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ORAL ADMINISTRATION OF *MELISSA OFFICINALIS* OR *BAHUINIA FORFICATA* INFUSIONS REDUCE THE POSTISCHEMIC RECOVERY OF HEARTS FROM HYPOTHYROID RATS

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Abstract

Drinking infusions of *Melissa officinalis* (Moff, "lemon balm") or *Bahuinia forficata* (Baf, "cow´s hoof") are clinically contraindicated in hypothyroidism (HypoT). However, it is not known whether they interfere in the cardiac response to coronary dysfunction. So, the aim of this work was to evaluate whether these plants interfere with the cardioprotection of HypoT after ischemia/reperfusion (I/R) in rats. In this model, HypoT was induced in rats by drinking methimazole for 15 days. Euthyroid (EuT) and HypoT rats drunk either Moff or Baf infusion during 7 days. Isolated perfused hearts were exposed to 20 min I/45min R inside a calorimeter, a model of stunning without infarct. Contractile pressure development (P, mmHg), diastolic pressure (LVEDP) and total heat rate (Ht, in mW/g) were measured. In EuT, Moff improved the postischemic contractile recovery (PICR) (105.0±16.4% of initial vs 69.4±6.0% in non-treated, $p<0.05$) and muscle economy (P/Ht). Baf slightly reduced PICR (40.4±6.7%) and significantly increased LVEDP. In HypoT hearts, Moff and Baf drastically reduced PICR (54.7±3.3% and 12.5±5.3% respectively vs 92.6±5.2% in non-treated, both $p<0.05$) as well as P/Ht, while increased LVEDP. Cyclosporine-A reversed the Moff dysfunction but not that of Baf, which was neither reduced by diazoxide. Results show that under HypoT Moff induced the opening of mPTP which reduce postischemic recovery, and Baf worsened the dysfunction although it was not related to mitochondrial calcium overload. Results suggest that the use of these plants in hypothyroid individuals with risk of cardiac ischemic episodes requires precaution.

Keywords: *heart; ischemia/reperfusion; stunning; hypothyroidism; lemon-balm; cow´s hoof*

Introduction

Hypothyroidism frequently appears in population over 30 years old and it is considered a risk factor for cardiovascular mortality [1, 2]. Even when it is easily treated with daily oral levothyroxine (T4) there are some interactions with medicines which worsen that endocrinological alteration. Among these, some medicinal plants interfere with the TSH binding to the receptor on the thyroid gland and with the basal and TSH-stimulated cyclic AMP [3]. One of these plants is *Melissa officinalis* L. (Lamiaceae), known as "lemon balm", which is frequently used in the phytotherapy of many countries as eupeptic, slightly sedative and antispasmodic, as well as for treating hypertension, dizziness, headache and palpitations [4, 5]. Other authors demonstrated *in vivo* inhibition of thyroid iodide transport, thyroid hormone secretion, and iodothyronine deiodination as well as antagonism to endogenous and exogenous TSH by extracts of *M. officinalis* [4, 6, 7]. Cardiotonic properties were also attributed to *M. officinalis* but not demonstrated [8]. A more recent work showed that the aqueous extract of *M. officinalis* at 50-200 mg/kg via i.p. induced a mild protective effect against severe arrhythmias consequent to episodes of cardiac ischemia and reperfusion in rats [9].

On the other hand, one of the most used plants for treating type-2 diabetes mellitus in South America belongs to the genus *Bauhinia* (Fabaceae) popularly known as "pata-de-vaca" or "cow´s hoof". The most commonly cited species is *Bahuinia forficata* L., whose leaves have the flavonoids kaempferitrina, kaempferol-3-O-α-diraminoside and the steroid sitosterol as the hypoglycemic active components [10, 11, 12]. It is widely distributed in semitropical and temperate regions of Brazil, Paraguay and Argentina, as well as in Africa [13]. The genus *Bauhinia* comprises 300-350 species, and it has been divided into 9 genera based on phylogenetic data, all of them with poliphenols and antioxidant properties [14]. *B. forficata* has been cited as a medicinal plant candidate to give thyroid hormone analogs according to ethnobotanical research in Salvador-Bahia, Brazil [12], as well as by the *in vitro* inhibition of thyroid peroxidase enzyme to about a half [15].

Drinking infusions of leaves from *M. officinalis* or *B. forficata* was contraindicated in hypothyroid patients because they potentiate the clinical endocrinological alteration [7]. Moreover, it is commonly thought that hypothyroidism is a risk factor for the cardiac diseases such as angor. The subclinical hypothyroidism in patients with cardiac diseases increased the mortality, but this was controlled by levothyroxine administration [2]. However, it was reported that hypothyroidism reduces the incidence of acute episodes of coronary syndrome in hospitalized patients of angor [16]. It is widely known that hearts reduce pressure development as a consequence of a short ischemic episode, and this condition is called "stunning". Accordingly, results from our laboratory showed that hypothyroidism improved the contractile recovery of isolated rat hearts exposed to no-flow ischemia and reperfusion (I/R) [17, 18]. So, the aim of this work was to evaluate whether these two medicinal plants have any effect on the postischemic cardiac stunning in interaction with the hypothyroidism, and which was the associated mechanism.

Methods

Ethical approval

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as was recommended in the Guide for Care and Use of Animals (NIH Nro publication # 85-23 revised in 1985 and 1996, National Academy Press, Washington DC, USA), according to the Resolution 1047 anexo II of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) de la República Argentina, 2005). The protocols were approved by the Laboratory Animals Care Commitee (CICUAL) of Facultad de Ciencias Exactas, Universidad Nacional de La Plata (Nr. 015-2015, approved on August 2015). Wistar rats were maintained in biotery with ad libitum food and water, and cycles light/dark 12 hs/12 hs.

Plant material and extracts

Leaves of both plants were obtained from a local herboristery, *Melissa officinalis* (Herboplata S.R.L., Argentinian Public Health certification 697/01) and *Bauhinia forficata* L. (Herbores S.R.L. lote 816/B,

Argentinian Public Health certification 12002-27007-130) and identified by MSc Agr. Ing. Martha Colares in the Herbarium of the Universidad Nacional de La Plata. Infusions of leaves from both plants were prepared at 5% w/v for oral administration.

Characterization of plant extracts

Preparation of samples

The extract for chromatographic assays was prepared from 1 gram of leaves (*Melissa officinalis* or *Bahuinia forficata*) in contact with 10 ml methanol during 5 minutes in a water bath at 60°C, and then filtered.

Chromatographic conditions

Samples were analyzed using a Dionex Ultimate 3000 UHPLC (Thermo Scientific, CA, USA) configured with a dual gradient tertiary pump (DGP-3000) and a DAD-3000 diode array detector. The stationary phase was a Thermo RP-8 column (250 x 4.6 mm, 5 µm, Thermo Scientific, USA) and the mobile phase was a 85:10:5 mixture of water : tetrahydrofuran : methanol adjusted to pH = 3 with phosphoric acid. The system was operated isocratically at room temperature. Flow rate was set at 1 ml/min, and the detection was performed at 264 nm. The extracts were diluted 1:2 with mobile phase and filtered through a 0.45 µm nylon membrane filter (13 mm, Osmonics Inc., USA) prior to their injection by triplicate (20 µl).

Qualitative analysis of the samples

Stock solutions of reference standards of gallic acid, chlorogenic acid, catechin, caffeic acid, isovitexin, rutin, vitexin and hyperoside were prepared in methanol. Then, adequate volumes were combined and diluted with mobile phase to yield a combined standard solution with approximate concentrations of 65, 10, 50, 30, 20, 20 and 10 mg/l, respectively.

Chromatography peaks present in the samples were confirmed by comparing their retention time and DAD spectra (200 – 400 nm) with those of the combined standard solution.

Pharmacological studies

In vivo treatments

Hypothyroidism (HypoT) was induced by adding methimazole (0.02%) in drinking water for 15 days in adult Wistar rats of either sex (230 to 280 g weight), while other rats were used as euthyroid controls (EuT). Hypothyroidism was tested by measuring the T4 free and TSH levels in blood of some of the rats before the ex vivo experiment.

Infusions 5% of *Melissa officinalis* or *Bahuinia forficata* leaves were orally administered ad-libitum, instead of drinking water, during 7 days to either euthyroid and hypothyroid rats (during the second week of methimazol treatment). After those periods, the experiment of isolated heart was performed as previously described.

Isolated heart preparation and contractile measurements

Wistar rats were heparinized (non-fractioned heparine 2000 IU) and anesthetized with pentobarbital overdose (60 mg/kg via i.p.) and tramadol (10-20 mg/kg via s.c.). Rats were spontaneously breathing during the general anesthesia, while the heart was rapidly excised. Retrograde perfusion was applied through coronary arteries by the Langendorff method, with control Krebs (C) at 37 °C and at a constant flow rate of 7 ml/min by gram, by a peristaltic pump (Gilson Minipuls, France), as previously described [19, 20]. The experimental conditions (working temperature, heart rate and I/R periods) were similar to those of previous models of cardiac stunning in rat hearts, without a significative infarct [20, 21]. Atria and the spontaneous beating were removed, and a latex balloon was introduced in the left ventricle, connected by a cannula to a Bentley DEL900 pressure transducer. While continuously perfused, the ventricles were introduced into the chamber of a flow calorimeter for little hearts, which was closed and submerged in a water bath kept at controlled temperature [19]. Ventricles were electrically stimulated with 5 V-5 ms at 3 Hz, by means of two electrodes connected to an electrical stimulator (Letica LE12406, Spain). The isovolumic left intraventricular pressure (LVP) was continuously recorded at optimal volume, as well as the total heat rate signal (both in mV) by a PowerLab 2/26 two channels digital acquisition system (AD Instruments, Australia). The LVP signal was calibrated in mmHg, and maximal pressure development (P) of a

contraction was calculated from the difference between LVP peak and the diastolic level or left ventricular end diastolic pressure (LVEDP). During I and R there were calculated the changes in diastolic pressure over the preischemic condition in Krebs-C (ΔLVEDP), as an estimation of diastolic contracture. Also, during I/R, the maximal pressure developed in contraction was expressed as a percentage of the steady initial P.

Calorimetrical measurements

The calorimeter was previously described [19, 22, 23]. The internal chamber has two ceramic modules with 127 thermosensitive units (Melchor Thermoelectrics, USA) each one, which detect changes in temperature between the inside (heart) and the outside (bath). Calibration and calorimetrical base lines in the abscence of heart and the presence and absence of perfusion were done as described before [19, 24]. The calorimeter was submerged in a water bath at 37.0 ± 0.01 °C in connection with other two baths which respectively control temperature and warm the perfusates. Cardiac heat output was recorded simultaneously to the signal of LVP in the same acquisition system. The total heat rate (Ht) of ventricle was calculated from that difference between cardiac signal and the base lines, and finally expressed in mW/g of wet weight. Moreover, the ischemic and post-ischemic measurements were expressed as a percentage of the steady initial Ht. Also, total muscle economy was calculated as the P/Ht ratio.

Experimental protocols

After about 40 min of stabilization with Krebs-C, an initial control value of P and Ht was recorded. Then hearts were exposed to different treatments followed by a period of 20 minutes of no-flow ischemia (I) and 45 minutes of reperfusion (R) in a model of moderate stunning, previously characterized [20, 21, 24].

Rats were distributed in the following basic treatment groups: control euthyroids (EuT, n = 7), control hypothyroids (HypoT, n = 5), euthyroids drinking *Melissa officinalis* (EuT-Moff, n = 4), euthyroid drinking *Bauhinia forficata* (EuT-Baf, n = 4), hypothyroids drinking *Melissa officinalis* (HypoT-Moff, n = 5), hypothyroids drinking *Bauhinia forficata* (HypoT-Baf, n = 4).

In order to understand whether cardiac dysfunction was associated to opening of the mitochondrial permeability transition pore (mPTP) 0.2 µmol/l cyclosporine-A was perfused during 15 minutes before ischemia and during reperfusion [25] in isolated hearts of two groups (HypoT-Moff-Cys-A, n = 4, and HypoT-Baf- Cys-A, n = 4). Moreover, in order to block the sarcorreticular Ca^{2+} leak by the RyR2, 100 µmol/l tetracaine [26] was perfused during 5 minutes before ischemia and then reperfused with Krebs-C in a group of EuT-Baf - Tetrac (n= 4). The role of mKATP channels was evaluated by perfusing hearts of EuT-Baf group with 30 µM diazoxide (EuT-Baf -Dzx, n= 4) before ischemia and then reperfused with Krebs-C. As a positive control, EuT hearts were also perfused with 30 µM diazoxide (EuT-Dzx, n= 4) before I/R as a cardioprotective.

Solutions and drugs

Hearts were perfused with Krebs-C (in mM): 1 MgCl2, 125 NaCl, 0.5 NaH2PO4, 7 KCl, 2 CaCl2, 25 NaHCO3, and 6 dextrose, bubbled with 95% O2-5% CO2. Methimazole (Sigma-Aldrich, St Louis, MO, USA) was dissolved to 0.02% w/v in drinking water. Cyclosporine-A (Cys-A, Fluka, Sigma-Aldrich, USA) was prepared in DMSO at 0.2 mM and diluted to 0.2 µM in Krebs-C. Tetracaine hydrochloride (Sigma-Aldrich, USA) was prepared at 0.1 mM solution in Krebs. Diazoxide (Sigma-Aldrich, USA) was prepared at 30 mM in DMSO and diluted in Krebs-C to 30 µM. All the drugs were diluted from their stock solutions or directly in Krebs-C at the moment of the experiment. In chromatographic assays all solvents used were HPLC grade. Standard compounds used in HPLC were: gallic acid (Merck, Darmstadt, Germany), chlorogenic acid, catechin, caffeic acid, isovitexin, rutin, vitexin and hyperoside (Sigma-Aldrich, USA).

Statistical analysis

Results were expressed as mean ± SEM. Multiple comparisons by two-way ANOVA for repeated measures (factors were treatment and time) were done for the respective groups of experiments. Tukey post-tests were done among the treatments when a significant difference was found by ANOVA, and their results are shown in each figure. Always a significance level of $p < 0.05$ was considered. All

statistical analyses were performed by using the Graph Pad Prism v.4 software.

Results

Chromatographic characterization of the plant extracts

Although the aim of this work was not to analyze in detail the phytochemistry of these plants, the general chromatographic profile was determined as a characterization. Figure 1 shows the chromatograms of the combined standard solution (a), *Melissa officinalis* extract (b) and *Bauhinia forficata* extract (c). Based on the identification criteria described (retention time and UV spectra), the presence of caffeic acid (peak #4) and rutin (peak #6) could be detected in the *Melissa officinalis* extract, while a high peak not identified by a standard suggests the presence of an isoflavanone (peak #9) according to the respective UV spectra [27, 28]. In *Bauhinia forficata* extract there were identified low quantities of vitexin (peak #7). According to the respective UV spectra, the main peaks not identified by standards suggest the presence of flavanones (peaks #10 and #11).

Effects of *Melissa officinalis* in euthyroid rat hearts

Control euthyroid rat (EuT-C) hearts developed an initial P of 95.0 ± 5.6 mmHg and Ht of 22.7 ± 1.5 mW/g. As usual, the 20 minutes period of no-flow ischemia reduced both, P and Ht, up to about zero while the diastolic tone was reduced. During the first minutes of reperfusion (R) the diastolic tone (ΔLVEDP) increased and then was gradually reduced (Fig. 2a). At 45 minutes R, P recovered up to 69.3 ± 6.0% of preischemic (Fig. 3a), and Ht recovered to 89.1 ± 6.0% of initial. In EuT-Moff rats, the hearts (initial P of 71.3 ± 4.6 mmHg and Ht of 14.0 ± 0.4 mW/g) increased their postischemic recoveries of P (to 105.0 ± 11.6%, $p<0.05$ vs EuT-C rats, Fig. 3a) and Ht (to 99.5 ± 3.3% of initial) with about the same reversible increase in the ΔLVEDP (Fig. 2a). Moreover, EuT-Moff increased the total muscle economy (P/Ht) during R respect to EuT-C hearts (Fig. 3b).

Effects of *Melissa officinalis* in hypothyroid rat hearts

Control hypothyroid rat (HypoT-C) hearts developed an initial P of 74.5 ± 9.1 mmHg and Ht of 14.9 ± 0.8 mW/g. As usual, the no-flow ischemia reduced P (Fig. 3c) and Ht. The diastolic tone was increased at the end of I but it was reversed during R (Fig. 2b). Comparing to EuT-C, the HypoT-C hearts recovered more the post-ischemic P and Ht, up to 105.0 ± 11.6% (Fig. 3c) and 107.7 ± 18.7% of initial, respectively at 45 minutes R. However, the hearts from HypoT rats which orally received Moff (initial P of 79.7 ± 3.7 mmHg and Ht of 15.9 ± 0.9 mW/g) reduced the post-ischemic contractile recovery (PICR) to 54.7 ± 4.4% of initial P ($p<0.05$ vs HypoT-C rats, Fig. 3c) and maintained the Ht recovery up to 101.2 ± 4.8% of initial. Consequently, Moff reduced the recovery of total muscle economy (P/Ht) during R in HypoT hearts (Fig. 3d). Perfusion of Cys-A to HypoT- Moff hearts (initial P of 79.7 ± 4.3 mmHg and Ht of 15.0 ± 0.8 mW/g) improved the recoveries of P and P/Ht (Figs. 3c and 3d) although increased the diastolic contracture respect to the HypoT-Moff group (Fig. 2b).

Effects of *Bahuinia forficata* in euthyroid rat hearts

The oral treatment of EuT rats with Baf (initial P of 76.6 ± 11.0 mmHg and Ht of 14.0 ± 2.6 mW/g) reduced the PICR of isolated hearts to 45.1 ± 10.4% ($p<0.05$ vs control rats, Fig. 4a) and maintained recovery of Ht up to 93.6 ± 17.0% of initial. The total muscle economy (P/Ht) was also reduced during R (Fig. 4b) and the diastolic contracture was increased during both I and R (Fig. 2a). The dysfunction of Baf was not reduced by perfusing hearts with 100 µM tetracaine (initial P of 65.0 ± 2.4 mmHg and Ht of 19.5 ± 0.1 mW/g) or with 30 µM diazoxide (initial P of 52.9 ± 17.2 mmHg and Ht of 12.2 ± 2.5 mW/g), because P and P/Ht remained low (Fig. 4a and b) and the diastolic contracture high (Fig. 2a).

Effects of *Bahuinia forficata* in hypothyroid rat hearts

The oral treatment of hypothyroid rats with Baf (initial P of 76.7 ± 12.7 mmHg and Ht of 11.9 ± 2.1 mW/g) also reduced the contractile recovery of isolated hearts to 12.0 ± 5.3% ($p<0.05$ vs control HypoT rats, Fig. 4c) and Ht to 96.5 ± 13.6% of initial at the end of R. The P/Ht ratio was also reduced during R (Fig. 4d) and the diastolic contracture was

increased (Fig. 2b). The dysfunction of Baf in HypoT rats was not reduced by perfusing hearts with Cys-A (initial P of 57.2 ± 5.4 mmHg and Ht of 9.6 ± 1.2 mW/g) (Figs. 2b, 4c and 4d).

Discussion

Results show that hypothyroid rats which drunk the acqueous crude extracts of *Melissa officinalis* or *Bahuinia forficata* suffered worsening of cardiac stunning during reperfusion, while in euthyroid rats *M. officinalis* was cardioprotector. Although these results are preclinical and not directly extrapolated to humans, they suggest having precaution in the use of these medicinal plants in patients of coronary disease and hypothyroidism. However, this work also suggests that drinking infusions of *Melissa officinalis* could be beneficial in euthyroid patients of coronary insufficience.

Our results show that oral administration of Moff to euthyroid rats (EuT) during one week improved the post-ischemic contractile recovery (PICR) and the muscle economy (P/Ht) (Fig. 3a-b). These results suggest that Moff has good cardiac properties when the thyroid state is normal, and agree with the only two reports about the cardiac properties of this aromatic medicinal plant traditionaly used for gastrointestinal and anxiety diseases. One report described that *Melissa officinalis* induced bradycardia in rats without changing the contractile force [8], and the other showed a mild protective effect against severe arrhythmias consequent to episodes of cardiac ischemia and reperfusion in rats [9]. Our study gives information about the contractile and energetic performance of the reperfused hearts in rats which received Moff during a subacute period of one week. The Moff-dependent increases in both, muscle economy (P/Ht) and contractile recovery, suggest that mitochondrial metabolism was improved and coupled to the cardiac demand. Other report has also shown that *Melissa officinalis* extract produces vasodilation dependent of endothelial nitric oxide, and rosmarinic acid, ester of caffeic acid, was one of the vasodilator compounds present in it [29]. Although our aim was not to identify the phytochemical components, because they had been previously described [30, 31, 32], the HPLC characterization let us to identify two compounds (caffeic acid and rutin) and suggested the presence of a flavanone. This finding would be in accordance with previous studies that described the presence of the flavanone hesperidin [30] and the flavonoid luteolin-3´-glucuronide [31] as important constituents of *M. officinalis*. Others reported also the presence of antioxidant triterpens in *M. officinalis*, such as ursolic, carnosic and oleanolic acids [33]. Both, the vasodilator and antioxidant activities of flavonoids and triterpens could contribute to protect heart from I/R dysfunction and maintain the mitochondrial metabolism. In fact, it was found that the *M. officinalis* extract reduces the lipid peroxidation [34]. Moreover, flavonoids demonstrated to be cardioprotective against I/R with mechanisms such as calcium influx blockade, mitochondrial K^+ channels activation and metabolism stimulation, which consequently reduce the mitochondrial calcium overload [35].

On the other hand, the group of HypoT rats drinking Moff reduced contractile recovery (Fig. 3c) and increased diastolic contracture (ΔLVEDP) with respect to the HypoT group (Fig. 2b). Consequently, the muscle economy (P/Ht) was reduced, suggesting a great energetic cost to maintain the contractile performance (Fig. 3d). We have previously described that HypoT hearts improve PICR and muscle economy (P/Ht) respect to the EuT hearts, by mechanisms which attenuated the mitochondrial Ca^{2+} overload [17, 18]. It is well known that cardiac ischemia reduces the ATP level, and so it reduces the cytosolic Ca^{2+} extrusion. Under reperfusion, the sarcorreticular Ca^{2+} is lost to cytosol when free H^+, Na^+ and Ca^{2+} are accumulated when the ATP level is gradually restored from metabolism [36]. Those events become in a diastolic contracture and slow recovery of systolic contraction (PICR) with high energy released. Those alterations were prevented by hypothyroidism, with a lower mitochondrial Ca^{2+} overload [17, 18]. Results of Moff on postischemic HypoT hearts suggests that this extract further increased the cytosolic [Ca^{2+}] during I/R (increase in LVEDP), so compromising the energetic metabolism (reduced P/Ht). It is also known that in severe conditions of ischemia, mitocohondria suffer Ca^{2+} overload and the opening of the permeability transition pore (mPTP) [37]. When this happens, mitochondria lose part of their

constituents and cell become in metabolic energetical collapse, increase in cytosolic Ca^{2+}, diastolic contracture and reduction of contractility. This dysfunction of HypoT rats drinking Moff infusion involved the activation of the mitochondrial permeability transition pore (mPTP), since cyclosporine-A (Cys-A) improved P and P/Ht. This mechanism was also described in other conditions of I/R [25].

B. forficata would also be contraindicated in hypothyroid patients [12]. Our results in both, EuT and HypoT rat hearts showed that drinking Baf infusion worsened the mechano-energetical performance during I/R (Figs. 2 and 4). The great increase in diastolic contracture suggest a cytosolic Ca^{2+} overload, by which it was suggested that the opening of mPTP could be responsible of dysfunction. However, the fact that dysfunction in HypoT-Baf rat hearts was not avoided by perfusing Cys-A suggests that it was not due to the mPTP opening. Alternatively, the diastolic contracture could be due to a sarcorreticular Ca^{2+} leak [26], but this hypothesis was also rejected because the inhibition of RyR2 channels with 100 µmol/l tetracaine did not attenuate the dysfunction in EuT-Baf rat hearts. Another possibility was that Baf induces the mitochondrial Ca^{2+} overload and metabolic disruption with depolarization, thus worsening the myocardial dysfunction during I/R. To evaluate this hypothesis, EuT rat hearts were perfused with diazoxide (Dzx) before I/R. This drug opens the mitochondrial K+ channels (mKATP) and consequently increases the mitochondrial potential ($\Delta\Psi m$) in a way that reduces the Ca^{2+} influx through the mitochondrial Ca^{2+} uniporter [38, 39]. However, results showed that Dzx neither prevented the post-ischemic mechano-energetical dysfunction triggered by Baf, suggesting that mitochondrial Ca^{2+} overload was not the origin of dysfunction. It would be possible that another mechanism related to the reactive oxygen species (ROS) production underlies the Baf effect, because it is a frequent cause of severe injury and infarct [40, 41]. The strong fall in P/Ht suggests that Baf induced a mitochondrial energetical uncoupling during R, because the metabolism (sensed as Ht) increased more than contractility (P). The uncoupling reduces the ATP synthesis, and consequently affects the cytosolic Ca^{2+} removal, generating diastolic contracture. Moreover, SERCA activity and sarcorreticular Ca^{2+} content are reduced, as well as the Ca^{2+} transients and contractility. Considering the phytochemistry of *Bahuinia* infusions, it was also reported the presence of flavonoids, β-sitosterol, proanthocyanidines and fatty acids of 18 C, steroids and polyphenoles as kaempferitrin [14, 42, 43]. All of them have been described as antiinflammatory [44] and antioxidants among other activities [14, 43]. Moreover, kaempferitrin seems to be responsible of the antidiabetic effect [45]. The chromatographic profile obtained here for *B. forficata* was consistent with the presence of several flavonoids in the extract. However, it was reported that the leaves have cytoplasmatic inclusions of calcium oxalate as druse formate [45], coumarins and tanins [46], which could cause the negative cardiac effects of the extract. In fact, it was shown that the extract of *B. forficata* was cytotoxic against FO-1 cancer cells, although it was non-toxic against Artemia salina and normal human lymphocytes [47].

Conclusions

This work shows that drinking infusions of *Melissa officinalis* was preventive of the stunning induced by cardiac I/R in euthyroid rats but increased the post-ischemic dysfuntion in hypothyroid rats, due to activation of the mitochondrial mPTP and Ca^{2+} overload. Contrarily, drinking infusions of *Bahuinia forficata* increased dysfunction in both euthyroid and hypothyroid rat hearts, and such mechano-energetical dysfunction was not associated to Ca^{2+} overload. These results give pharmacological basis to the clinical precaution of using these medicinal plants in patients with coronary disease and hypothyroidism.

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References

1. McQuade C, Skugor M, Brennan DM, Hoar B, Stevenson C, Hoogwerf BJ. Hypothyroidism and moderate subclinical hypothyroidism are associated with increased all-cause mortality independent of coronary heart disease risk factors: a PreCIS database study. Thyroid 2011; 21: 837-843.

2. Jabbar A, Ingoe L, Pearce S, Zaman A, Razvi S. Thyroxine in acute myocardial infarction (ThyrAMI) - levothyroxine in subclinical hypothyroidism post-acute myocardial infarction: study protocol for a randomised controlled trial. Trials 2015; 16: 115.

3. Auf´mkolk M, Ingbar JC, Amir SM, et al. Inhibition by certain plant extracts of the binding and adenylate cyclase stimulatory effect of bovine thyrotropin in human thyroid membranes. Endocrinol 1984; 115: 527-534.

4. Brendler T, Gruenwald J, Kligler B, et al. Lemon Balm (Melissa officinalis L.): an evidence-based systematic review by the natural standard research collaboration. J Herb Pharmacother 2005; 5: 71-116.

5. Noguchi-Shinohara M, Ono K, Hamaguchi T et al. Pharmacokinetics, safety and tolerability of Melissa officinalis extract which contained rosmarinic acid in healthy individuals: a randomized controlled trial. PLoS One 2015; 10(5): e0126422. doi: 10.1371/journal.pone.0126422

6. Sourgens H, Winterhoff H, Gumbinger HG, Kemper FH. Antihormonal effects of plant extracts: TSH- and prolactin suppressing properties of Lithospermum officinale and other plants. Planta Med 1982; 45: 78.

7. Winterhoff H, Sourgens H, Kemper FH. Antihormonal effects of plant extracts: pharmacodynamic effects of Lithospermum officinale on the thyroid gland of rats; comparison with the effects of iodide. Horm Metab Res 1983; 15: 503-507.

8. Gazola R, Machado D, Ruggiero C, Singi G, Macedo Alexandre M. Lippia alba, Melissa officinalis and Cymbopogon citratus: effects of the aqueous extracts on the isolated hearts of rats. Pharmacol Res 2004; 50: 477-480.

9. Joukar S, Zarisfi Z, Sepehri G, Bashiri A. Efficacy of Melissa officinalis in suppressing ventricular arrhythmias following ischemia-reperfusion of the heart: a comparison with amiodarone. Med Princ Pract 2014; 23: 340-345.

10. da Silva KL, Cechinel Filho V. Plants of the genus Bauhinia: chemical composition and pharmacological potential. Química Nova 2002; 25: 449-454.

11. Pepato MT, Keller EH, Baviera AM, Kettelhut IC, Vendramini RC, Brunetti IL. Anti-diabetic activity of Bauhinia forficata decoction in streptozotocin-diabetic rats. J Ethnopharmacol 2002; 81: 191-197.

12. Cunha Lima ST, Rodrigues ED, Melo T, et al. Levantamento da flora medicinal usada no tratamento de doenças metabólicas em Salvador, BA- Brasil. Rev Bras Plantas Med 2008; 10: 83-89.

13. Alonso J, Desmarchelier C. Plantas Medicinales Autóctonas de la Argentina. Ed. LOLA, 2005; pp. 432-438.

14. Farag MA, Sakna ST, El-fiky NM, Shabana MM, Wessjohann LA. Phytochemical, antioxidant and antidiabetic evaluation of eight Bauhinia L. species from Egypt using UHPLC–PDA–qTOF-MS and chemometrics. Phytochem 2015; 119: 41-50.

15. Neto J, Ferreira A, Kuster R, Amorin M, Carvalho D. Inhibicao da peroxidase tireoidea pelo extrato de pata de vaca (Bahuinia forficata) e de pedra hume kaá (Myrcia uniflora). Inst Biofísica Carlos Chagas Filho e Núcleo de Pesquisas de Productos Naturais, Univ. Federal do Rio de Janeiro. 2001; Resumo P131. http://www.tiroides2000.org

16. Dhital R, Basnet S, Poudel DR. Impact of hypothyroidism on occurrence and outcome of acute coronary syndrome from the National Inpatient Sample. Am J Cardiol 2017; 120: 2160-2163.

17. Ragone MI, Lazarte ML, Consolini AE. Hypothyroidism reduces cardiac stunning with a mitochondrial regulation of sarcoreticular Ca2+ leak: a mechano-energetical study. XXII World congress

of the International Society for Heart Research (ISHR). J Mol Cell Cardiol 2016; 98 Suppl: 86-95, abstract 304.

18. Consolini AE, Ragone MI, Bonazzola P, Colareda GA. Mitochondrial bioenergetics during ischemia and reperfusion. Adv Exp Med Biol 2017; 982: 141-167.

19. Consolini AE, Ragone MI, Conforti P, Volonté MG. Mitochondrial role in ischemia-reperfusion of rat hearts exposed to high-K cardioplegia and clonazepam: energetic and contractile consequences. Can J Physiol Pharmacol 2007; 85, 483-496.

20. Ragone MI, Bonazzola P, Colareda GA, Lazarte ML, Bruno F, Consolini AE. Cardioprotection of stevioside on stunned rat hearts: a mechano-energetical study. Phytomed 2017; 35: 18-26.

21. Colareda GA, Ragone MI, Consolini AE. Sex differences in the mechano-energetical effects of genistein on stunned rat and guinea-pig hearts. Clin Exp Pharmacol & Physiol 2016; 43: 102-115.

22. Ponce-Hornos JE, Ricchiuti NV, Langer GA. Online calorimetry in arterially perfused rabbit interventricular septum. Am J Physiol- Heart Circ Physiol 1982; 243: H289-H295.

23. Ponce-Hornos JE, Bonazzola P, Marengo FD, Consolini AE, Márquez MT. Tension-dependent and tension-independent energy components of heart contraction. Pflugers Arch 1995; 429(6): 841-51.

24. Ragone MI, Bonazzola P, Colareda GA, Consolini AE. Cardioprotection of hyperthyroidism on the stunned rat heart under ischaemia/reperfusion: energetics and role of mitochondria. Exp Physiol 2015; 100.6: 680-697.

25. Ragone MI, Consolini AE. Role of the mitochondrial Ca2+ transporters in the high-[K+]o cardioprotection of rat hearts under ischemia and reperfusion: a mechano-energetic study. J Cardiovasc Pharmacol 2009; 54 (3): 213-223.

26. Sankaranarayanan R, Li Y, Greensmith DJ, Eisner DA, Venetucci L. Biphasic decay of the Ca transient results from increased sarcoplasmic reticulum Ca leak. J Physiol 2016; 594: 611-623.

27. Gattuso G, Barreca D, Gargiulli C, Leuzzi U, Caristi C. Flavonoid Composition of Citrus Juices. Molecules 2007; 12: 1641-1673.

28. Tsimogiannis D, Samiotaki M, Panayotou G, Oreopoulou V. Characterization of Flavonoid Subgroups and Hydroxy Substitution by HPLC-MS/MS. Molecules 2007; 12: 593-606.

29. Ersoy S, Orhan I, Turan NN, Sahan G, Ark M, Tosun F. Endothelium-dependent induction of vasorelaxation by Melissa officinalis L. ssp. officinalis in rat isolated thoracic aorta. Phytomed 2008; 15: 1087-1092.

30. Carnat AP, Carnat A, Fraisse D, Lamaison JL. The aromatic and polyphenolic composition of lemon balm (Melissa officinalis L. subsp. officinalis) tea. Pharm Acta Helv 1998; 72: 301-305.

31. Caniova A, Brandsteterova E. HPLC analysis of phenolic acids in Melissa officinalis. J Liq Chrom Rel Technol 2001; 24: 2647-2659.

32. Carocho M, Barros L, Calhelha RC, et al. Melissa officinalis L. decoctions as functional beverages: a bioactive approach and chemical characterization. Food Funct 2015; 6: 2240-2248.

33. Herodez SS, Hadolin M, Skerget M, Kne Z. Solvent extraction study of antioxidants from Balm (Melissa officinalis L.) leaves. Food Chem 2003; 80: 275-282.

34. Hohmann J, Zupko I, Redei D, et al. Protective effects of the aerial parts of Salviaofficinalis, Melissa officinalis and Lavandula angustifolia and their constituents against enzyme-dependent and enzyme- independent lipid peroxidation. Planta Med 1999; 65: 576-578.

35. Testai L. Flavonoids and mitochondrial pharmacology: a new paradigm for cardioprotection. Life Sci 2015; 135: 68-76.

36. Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 1999; 79: 609-634.

37. Bernardi P, Di Lisa F. The mitochondrial permeability transition pore: molecular nature and role as a target in cardioprotection. J Mol Cell Cardiol 2015; 78: 100-106.

38. Garlid KD, Paucek P, Yarov-Yarovoy V, et al. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K+ channels. Possible mechanism of cardioprotection. Circ Res 1997; 81: 1072-1082.

39. Murata M, Akao M, O`Rourke B, Marbán E. Mitochondrial ATP-sensitive potassium channels attenuate matrix Ca2+ overload during simulated ischemia and reperfusion. Possible mechanism of cardioprotection. Circ Res 2001; 89: 891-898.

40. Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev 2008; 88: 581-609.

41. Jang S, Lewis TS, Powers C, et al. Elucidating mitochondrial electron transport chain supercomplexes in the heart during ischemia-reperfusion. Antioxid. Redox Signal 2017; 27: 57-69.

42. Cechinel-Filho V. Chemical composition and biological potential of plants from the genus Bauhinia. Phytother Res 2009; 23: 1347-1354.

43. Ferreres F, Gil-Izquierdo A, Vinholes J, Silva ST, Valentao P, Andrade PB. Bauhinia forficata Link authenticity using flavonoids profile: Relation with their biological properties. Food Chem 2012; 134: 894–904.

44. Handa SS, Chawla AS, Sharma AK. Plants with antiinflammatory activity. Fitoterapia 1992; 63: 3-31.

45. da Silva KL, Weber Biavatti M, Leite SN, Yunesb, RA, Delle Monachec F, Cechinel Filho V. Phytochemical and pharmacognostic investigation of Bauhinia forficata Link (Leguminosae). Z Naturforsch 2000; 55c: 478-480.

46. Marques GS, Marques Lyra MA, Santos Peixoto M, et al. Caracterização fitoquímica e físico-química das folhas de Bauhinia forficata Link coletada em duas regiões brasileiras. Rev Ciênc Farm Básica Apl 2012; 33: 57-62.

47. Miceli N Buongiorno, L.P., Celi, M.G.,et al. Role of the flavonoid-rich fraction in the antioxidant and cytotoxic activities of Bauhinia forficata Link. (Fabaceae) leaves extract. Nat Prod Res 2016; 30,1229-1239.

Figure 1. High-performance liquid chromatography profiles of the combined standard solution (a), Melissa officinalis (b) and Bauhinia forficata (c) extracts. Peak numbers corresponding to standard compounds: (1) Gallic acid; (2) Chlorogenic acid; (3) Catechin; (4) Caffeic acid; (5) Isovitexin; (6) Rutin; (7) Vitexin; (8) Hyperoside. UV spectra of the identified peaks and those from the main non-identified flavonoids are shown at the right side

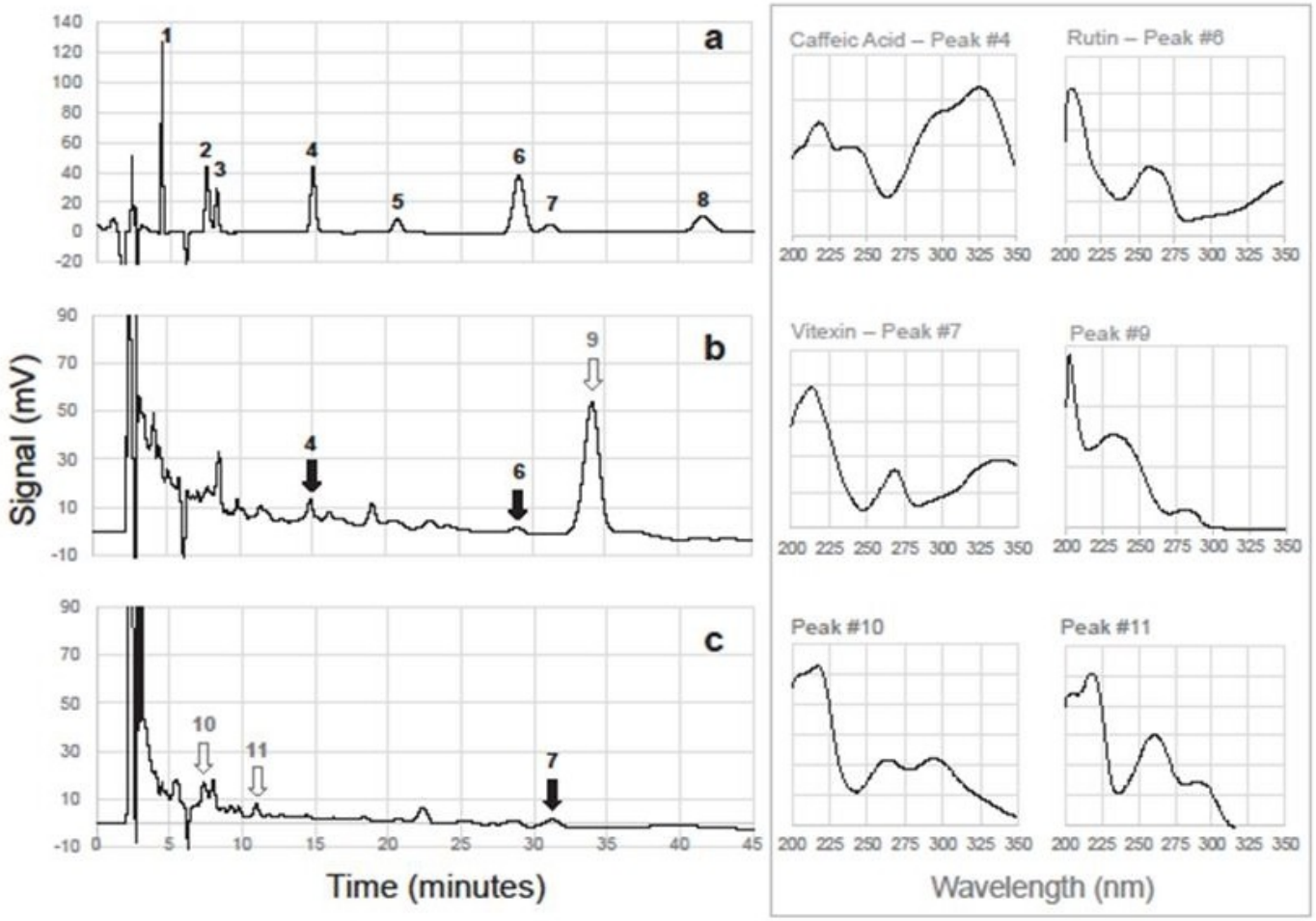

Figure 2. Effects of treatments with oral 5% infusion of *Melissa officinalis (Moff)* and *Bahuinia forficata (Baf)* on the changes in diastolic pressure (ΔLVEDP) suffered by isolated hearts from euthyroid (EuT, a) and hypothyroid (HypoT, b) rats exposed to I/R, at the following times: 5 min I, 20 min I, 5 min R and 45 min R. Moreover, the effects of perfusing the following drugs: 0.2 µmol/l cyclosporine-A (Cys-A), 100 µmol/l tetracaine (Tet) or 30 µmol/l diazoxide (Dzx) are shown. Two-way ANOVA: by treatment: F = 68.54 in (a) and 13.86 in (b); by time: F = 26.67 in (a) and 9.09 in (b), all p < 0.0001; Tukey´s tests: * p < 0.05 vs EuT-C in (a) and * p < 0.05 vs HypoT-C in (b), n is in brackets in each group.

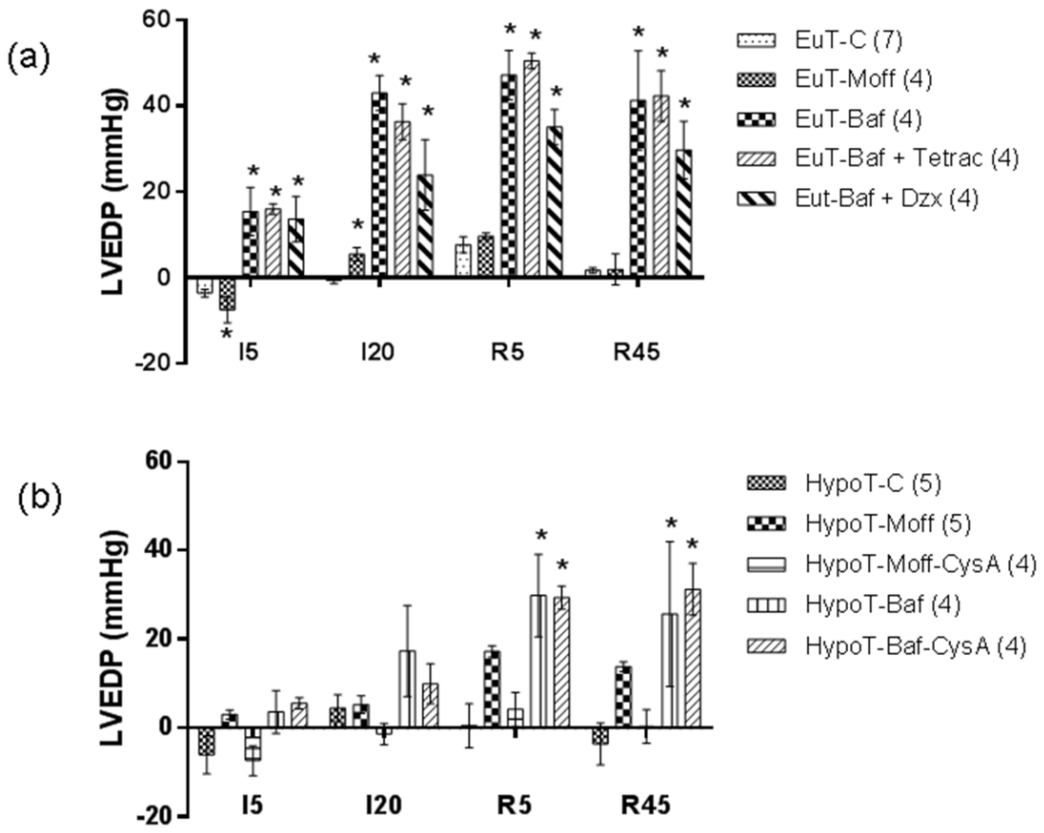

Figure 3. Effects of oral treatment with 5% infusion of Melissa officinalis on the cardiac pressure development (P) and the total muscle economy (P/Ht) during ischemia and reperfusion (I/R) of euthyroid rats (EuT-Moff) versus non-treated EuT rats (EuT-C) (a and b), and in hypothyroid rats (HypoT-Moff) in the absence and the presence of 0.2 µmol/l cyclosporine-A perfusion (HypoT-Moff-Cys-A) versus non-treated hypothyroid rats (HypoT-C) (c and d). See two-way ANOVA in Table 1; Tukey´s tests: * $p < 0.05$ vs EuT-C in (a/b) and * $p < 0.05$ vs. HypoT-C in (c/d), # $p < 0.05$ vs HypoT-Moff; n is in brackets in each group in (a) and (c).

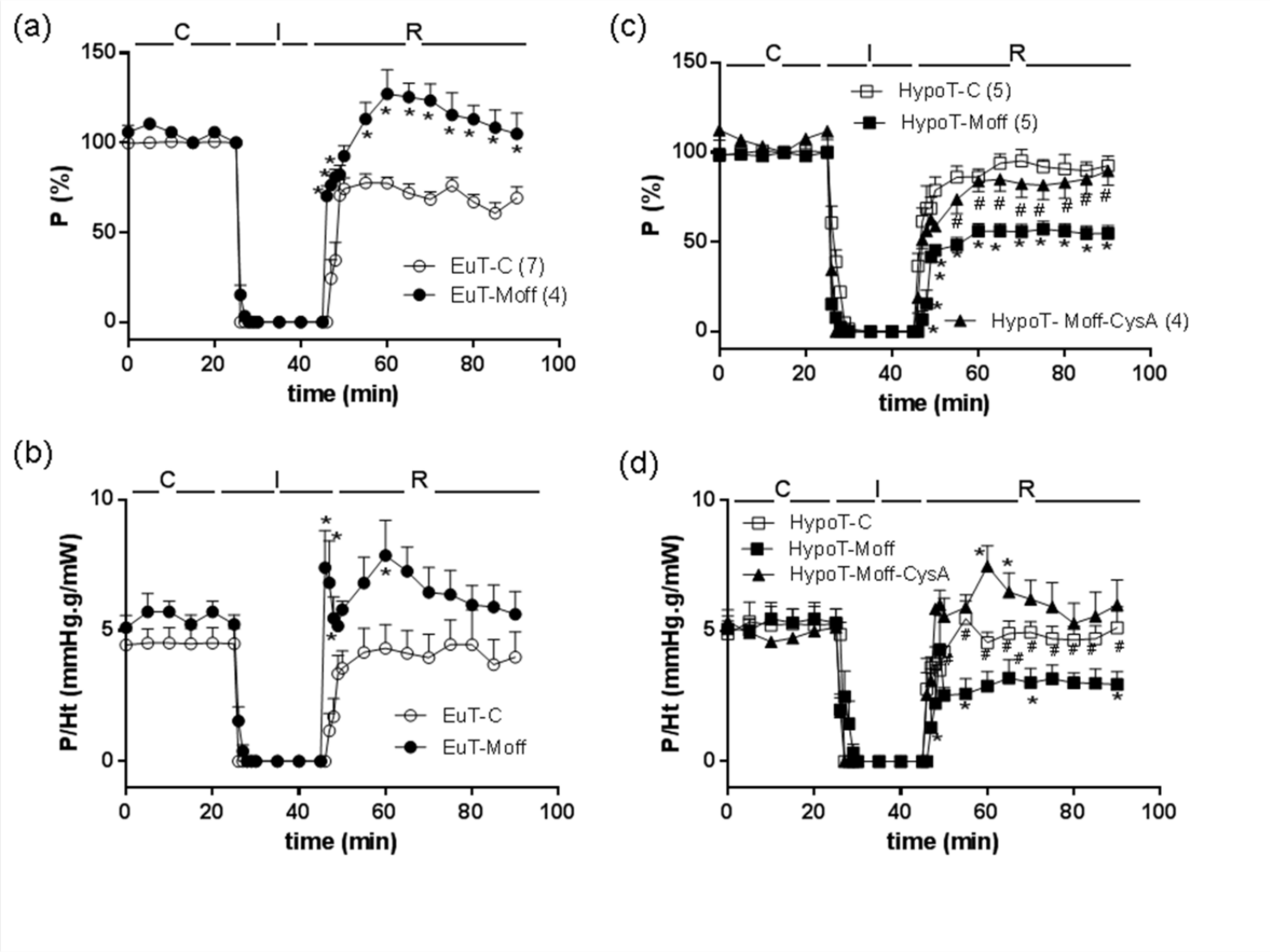

Figure 4. Effects of oral treatment with 5% infusion of Bahuinia forficata on the cardiac pressure development (P) and the total muscle economy (P/Ht) during ischemia and reperfusion (I/R) in euthyroid rats (EuT-Baf), in the absence and the presence of 100 µmol/l tetracaine (EuT-Baf-Tet) or 30 µmol/l diazoxide (EuT-Baf-Dzx) versus non-treated (EuT-C) rats (a and b); and in hypothyroid rats (HypoT-Baf) in the absence and the presence of 0.2 µmol/l cyclosporine-A (HypoT-Baf-Cys-A) vs versus non-treated HypoT-C rats (c and d). See two-way ANOVA in Table 2; Tukey´s tests: * $p < 0.05$ vs EuT-C in (a/b) and * $p < 0.05$ vs. HypoT-C in (c/d), # $p < 0.05$ vs HypoT-Baf, n is in brackets in each group in (a) and (c).