola* leaves (AqEx) reversibly reduced the contractility of guinea pig atrium in a concentration-dependent manner. When acting on the natural right atrial pacemaker, this extract promoted a significant bradycardia (Vasconcelos et al., 2005). In guinea pig isolated heart, it was observed that AqEx induced different degrees of atroventricular block (Vasconcelos et al., 2006). It is well known that drugs that reduce the membrane calcium inward current, besides exerting a negative inotropic effect on the myocardium, can also depress the atrial pacemaker activity and reduce the conduction velocity through the atroventricular node. To shed light on this theme the present work sought to evaluate the effect produced by extracts of *A. carambola* leaf on the cellular calcium inward current. The investigation was performed on both guinea pig atrial tissue and GH3 neuroendocrine cells.

To evaluate the effect of AqEx on the L-type calcium current several experiments were performed by submitting the guinea pig left atrium to different concentrations of BAY K 8644. This agonist acts as a positive inotropic modulator by increasing the calcium channel opening time (Näbauer et al., 1988). Such effect is not mediated by the intracellular AMPc.
concentration (Böhm et al., 1985). BAY K 8644 increases the myocardial contractile force due to its positive action on the L-type calcium current (Fassina et al., 1991). These findings allowed us to investigate if the AqEx could exert its inotropic action by changing the calcium inward current in the mammalian myocardium. To do so, atrial concentration-response curves were obtained by progressively increasing the BAY K 8644 concentration in the organ bath. This protocol was firstly executed before and then after adding the AqEx to the bath. The results showed that AqEx shifted that curve to the right leading to an increase of the EC_{50} from 7.8 ± 0.38 to 115.1 ± 0.44 nM. Furthermore, it also reduced in 90 % the maximum inotropic effect of BAY K 8644. These data suggest the AqEx is able to reduce the calcium inward current explaining thus, at least in part, the negative inotropic effect of this leaf extract on the guinea pig atrial myocardium.

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**REFERENCES**


