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Communication

[*Comunicação*]

Extract from *Arrabidaea chica* (*Fridericia chica*) leaves show preventive action for the mitigation of doxorubicin-induced cardiotoxicity

[*Extrato das folhas de Arrabidaea chica (Fridericia chica) mostrou potencial ação no combate à cardiotoxicidade induzida pela doxorrubicina*]

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Doxorubicin (dox) is used for treatment of several types of cancer in humans and dogs; however, it shows important off-target cardiotoxic effects, which appear even months after completing the treatment in both species (Almeida *et al*., 2015; Hallman *et al*., 2019). Thus, it is imperative to find a treatment that reduces dox cardiotoxicity maintaining its anticancer efficacy. Considering the oxidative stress induced by dox, an antioxidant agent is an option. Green tea from *Camellia sinensis* (L.) Kuntze has been studied and demonstrated cardioprotective potential against dox toxicity (Aboulwafa *et al*., 2019; Saeed *et al*., 2015). In this scenario, a plant extract which has high flavonoid content, such as *Arrabidaea chica* (Bonpl.) Verl. [*Fridericia chica* (Bonpl.) L.G.Lohman] of the Bignoniaceae family, should be considered to prevent dox-induced oxidative stress in the myocardium. Due to its high flavonoid content (Barbosa *et al*., 2008), we hypothesized that *A. chica* has a previously uninvestigated cardioprotective role. Thus, the present study aimed to evaluate the cardioprotective effect of *A. chica*, compared with *C. sinensis* (a known positive control), and the maintenance of dox anticancer activity in the presence of both extracts.

A. chica dry leaves (20g) were macerated with 100ml of a mixture ethanol:water (7:3) and took to ultrasonic bath at room temperature for 18h. The *A. chica* extract (AC) obtained was submitted to partition with solvents (Urbonaviciūte *et al*., 2006) to obtain a rich flavonoid content extract (2g). Voucher specimens of *A. chica* were deposited at the herbarium of the Universidade Estadual de Campinas (Campinas, SP, Brazil) under the code UEC 145.956. Mass spectrometry analyses of AC and standards were carried out on a Shimadzu LCMS-IT-TOF system. Among several peaks, the analysis showed one which was identified at 287.079 $[M+h]^+$m/z corresponding to kaempferol. A commercially *C. sinensis* extract (CS) was used (Sunphenon DCF®, Taiyo Kagaku Co.), containing total polyphenols (>80%), catechins (>80%), epigallocatechins (>45%), and caffeine (<1%) detected by high performance liquid chromatography (HPLC), as informed by the manufacturer.

Ventricular cardiomyocyte isolation from neonatal Wistar rats was performed as described earlier (Ott *et al*., 2008) and human mammary adenocarcinoma cell line MDA-MB-231 was used. Both cell cultures were maintained in a humidified incubator with 5% CO_2 at 37°C. Cell viability was assessed through the MTT assay (Mosmann, 1983) and cellular death was evaluated by the propidium iodide (PI) nuclear staining (Riccardi & Nicoletti, 2006) analyzed with a FacScan (BD Biosciences) and CellQuest to determine percentages of viable and apoptotic/necrotic cells. For MTT, cardiomyocytes were plated at $2.6x10^4$, $5.2x10^5$

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or $2.6x10^5$ cells/cm^2 and MDA-MB-231 cells were plated at $1.3x10^4$ cells/cm^2 in 24-well plates. For PI, $6x10^5$ cardiomyocytes and $1x10^5$ MDA-MB-231 cells were plated in 6-well plates.

Both cell cultures were subjected to the following treatments, in three independent experiments performed in triplicates: control group (cells incubated only with media, no plant extract, no dox); AC group (cells incubated with 0, 12.5, 25, 50, 100, and 200µg/ml of AC; without dox); CS group (cells incubated with 0, 12.5, 25, 50, 100, and 200µg/ml of CS; without dox); Dox group (no herbal extracts; 5µmol/l dox incubation for 2, 4, 16, and 24h); AC-Dox group (cells incubated with 0, 12.5, 25, 50, 100, and 200µg/ml of AC; 5.0µmol/l dox) and CS-Dox group (cells incubated with 0, 12.5, 25, 50, 100, and 200µg/ml of CS; 5.0µmol/l dox). After 24h of seeding, fresh media was added to each cell type containing the mentioned concentrations of herbal extract. After 48h of seeding, 5.0µmol/l dox (Adriblastina®, Pfeizer) was added to each well. After 58h of seeding, cell viability and death analysis were performed. Statistical analyses were carried out using R program for one-way analysis of variance (ANOVA) followed by Scott-knott test, and significance of 5%.

Data of MTT assay demonstrated that AC and CS were not toxic to the cells at a concentration rate from 12.5 up to 50µg/ml, as cell viability was not different from control (P>0.05). However, extracts at higher concentrations of 100 and 200µg/ml were toxic (lower cell viability compared to control, P<0.05) and, therefore, excluded from further investigations. These findings are explained byhormesis, a dose-response relationship that is characterized by low-dose stimulation and high-dose inhibition. Such pro-oxidative property was also observed with resveratrol (Plauth *et al*., 2016). Flavonoids are considered antioxidants, but they can become pro-oxidative as described here for high doses. This paradoxical effect highlights the misleading concept of considering antioxidant agents always beneficial (Lei *et al*., 2016).

The percentage of death cardiomyocytes significantly increased after incubation with dox, compared to control (p=0.015), as expected. However, cellular death was lower for cardiomyocytes incubated with dox+plant extracts (P<0.05 for all dosage tested), compared to dox only (Figure 1A), indicating a protective effect of AC and CS against dox over cardiomyocytes. Such protection was not observed for MDA-MB-231 cells, which is desirable, because the anti-neoplastic property of dox must be kept over cancer cells (Figure 1B). In fact, the association of Dox with AC at 12.5µg/ml and Dox with CS at 25 and 50µg/ml resulted in more cell death to breast cancer cells, compared to Dox only (P<0.05), indicating a potentializing action of dox by these extracts in cancer cells. All data obtained for PI assay were confirmed by MTT assay.

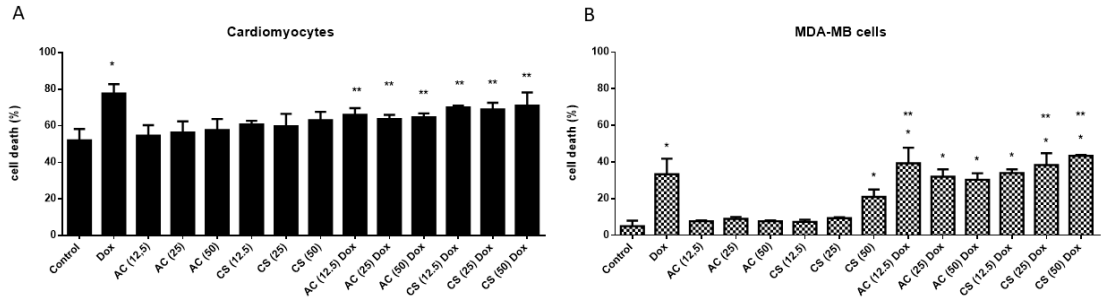

Figure 1. Cell death of cardiomyocytes isolated from neonatal Wistar rats (A) and human mammary adenocarcinoma cell line MDA-MB 231 (B) following treatment with doxorubicin (Dox) for 24h, as assessed by propidium iodide incorporation. Data is represented as %, according to untreated control. Dox was used at 5µmol/l and *Arrabidaea chica* (*Fridericia chica* - AC) and *Camellia sinensis* (CS) extracts at 12.5, 25 and 50 µg/ml. Results are presented as mean ± SEM of three experiments. *P<0.05 *vs* control. **P<0.05 *vs* Dox.

The CS used had high concentration of epigallocatechin (> 45%), which was proved to be cardioprotective against dox (Saeed *et al*., 2015), explaining the preservation of cardiomyocyte viability. Mass spectrometry analyses of the AC identified the presence of kaempferol. In ischemia-reperfusion injury in the heart, kaempferol decreased levels of inflammatory markers (TNF-alpha and IL-6) and proapoptotic proteins (Bax and caspase-3), and increased expression of the antiapoptotic protein Bcl-2 (Suchal *et al*., 2016). In dox-induced toxicity, kaempferol protected heart cells by inhibiting the p53-mediated mitochondria-dependent intrinsic apoptotic signaling (Xiao *et al*., 2012). While dox promotes apoptosis by increasing Bax and decreasing Bcl-2, kaempferol inhibits apoptosis by showing an opposite action (Xiao *et al*., 2012). In this context, at least part of AC effectiveness demonstrated here is due to the presence of kampferol.

Despite the potential therapeutic properties of *A. chica*, described with antineoplastic, anti-inflammatory and antimicrobial properties and useful for treating osteoarthritis (Barbosa *et al*., 2008; Michel *et al*., 2015; Vasconcelos *et al*., 2019), there is no previous study evaluating its effect against oxidative injury to the heart. Present data demonstrated a novel antioxidant activity of *A. chica* on dox-induced cardiotoxicity. The *A. chica* and *C. sinensis* treatments were shown to efficiently and selectively preserve the viability of cardiomyocytes, while maintaining dox anticancer activity, making them promising alternatives to be used by oncology patients under dox treatment.

Keywords: antioxidant, cardiotoxicity, flavonoids, oncology

RESUMO

A doxorrubicina (dox) é um medicamento antineoplásico que induz cardiotoxicidade por estresse oxidativo. Os flavonoides são antioxidantes extraídos de plantas como Camellia sinensis e Arrabidaea chica (Fridericia chica). Esta pesquisa objetivou avaliar efeitos protetores do extrato de A. chica (AC), comparado ao de C. sinensis (CS), frente ao estresse oxidativo induzido pela dox, no coração. Cardiomiócitos e células neoplásicas MDA-MB 231 foram incubados com AC e CS. Depois, adicionou-se dox e avaliaram-se taxas de viabilidade e morte celular. A citometria de fluxo para o ensaio de iodeto de propídeo (IP) em cardiomiócitos mostrou as seguintes taxas de morte celular: controle 53%; dox 78% (maior que controle, P=0,015); AC_12,5μg/mL + dox 65% (menor que dox, P=0,031); AC_25μg/mL + dox 62% (menor que dox, P=0,028); AC_50μg/mL + dox 63% (menor que dox, P=0,030); CS_12,5μg/mL + dox 71% (menor que dox, P=0,040); CS_25μg/ml + dox 69% (menor que dox, P=0,037); CS_50μg/mL + dox 74% (menor que dox, P=0,044). Resultados das células MDA-MB 231 mostraram que nenhum extrato interferiu na atividade antitumoral da dox. Os dados de IP foram corroborados pelos de MTT. Este estudo reporta promissora utilização de A. chica na prevenção da cardiotoxicidade induzida pela dox.

Palavras-chave: antioxidante, cardiotoxicidade, flavonoides, oncologia

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