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Article

Doxorubicin cardiotoxicity and *Camellia sinensis* cardioprotection determined by speckle-tracking echocardiography

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Doxorubicin (Dox) is a medication used in the treatment of cancerous tumors and hematologic malignancies with potentially serious side effects, including the risk of cardiotoxicity. Flavonoids are plant metabolites with antioxidant properties and can be extracted from *Camellia sinensis* (CS). The aim of this study is to evaluate the possible cardioprotective effect of CS against injuries induced by Dox in rats. A total of 32 animals were distributed into four groups: (1) control - intraperitoneal injection (I.P.) of 0.5 mL saline weekly and 1.0 mL water by gavage daily; (2) CS - 0.5 mL saline I.P. weekly and 200 mg/kg CS by gavage daily; (3) Dox - 5.0 mg/kg Dox I.P. weekly and 1.0 mL water by gavage daily; and (4) Dox + CS -5.0 mg/kg Dox I.P. weekly and 200 mg/kg CS by gavage daily. Clinical examinations, blood profiles, electrocardiograms, echocardiograms, and histological analyses of hearts were performed over 25 days. The animals in the Dox group showed changes in body weight and in erythrogram, leukogram, electrocardiography, and echocardiography readings. However, animals from the dox+CS group had significantly less change in body weight, improved cardiac function, and showed more preserved cardiac tissue. This study demonstrated that CS prevents dox-induced cardiotoxicity, despite enhancing the cytotoxic effect on blood cells.

Keywords: Flavonoids. Green tea. Heart injury. Radial strain. Longitudinal strain.

INTRODUCTION

Doxorubicin (Dox) is an anticancer drug largely used for treating several types of cancer, but it is closely associated with important off-target cardiotoxic effects, which may appear years after completing the treatment (Al-Malky, Al Harthi, Osman, 2020; Almeida *et al.*, 2015). The pharmacological action mechanisms of Dox include inhibition of DNA and RNA synthesis, inhibition of topoisomerase II, release of apoptotic proteins, and production of reactive oxygen species (Elliott, 2006). Considering the oxidative stress induced by Dox, the use of an antioxidant agent to protect the cardiomyocytes is promising. Flavonoids belong to the polyphenol group of plant secondary metabolites. They are extracted from a number plant species, such as green tea from *Camellia sinensis* (L.) Kuntze (Chacko *et al.*, 2010) and show cardioprotective potential. *C. sinensis* green tea is very popular because of its health

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benefits, including its anticancer, anti-oxidant, and antimicrobial properties. This is due to the complex chemical composition of green tea, which comprises different classes of chemical compounds, such as polyphenols, specifically flavonoids, proteins, minerals, vitamins, and amino acids. Flavonoids constitute a class of compounds that could mitigate the Dox toxicity due to their antioxidant qualities, which may directly or indirectly counteract the oxidative stress and apoptosis induced by the antineoplastic drug.

Echocardiography is the most noninvasive technique for assessing myocardial function, with an accuracy similar to that of computed tomography and magnetic resonance imaging, which are considered to be the gold standard in the evaluation of heart function (Nagueh, Shah, 2020). The introduction of two-dimensional speckle- tracking echocardiography (ST-Echo) allowed the addition of two new variables in the assessment of myocardial function: strain and strain rate. Strain represents the percentage of myocardial deformation during the cardiac cycle, and strain rate is defined as the velocity at which deformation occurs (Del Castillo *et al*., 2010). The advantages of the ST-Echo method over conventional methods are minimal influence of preload and volume status, angle independence, and greater sensitivity in detecting myocardial dysfunction, which allows earlier diagnosis (Singh *et al*., 2022). Two-dimensional ST-Echo has shown good repeatability and reproducibility in humans and laboratory animals (Oliveira *et al*., 2013; Almeida *et al*., 2015).

Since cytotoxic chemotherapy is essential to treating cancer patients, it is imperative to look for strategies that reduce its side effects. Despite multiple studies and strong evidence from many experimental models that anti-oxidant therapies can reduce or prevent Dox-induced cardiotoxicity, results have not been as promising in the clinical setting, particularly in the cardiac function context, as assessed by two-dimensional speckle tracking. Thus, the present study aimed to investigate the potential cardioprotective role of herbal *C. sinensis* and an echocardiographic evaluation in the presence of such extract.

MATERIAL AND METHODS

Camellia sinensis (CS) extract

A commercially available brand of 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. The CS extract dosage was 200 mg/kg body weight per day, according to Lustosa et *al.* (2016).

Doxorubicin

Four weekly applications of Dox (Adriblastine doxorubicin hydrochloride) were performed using the reference drug (Adriblastina®, Pfizer), 5 mg/kg (Oliveira *et al*., 2014).

Animals

Adult male Wistar rats (n=32), two months old, weighing between 290 and 330 g, were used as the experimental model. The animals were obtained from the Central Vivarium of the Federal University of Minas Gerais (Brazil), kept in standard conditions (alternating cycles of 12 hours of light with 12 hours of darkness) at 22 ± 2ºC, and fed with a standard laboratory diet (Nutropica®) and water *ad libitum*. All animals were used in accordance with experimentation ethics, respecting their welfare and minimizing any discomfort. All experimental protocols were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee in place at the University.

Experimental design (protocol of doxorubicin-induced cardiotoxicity in rats)

The animals were randomly distributed into four groups (8 animals/group): control group [intraperitoneal injection (I.P.) of saline weekly, and 1.0 mL water by gavage daily]; CS group (saline I.P. weekly, and 200 mg/kg CS by gavage daily); Dox group (5.0 mg/kg I.P. weekly, and 1.0 mL water by gavage daily); and Dox+CS

group (5.0 mg/kg Dox I.P. weekly, and 200 mg/kg CS by gavage daily).

First, CS and water gavage were given 48 h prior (T0) to the first Dox injection (T2). Animals were weighed weekly and the Dox and CS dosage was properly calculated for each animal. The injections were given once a week for four weeks. Therefore, a total of four Dox injections every seven days (T2, T9, T16, T23), and 25 daily gavages were given.

The experimental protocol ended 48 h after the last Dox injection was administered,which occurred on the same day of the last gavage (T25).

Clinical evaluation

Daily (from T0 to T25) clinical examination included signs of lethargy, diarrhea, dehydration, and death. Body weight was evaluated weekly and the dosages of Dox and CS were updated as needed.

Echocardiography (Echo)

Cardiac morphology and function in adult male Wistar rats were assessed noninvasively at T0 and T25 using a high-frequency, high-resolution echocardiography (Echo) system consisting of a VEVO 2100 ultrasound machine equipped with a 30- to 40-MHz bifrequential transducer (Visual Sonics, Toronto, ON, Canada). The rats were anesthetized with 5% isoflurane for 1 min for induction and then placed in the supine position on an imaging stage equipped with built-in electrocardiographic electrodes for continuous heart rate monitoring and a heater to maintain body temperature at 37°C. The anesthesia was maintained with 1.0-1.25% isoflurane. High-resolution images were obtained in the right and left parasternal long and short axes and apical orientations. Standard B-mode images at 235 frames/s were acquired. Left ventricular (LV) dimensions and wall thickness were measured by M-mode at the level of the papillary muscles in the left parasternal short axis during end systole and end diastole. All measurements and calculations were made in accordance with the American Society of Echocardiography guidelines. The following M-mode measurements were made: LV internal end-diastolic and end-systolic dimensions, LV posterior wall thickness at end diastole and end systole, and interventricular septal thickness at end diastole and end systole. Based on these parameters, end-diastolic LV volume, end-systolic LV volume, fractional shortening, ejection fraction, stroke volume, and cardiac output were calculated by the Teichholtz method. The evaluation of cardiac deformation was also performed using VevoStrain software (version 1.4.0, Visual Sonics). Long- and short axis images were acquired and recorded for offline analysis, measuring longitudinal and radial ST-Echo. Velocity, displacement, strain, and strain rate were measured by applying the speckle-tracking technique to B-mode recordings, which permits tracking of the movement of cardiac walls along the cardiac cycle from a semiautomatically traced LV. The curves of cardiac muscle movement were also used for asynchronous analysis using the same software.

Electrocardiographic Examination (ECG)

For electrocardiography (ECG), Wistar rats were anesthetized using 1.0-1.25% isoflurane and were placed in supine position for the examination which last 10 min and was performed at T0 and T25. Computer ECG (ECG-PC TEB, São Paulo, SP, Brazil) examinations were taken prior to the experiment and again at the end. Six leads (bipolar limb and unipolar augmented limb leads) were simultaneously recorded at a speed of 50 mm/s, sensitivity of 20 mm/1 mV, and lead II was considered for analysis. Heart rate (HR), amplitude (mV) and length (ms) of the P wave, QRS complex and T wave, the ST segment, PR and QT intervals, and the presence of arrhythmias and conduction disturbance were evaluated.

Blood profile

After performing the ECG at T25, still under anesthesia (1.0-1.25% isoflurane), blood samples were collected by cardiac puncture while the animals were still under anesthesia. A complete blood count was performed with the sample from the EDTA tube,

which was homogenized and processed in an automatic blood cell analyzer (PocH-100iV - Sysmex), thus providing white blood cell count (WBC), red blood cell count (RBC), packed cell volume, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets count (PLT). The white cell differential counting was done under light microscopy.

Biochemical analysis

The total protein concentration was calculated by a refractometer (Ningbo Utech International CO LTD) and the fractionated albumin, alpha (α1 and α2), beta β1 and β2) proteins, whereas gamma globulins (γ) were evaluated by electrophoresis in agarose gel (CELMGEL) (30 min) in a TRIS buffer. The gels were stained for 5 minutes in 200 mL of Amido black and destained in an acid acetic solution (7%) until the gel background was completely clear. The concentration of protein fractions was determined by the use of computer-assisted software CELM SE- 250.

Histological Analysis

At the end of the treatment period (last gavage), all rats were euthanized under anesthesia. Euthanasia was performed at T25 by deepening of isoflurane anesthesia, followed by IP administration of thiopental (150 mg/kg). Necropsy was then performed, and heart samples were obtained. The hearts were weighed and the heart weight/body weight ratio was obtained to infer cardiac enlargement. Heart fragments were fixed in 10% neutral buffered formalin, paraffin embedded, and 5-µm sections were stained with hematoxylin and eosin.

Statistical Analysis

An experimental randomized design was used, with the data presented as mean value and standard deviation. Quantitative variables were analyzed with the normality distribution test (Kolmogorov-Smirnov), followed by ANOVA and Tukey test ($p<0.05$). All the data were evaluated with Instat GraphPad and GraphPad Prism 7. The significance level was established at $p<0.05$.

RESULTS

ECG recordings were compared from T25 to T0 for each animal and between groups at T25. There was no difference in heart rate, which ranged from 202 bpm to 398 bpm. All animals showed normal sinus rhythm either at T0 or T25. No difference in ECG variables was observed between animals from the Control, CS, and Dox+CS groups. However, animals that received Dox showed changes (Figure 1), such as increased amplitudes of the P, R, and T waves ($p < 0.05$). No arrhythmias or conduction abnormalities were detected.

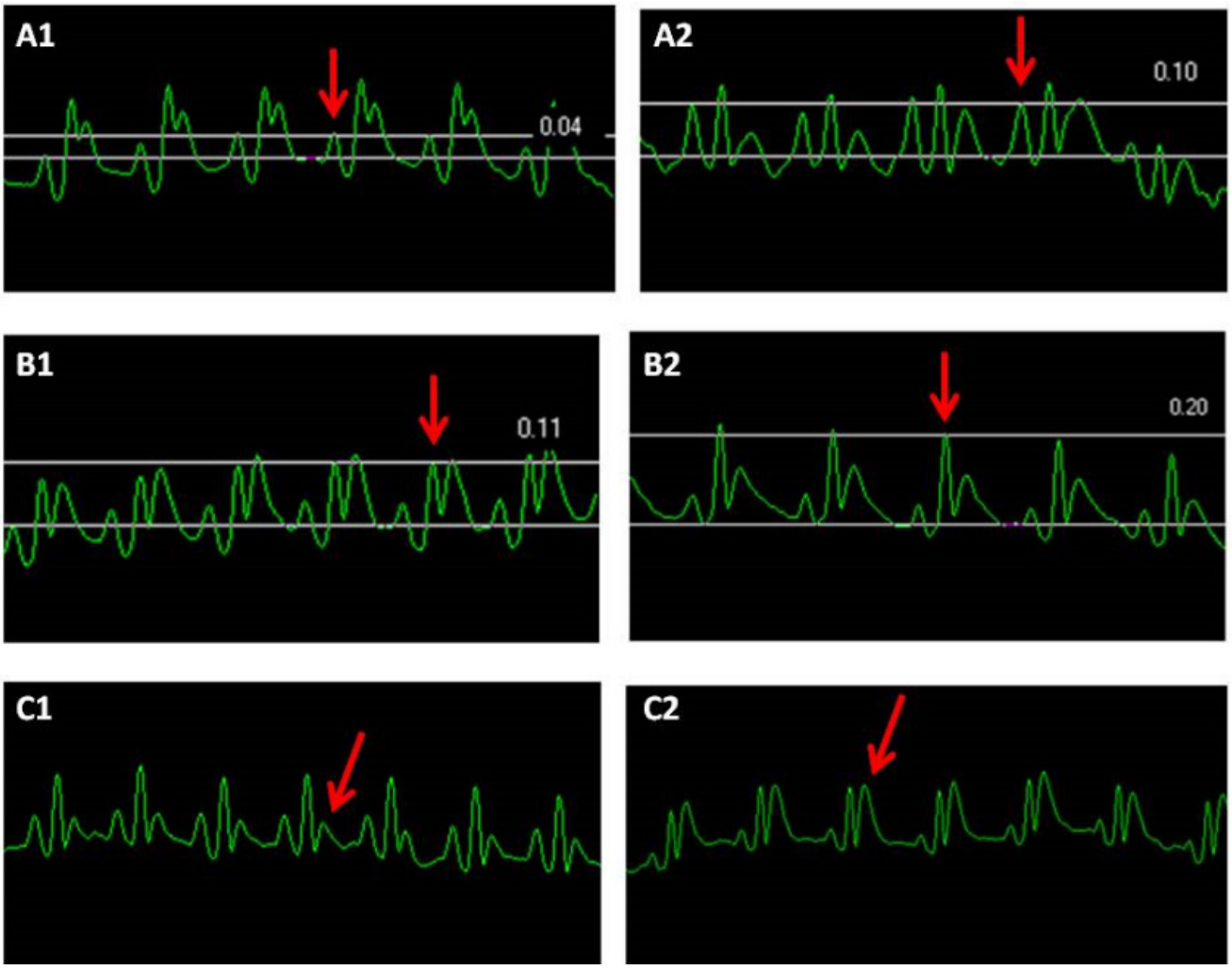

FIGURE 1 - Representative electrocardiographic examinations obtained from Wistar rats of the doxorubicin-treated group (DII, velocity 50 mm/s, sensitivity 2N). Tracings on the left side of the panel were taken at the beginning of the study (T0), while the ones on the right side were taken at the end (T25). Observed changes were increased amplitudes of the P wave (from 0.04 mV [A1] to 0.10 mV [A2]), the R wave (from 0.11 mV [B1] to 0.20 mV [B2]), and the T wave (T waves with amplitude greater than R wave [C2], which were not observed in the beginning for the same animal [C1]).

Echocardiography at T0 for all animals had statistically similar values for M-mode and ST-Echo. By the end of experimental period, only animals from the Dox group showed significantly lower values for almost all echo parameters (Table I). In addition, the myocardial movement evaluated by M-mode was asynchronous in the Dox group (Figure 2). Considering the longitudinal and radial parameters in ST-Echo, only the radial strain showed a statistical difference, with the Dox group having the lowest values (Figures 3 and 4). The values in the Dox+CS animals were similar to those of the Control and CS groups.

TABLE I - Echocardiography data (mean and standard deviation) from Wistar rats obtained at the end of the experiment (T25)

Variables	Groups				
	Control	CS	Dox	Dox+CS	p-value
Heart rate (bpm)	332 (9.9)	362 (32.4)	332 (49.9)	355 (55.5)	0.313
Cardiac output (ml/min)	68.1 (9.7)[a]	70.7 (8.2)[a]	48.2 (16.8)[b]	48.8 (7.3)[b]	0.001
Ejection Fraction (%)	68.9 (1.9)[a]	69.9 (5.9)[a]	54.4 (4.4)[b]	68.7 (7.4)[a]	0.005
Fractional Shortening (%)	36.9 (2.1)[a]	37.4 (2.7)[a]	28.8 (6.1)[b]	39.8 (6.1)[a]	0.008
Stroke volume (µL)	205 (29.0)[a]	198 (15.6)[a]	139 (31.1)[b]	129 (37.6)[b]	0.0004
Radial srain (%)	17.1 (6.1)[a]	24.8 (7.9)[a]	8.1 (1.1)[b]	16.7 (5.2) [a]	0.044
Longitudinal strain (%)	15.3 (4.2)[a]	15.7 (3.2)[a]	7.09 (2.03)[b]	9.78 (1.11)[b]	0.003

CS: *Camellia sinensis*; Dox: doxorubicin
Different lowercase letters in the same row indicate statistically different values.

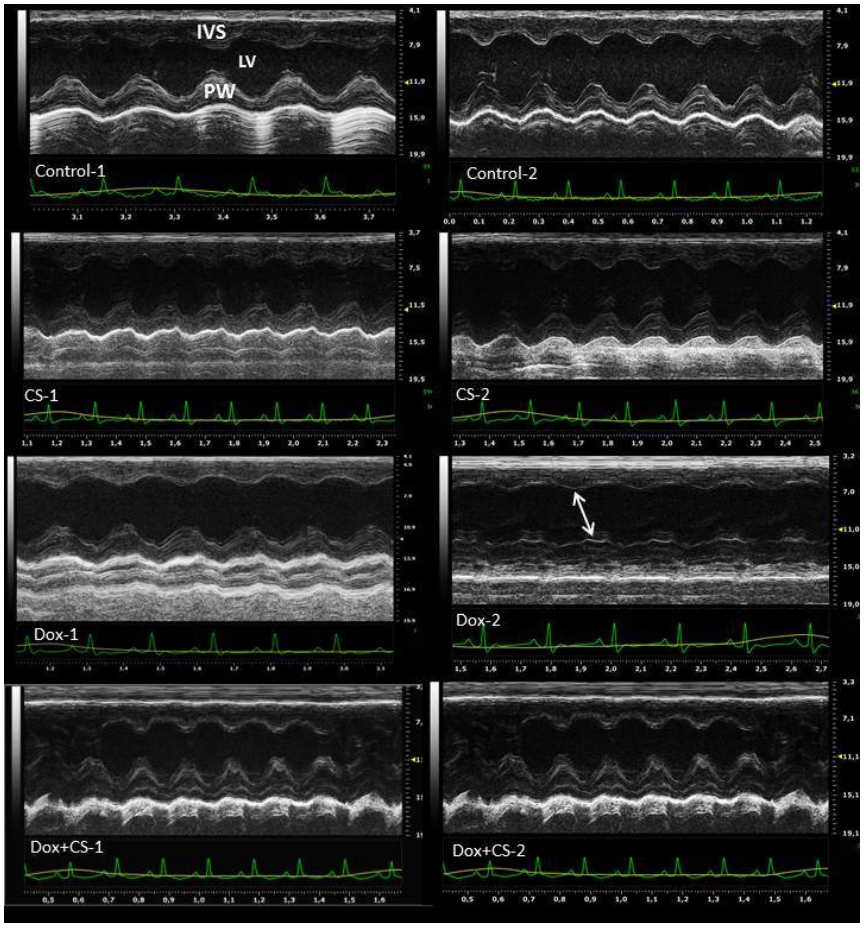

FIGURE 2 - Echocardiography M-mode images of the left ventricle obtained at the level of the papillary muscles in the left parasternal short axis during end systole and end diastole. Illustrations on the left side of the panel (1) were taken at the beginning (T0) whereas those on the right side (2) were taken at the end (T25) of the study. Before (1), all groups showed similar patterns for contraction and movement of the ventricular muscle. After (2), the doxorubicin (Dox) group had decreased contraction and showed asynchrony (arrow) between interventricular septum (IVS) and left ventricle posterior wall (PW) movement.

CS: *Camellia sinensis*; LV: left ventricle.

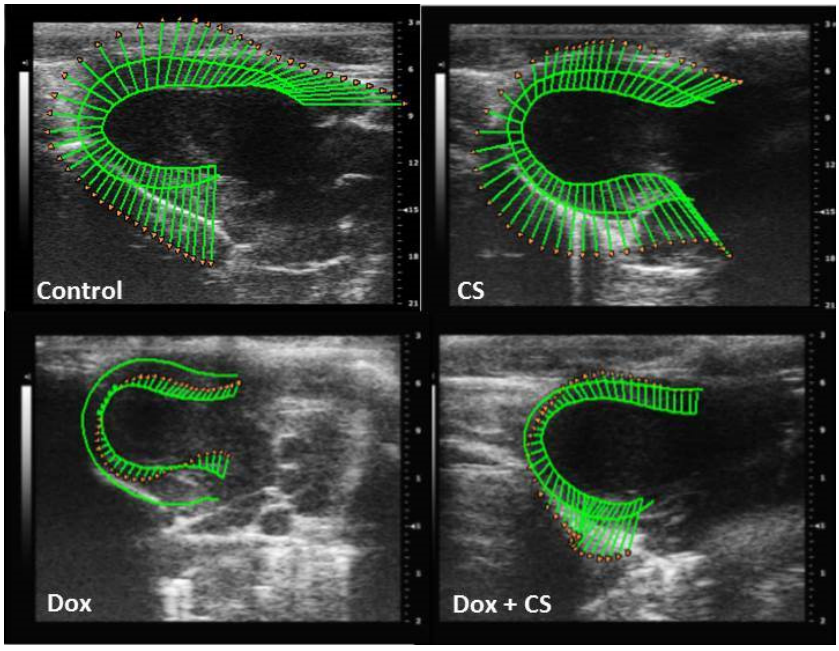

FIGURE 3 - Strain-based vectorial representation of cardiac function measured by applying the speckle-tracking technique to B-mode recordings of the left ventricle obtained at right parasternal long axis view at diastole. Note the different sizes of the maximum vectors between the groups.

CS: *Camellia sinensis*; Dox: doxorubicin.

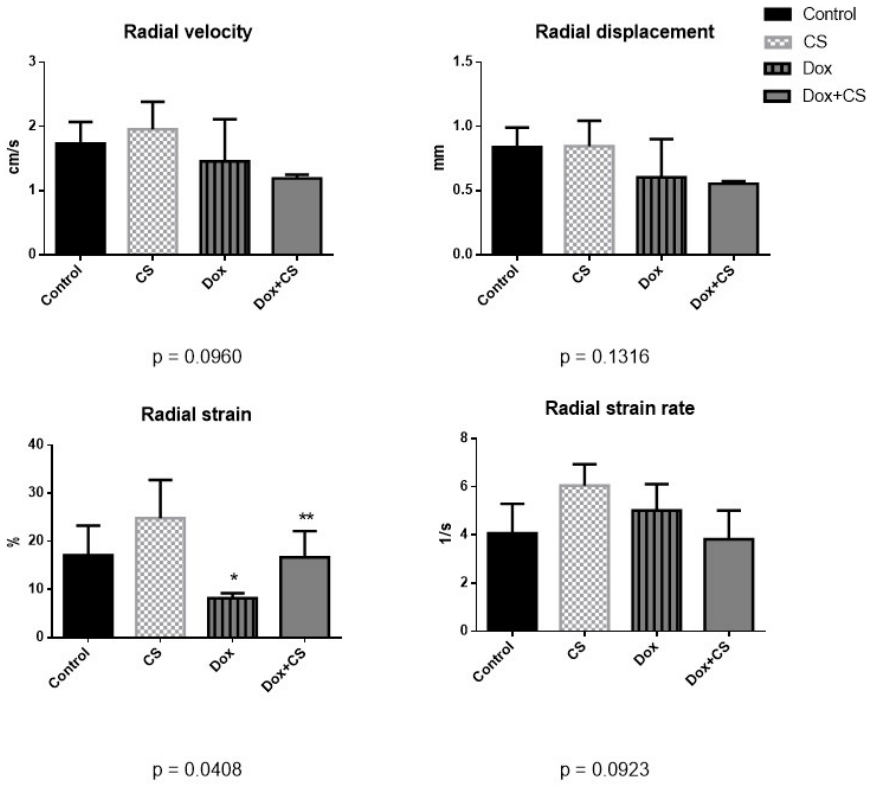

FIGURE 4 - Radial parameters of two-dimernsional speckle-tracking echocardiography obtained at the end of the study (T25).

CS: *Camellia sinensis*; Dox: doxorubicin; **vs* Control; ***vs* Dox (p-value is indicated for each variable).

Eleven animals died during the experimental period, six from the Dox group and five from the Dox+CS group. One animal from the Dox groups died on the last day, just after being evaluated for ECG and echo. In addition, Dox and Dox+CS animals showed signs of diarrhea, dehydration, apathy, and lethargy. The Control and CS groups gained weight during the four weeks of the experiment (60 g and 33 g, respectively), while animals from the Dox and Dox+CS groups lost weight (-55 g and -62 g, respectively) (Table II).

TABLE II - Body weight and delta body weight (mean and standard deviation) from Wistar rats obtained at the beginning (T0) and the end (T25) of the experimental study

Variables	Groups				
	Control	CS	Dox	Dox+CS	p-value
BW (g) at T0	314(17.5)	319(29.1)	308(26.6)	307(21.5)	0.089
BW (g) at T25	374 (24.5)[a]	352(23.7)[a]	253(46.7)[b]	245(24.8)[b]	0.011
Delta BW (T25-T0)	+60(21.3)[a]	+33(45.8)[b]	-55(49.0)[c]	-62(27.1)[c]	0.002

BW: body weight; CS: *Camellia sinensis*; Dox: doxorubicin.
Different lowercase letters in the same row indicate statistically different values.

At hematological tests (Table III), The CS group presented similar levels of all the hematological test parameters (Table III) as compared to the Control group. Conversely, the Dox-treated groups (Dox and Dox+CS) showed interference in the red blood cell count with a decrease showing up on the erythrogram. However, in relation to total leukocytes, there was a significant decrease only in the group challenged with doxorubicin and treated with CS ($p<0.05$). The lymphocytes showed a significant reduction in number in the groups that received doxorubicin overdose, regardless of treatment.

As shown in Table III, there was a significant reduction in the serum total protein value between the Dox+CS group (6.7 g/dL) and others groups, most likely due to a drop in the albumin value, α1-globulin fraction, and γ- globulin (Table III).

TABLE III - Blood test results, mean and standard deviation, from Wistar rats obtained at the end of the experimental study (T25)

Variables	Groups			
	Control	CS	Dox	Dox+CS
RBC (x 10^6/µL)	8.1 (0.9)	8.4 (0.7)	7.4 (0.8)*	6.1 (0.8)*
Hemoglobin (g/dL)	13.4 (0.4)	13.7 (0.6)	12.2 (1.6)*	11.9 (0.3)*
Packed cell volume (%)	39.1 (2.7)	39.3 (2.8)	34.5 (4.9)*	26.5 (9.5)*

TABLE III - Blood test results, mean and standard deviation, from Wistar rats obtained at the end of the experimental study (T25)

Variables	Groups			
	Control	CS	Dox	Dox+CS
MCV (fL)	48.3 (6.6)	46.8 (5.1)	46.6 (1.9)	43.4 (9.5)
MCH (%)	16.5 (2.1)	16.3 (1.6)	16.5 (0.5)	19.5 (2.5)
MCHC (%)	34.3 (2.1)	34,9 (1.7)	35.4 (0.3)	44.9 (17.8)
WBC (x 10^3/µL)	6.2 (1.4)	5.5 (1.1)	5.9 (5.1)	1.1 (0.5)*
Lymphocytes (x 10^3/µL)	4.6 (1.3)	4.1 (0.8)	1.3 (1.7)*	0.7 (0.3)*
Neutrophils (x 10^3/µL)	1.3 (0.3)	1.2 (0.4)	2.5 (3.4)	0.2 (0.1)
Monocytes (x 10^3/µL)	0.2 (0.04)	0.1 (0.05)	0.4 (0.5)	0.04 (0.02)
Eosinophils (x 10^3/µL)	0.1 (0.06)	0.1 (0.1)	0.08 (0.1)	0 (0)
Basophils (x 10/µL)	2.0 (3.7)	0.7 (1.8)	0 (0)	0 (0)
Total proteins (g/dL)	8.0 (0.5)	7.6 (0.4)	7.9 (0.1)	6.7 (0.5)*
Albumin (g/dL)	3.2 (1.0)	2.1 (1.1)	2.9 (0.9)	2.0 (0.4)
α-1 globulin (g/dL)	1.1 (0.6)	1.3 (0.5)	1.7 (0.3)	0.8 (0.4)
α-2 globulin (g/dL)	0.6 (0.5)	0.7 (0.2)	1.1 (0.6)	1.3 (0.5)
β-1 globulin (g/dL)	1.0 (0.5)	0.9 (0.6)	0.7 (0.1)	1.0 (0.2)
β-2 globulin (g/dL)	1.3 (0.5)	1.5 (0.5)	1.3 (0.4)	1.0 (0.2)
γ- globulins (g/dL)	0.8 (0.3)	1.1 (0.7)	0.2 (0.06)*	0.6 (0.1)*
A/G	0.7 (0.3)	0.4 (0.2)	0.6 (0.2)	0.4 (0.1)

*in the same row indicates statistical difference ($p<0.05$).

Histological analyses revealed local spots of fibrosis, necrosis, and hyaline degeneration in the Dox group. The Dox+CS group showed a different lesion pattern in which hemorrhage, vascular dilation, and interstitial edema were observed in a diffuse pattern (Figure 5). No lesion was observed in the hearts from the Control and CS groups.

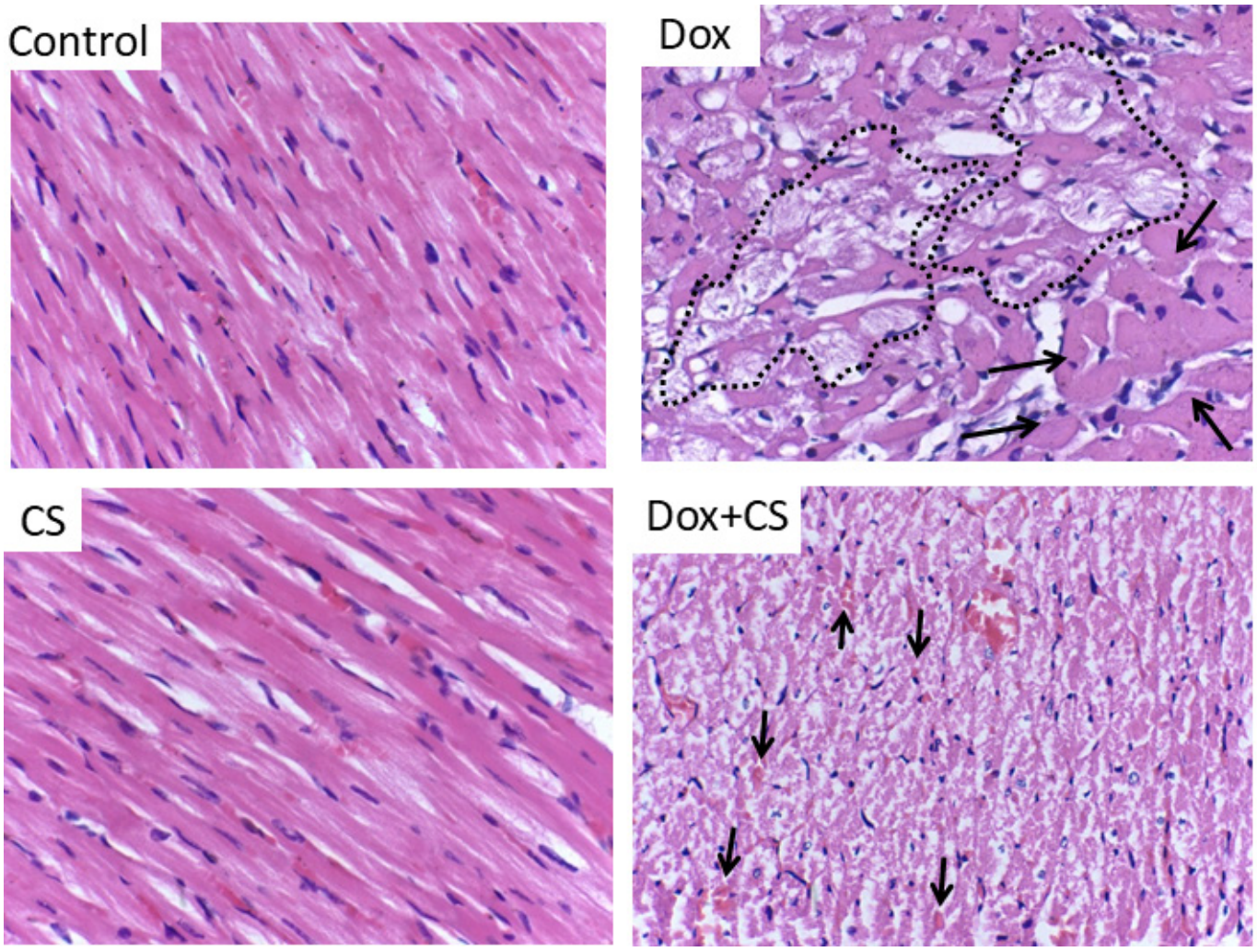

FIGURE 5 - Photomicrographs of Wistar rat hearts in longitudinal views (Hematoxylin and Eosin staining, 60 X). Normal hearts with no lesions (Control and CS groups). Hyaline degeneration areas (arrow) and necrosis (black dots) were observed in the Dox group. Hemorrhage (arrows) and interstitial edema, noted by dissociated muscle fiber, were observed in the Dox+CS group.

CS: *Camellia sinensis*; Dox: doxorubicin.

DISCUSSION

The present study investigated the effect of a flavonoid-rich extract from CS and its possible role in improving ventricular function as evaluated by ST-Echo, standard echocardiography, and ECG in a rat model of Dox-induced cardiotoxicity. Dox is used in cancer treatment for humans and companion animals. It is used in dogs to treat lymphoma (University of Wisconsin-Madison Protocol), osteosarcoma, hemangiosarcoma, and squamous cell carcinoma (Gallay-Lepoutre, Bélanger, Nadeau, 2016). In this scenario, and considering the widespread use of Dox in routine clinical practice, the search for ways to protect heart cells from its adverse effects is imperative.

The conventional echocardiography variables, ejection fraction, fractional shortening, cardiac output, and stroke volume indicated cardiac impairment in the Dox group, as demonstrated by other authors (Oliveira *et al.*, 2013; Almeida *et al.*, 2015; Gallay-Lepoutre, Bélanger, Nadeau, 2016). However, only half of these parameters in the Dox+CS group demonstrated ventricular improvement. Given the conflicting data, a more advanced technique, such as the ST-Echo, is needed to avoid misinterpretation.

ST-Echo is considered a more reliable technique for evaluating cardiac function, particularly for an early and more precise diagnosis of heart failure (Singh *et al*., 2022). In the present study,sboth radial and longitudinal strain were lower in the Dox group as compared to the control and CS groups. However, improvement in ventricular function was observed in the Dox+CS group only for the radial strain. This finding suggests that, in the context of Dox cardiotoxicity, the radial direction of the ventricular motion was the first to recover in Wistar rats while the longitudinal motion of the fibers may improve later. A mid-term recovery of global longitudinal strain is reported for other cardiac insults as myocardial infarction (Eslami *et al*., 2021) and coronavirus disease 2019 (Ramadan *et al*., 2021).

In a hypertensive rodent model (Leader *et al*., 2019), the animals displayed a reduced ejection fraction and impaired global longitudinal strain, as demonstrated in this study. The authors also demonstrated that the global longitudinal strain showed a stronger correlation with cardiac interstitial fibrosis than the standard parameter ejection fraction. In this study, the Dox+CS animals had histological changes in the ventricular myocardium while retaining an impaired longitudinal strain. The histological analysis showed that important changes were detected in both groups that received Dox, with the worst ones found in the Dox group and milder ones in the Dox+CS group, whose animals also showed improvement in the global radial strain and conventional parameters (ejection fraction and fractional shortening), yet reduced longitudinal strain. No lesion was observed in the Control and CS groups, indicating CS caused no damage to the myocardium.

An ECG examination of the Dox animals showed abnormalities in the P, R, and T waves. Increased P and R waves indicate atrial and ventricular remodeling, which were not detected in the Dox+CS group, suggesting a preventive effect by CS. Abnormalities in the T wave have been associated with the development of polymorphic ventricular tachycardia (Kashiura *et al*., 2019) by disturbing ventricular repolarization. Therefore, an increase in T wave amplitude may be attributed to the Dox cytotoxic effect on the heart's electrical system.

The CS that was used had high concentrations of catechins (>80%), particularly epigallocatechins (>45%), which have been proven to be cardioprotective against Dox (Saeed *et al*., 2015). As such, it is reasonable to hypothesize that epigallocatechins strongly contributed to the preservation of cardiomyocyte viability. In this way, Dox led to clinical disturbances and mortality of animals in the Dox and Dox+CS groups. Such effects have also been found by others researchers (Xiang et al., 2009) amd can be attributed to Dox administration as the animals that received only CS did not show any of these findings. Although CS green tea has several beneficial health effects, its constituents may be beneficial up to a certain dose, with higher doses possibly causing unknown side effects. Moreover, the effects of green tea catechins may not be the same in all individuals. Epigallocatechin gallate (EGCG) of green tea extract is cytotoxic, and the excessive consumption of green tea could trigger acute cytotoxicity in liver cells (Schmidt *et al*., 2005).

Doxorubicin toxicity was observed in the cardiovascular system and also systemically, with multiple changes documented in rats receiving chemotherapy. Environmental conditions were the same for all animals, and those in the Control and CS groups gained weight and had a healthy appearance, with no documented deaths. Thus, adverse side effects were inferred to have been caused by Dox. Studies using the same experimental model reported deaths among the animals that received Dox (Xiang *et al*., 2009; Kenk *et al*., 2010).

Weight loss with the use of Dox was also reported by other researchers (Karagoz *et al*., 2008; Chandran *et al*., 2009; Hazari *et al*., 2009; Xiang *et al*., 2009; Kenk *et al*., 2010), being a recurrent finding during chemotherapy. Animals that did not receive Dox gained weight throughout the experimental period, as was observed in this study. However, those that received Dox alone or in association with other drugs had significant weight loss, which reveals another adverse effect, despite its cardiac function preservation attributes. This highlights the importance of close follow-up in patients using Dox, not only to assess cardiac function but also to monitor their general clinical condition, always with the intent of maintaining their quality of life. In addition to Dox's toxic effect, it must be remembered that as this is a cancer patient and weight loss can also be a sign of paraneoplastic syndrome.

It should be noted that rats receiving Dox consumed less food. Every morning, the amount of food and water in the animals' cages was evaluated and there was always food left over in those that were being treated with Dox,, unlike the other cages where it was necessary to replace food daily. Thus, a reduction in food consumption became evident, even though it was not being weighed for a quantitative assessment. The animals that received Dox and being treated with the plant extract (CS group) showed an even more significant weight loss than those that received Dox alone. In addition, the animals that received the plant extract (CS group) gained less weight than did the Control group. It was hypothesized that the weight loss (Dox+CS) and lower weight gain (CS) of these animals could be attributed to either an increased metabolic rate or urinary output, the latter being a result of the green tea (Basu *et al*., 2010). However, none of these mechanisms has been well studied or, as yet, confirmed.

There were slight changes in the erythrogram at the end of the experiment (T25), possibly associated with vasculitis and hemorrhage. Although green tea catechins may have an affinity for iron, and green tea infusions can cause a significant decrease in iron bioavailability from the diet, it did not result in iron deficiency anemia.

Conversely, s severe reduction in the white cell count was observed in the Dox+CS group. Hematological toxicity is the most common complication during chemotherapy treatment (Botelho *et al*., 2020), and the cytopenia that occurs can be severe and potentially life-threatening, often leading to the temporary or permanent suspension of the drug (Sleijfer *et al.*, 2018). Hou *et al.* (2009) reported that animals receiving doxorubicin presented anemia and lymphopenia, similar to what was observed in this experiment. However, *C. sinensis* could exacerbate the cytotoxic effect of doxorubicin or it might contain some other substance that would have a specific effect on white blood cells.

Although CS green tea has a number of health benefits, its constituents may be beneficial up to a certain dose, with higher doses possibly causing unknown side effects. Moreover, the effects of green tea catechins may not be the same in all individuals. Epigallocatechin gallate (EGCG) from green tea extract is cytotoxic and the excessive consumption of green tea can trigger acute cytotoxicity in liver cells (Schmidt *et al.*, 2005). Another study involving hamsters found that excessive intake of green tea might cause oxidative DNA damage in the pancreas and liver (Takabayashi et al., 2006). Yun, Kim and Song (2006) explained that EGCG acts as a pro-oxidant rather than an antioxidant in pancreatic β cells *in vivo*. Consequently, high intake of green tea may be detrimental to the hypoglycemic control in diabetic animals.

Some of the animals submitted to Dox also showed a decline in total proteins with a drop in the γ- globulins fraction, which is composed of immunoglobulins. It is known that a decrease in γ- globulins occurs in immunodeficiencies of different causes (Eckersall, Bell, 2014). Thus, Dox toxicity is attributed to the occurrence of these findings that complement lymphopenia and gamma globulinemia. Immunoglobulin (IgA and IgG) is normally the last fraction viewed in electrophoresis, primarily the IgG immunogamaglobulin. Accordingly, a significant reduction of γ-globulin in the Dox+CS group when compared to other groups was due to a decrease in IgG, a common occurrence in animals treated with Dox.

Albumin is a negative acute phase protein whose concentration gradually falls during infectious and inflammatory diseases. The secretion of albumin is stimulated by a drop in osmotic pressure but it can also be activated by pathophysiological changes, such as during an infectious or inflammatory disease when the secretion is reduced, which is caused by proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor- α (TNF). These cytokines are simultaneously responsible for the increased synthesis and secretion of the acute phase proteins (Ceron, Eckersall, Martýnez-Subiela, 2005).

The results of this study demonstrated that Dox induces cardiotoxicity by interfering with both radial and longitudinal twist and motion of the left ventricle in Wistar rats. In addition, the flavonoid-rich extract from *C. sinensis* was able to quickly restore radial ventricular orientation of the myocardial fibers, promoting improvement in the global radial strain. No short-term improvement was observed in the longitudinal strain due to the toxicity induced by the Dox assembly to other cardiac insults.

CONCLUSION

The results of this study suggest that *C. sinensis* could be administrated in patients who are receiving Dox treatment to preserve the viability of cardiomyocytes, but their blood profile should be frequently evaluated.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interest.

REFERENCES

Al-Malky HS, Al Harthi SE, Osman AM. Major obstacles to doxorubicin therapy: Cardiotoxicity and drug resistance. J Oncol Pharm Pract. 2020;26(2):434-44.

Almeida ALC, Silva VA, Souza Filho AT, Rios VG, Lopes JRP, Afonseca SO, et al. Subclinical ventricular dysfunction detected by speckle tracking two years after use of anthracycline. Arq Bras Cardiol. 2015;104(4):274-83.

Basu A, Sanchez K, Leyva MJ, Wu M, Betts NM, Aston CE, et al. Green tea supplementation affects body weight, lipids, and lipid peroxidation in obese subjects with metabolic syndrome. J Am Coll Nutr. 2010;29(1):31-40.

Botelho AFM, Lempeck MR, Branco SEMT, Nogueira MM, de Almeida ME, Costa AG, et al. Coenzyme Q10 cardioprotective effects against doxorubicin-induced cardiotoxicity in Wistar rat. Cardiovasc Toxicol. 2020;20(3):222-34.

Ceron JJ, Eckersall PD, Martýnez-Subiela S. Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet Clin Pathol. 2005;34(2):85-9.

Chacko SM, Thambi PT, Kuttan R, Nishigaki I. Beneficial effects of green tea: a literature review. Chin Med. 2010;5(13):1–9.

Chandran K, Aggarwal D, Migrino RQ, Joseph J, McAllister D, Konorev EA, et al. Doxorubicin inactivates myocardial cytochrome c oxidase in rats: cardioprotection by Mito-Q. Biophys J. 2009;96(4):1388-98.

Del Castillo JM, Herszkowicz N, Ferreira C. Speckle tracking - a contratilidade miocárdica em sintonia fina. Rev Bras Ecocardiogr Imagem Cardiovasc. 2010;23(3):46-4.

Eckersall PD, Bell R. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. Vet J. 2010;185(1):23-7.

Elliott P. Pathogenesis of cardiotoxicity induced by anthracyclines. Semin Oncol. 2006;33(3 Suppl 8), S2–S7.

Eslami V, Bayat F, Asadzadeh B, Saffarian E, Gheymati A, Mahmoudi E, et al. Early prediction of ventricular functional recovery after myocardial infarction by longitudinal strain study. Am J Cardiovasc. 2021;11(4):471-77.

Gallay-Lepoutre J, Bélanger MC, Nadeau ME. Prospective evaluation of Doppler echocardiography, tissue Doppler imaging and biomarkers measurement for the detection of doxorubicin-induced cardiotoxicity in dogs: A pilot study. Res Vet Sci. 2016;105:153-59.

Hazari MS, Haykal-Coates N, Winsett DW, Costa DL, Farraj AK. Continuous electrocardiogram reveals differences in the short-term cardiotoxic response of Wistar-Kyoto and spontaneously hypertensive rats to doxorubicin. Toxicol Sci. 2009;110(1):224-34.

Hou XW, Jiang Y, Wang LF, Xu HY, Lin HM, He XY, et al. Protective role of granulocyte colony-stimulating factor against adriamycin induced cardiac, renal and hepatic toxicities. Toxicol Lett. 2009;187(1):40-4.

Karagoz B, Suleymanoglu S, Uzun G, Bilgi O, Aydinoz S, Haholu A, et al. Hyperbaric oxygen therapy does not potentiate doxorubicin-induced cardiotoxicity in rats. Basic Clin Pharmacol Toxicol. 2008;102(3):287-92.

Kashiura M, Fukushima F, Tamura H, Moriya T. T-wave alternans: A harbinger of polymorphic ventricular tachycardia. Oxf Med Case Rep. 2019;9:410–11.

Kenk M, Thackeray JT, Thorn SL, Dhami K, Chow BJ, Ascah KJ, et al. Alterations of pre- and postsynaptic noradrenergic

signaling in a rat model of adriamycin-induced cardiotoxicity. J Nucl Cardiol. 2010;17(2):254-63.

Leader CJ, Moharram M, Coffey S, Sammut IA, Wilkins GW, Walker RJ. Myocardial global longitudinal strain: An early indicator of cardiac interstitial fibrosis modified by spironolactone, in a unique hypertensive rat model. Plos One. 2019;14(8):e0220837.

Lustosa BB, Polegato B, Minicucci M, Rafacho B,Santos PP, Fernandes AA, et al. Green tea (*Camellia sinensis*) attenuates ventricular remodeling after experimental myocardial infarction. Int J Cardiol. 2016;225:147-53.

Nagueh SF, Shah DJ. Echocardiography first but here comes CMR for grading left ventricular diastolic function. ACC Cardiovasc Imaging. 2020;13(12):2543-45.

Oliveira MS, Carvalho JL, Campos ACA, Gomes DA, Goes AM, Melo MM, Doxorubicin has *in vivo* toxicological effects on *in vivo* cultured mesenchymal stem cells. Toxicol Lett. 2014; 224(3):380-86.

Oliveira MS, Melo MB, Carvalho JL, Melo IM, Lavor MSL, Gomes DA, et al. Doxorubicin cardiotoxicity and cardiac function improvement after stem cell therapy diagnosed by strain echocardiography. J. Cancer Sci. Ther. 2013;5(2):52-7.

Ramadan MS, Bertolino L, Zampino R, Durante-Mangoni E. Cardiac sequelae after coronavirus disease 2019 recovery: a systematic review. Clin Microbiol Infect. 2021;27(9):1250-61.

Saeed NM, El-Naga RN, El-Bakly WM, Abdel-Rahman HM, Salah ElDin RA, El-Demerdash E. Epigallocatechin-3-gallate pretreatment attenuates doxorubicin-induced cardiotoxicity in rats: A mechanistic study. Biochem Pharmacol. 2015;95(3):145-55.

Schmidt M, Schmitz HJ, Baumgart A, Guedon D, Netsc, MI, Kreuter MH, et al. Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. Food Chem Toxicol. 2005;43(2):307-14.

Singh RB, Sozzi FB, Fedacko J, Hristova K, Fatima G, Pella D, et al. Pre-heart failure at 2D- and 3D-speckle tracking echocardiography: A comprehensive review. Echocardiography. 2022;39(2):302-09.

Sleijfer S, Rizzo E, Litière S, Mathijssen RHJ, Judson IR, Gelderblom H, et al. Predictors for doxorubicin-induced hematological toxicity and its association with outcome in advances soft tissue sarcoma patients; a retrospective analysis of the EORTC-soft tissue and bone sarcoma group database. Acta Oncol. 2018;57(8):1117-26.

Takabayashi F, Tahara S, Kaneko T, Harada N. Effect of green tea catechins on oxidative DNA damage of hamster pancreas and liver induced by N-nitrosobis (2-oxopropyl) amine and/or oxidized soybean oil. Biofactors. 2004;21(1-4):335-37.

Xiang P, Yan H, Karen D, Huang LG, Chen Y, Tu L, et al. Dexrazoxane protects against doxorubicin-induced cardiomyopathy : upregulation of Akt and Erk phosphorylation in a rat model. Cancer Chemother Pharmacol. 2009;63(2):343-49.

Yun SY, Kim SP, Song DK. Effects of (-)-epigallocatechin-3-gallate on pancreatic beta-cell damage in streptozotocin-induced diabetic rats. Eur J Pharmacol. 2006;541(1-2):115-21.

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