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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

Acute Effects of Origanum compactum on the Cardiovascular System

Eva Jaeken

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Molecular Mechanisms in Health and Disease

SUPERVISOR :

dr. Dorien DELUYKER

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Acute Effects of *Origanum compactum* on the Cardiovascular System*

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*Running title: Cardiovascular Effects of *O. compactum*

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ABSTRACT

Cardiovascular diseases (CVD) are the number one cause of death globally, affecting many people in low- and middle-income countries. Recent studies suggested that high-molecular-weight advanced glycation end-products (HMW-AGEs) contribute to CVD development. Despite the lack of scientific knowledge, plant extracts of *Origanum compactum* (*O. compactum*) are commonly used by the Moroccan population to treat diseases like diabetes and hypertension. In this study, we investigated the acute effects of *O. compactum* on the cardiovascular system and whether it can prevent the harmful effects caused by HMW-AGEs. To investigate this, cardiomyocytes were isolated from healthy Sprague-Dawley rats by enzymatic dissociation through retrograde perfusion of the aorta. Cardiomyocytes were used as control or pre-incubated for 30 minutes with *O. compactum* (1, 5, 10 or 100 µg/mL). Unloaded cell shortening was measured before and after application of HMW-AGEs (200 µg/mL) for 7 minutes. Whole-cell patch-clamp was performed to measure the electrophysiological properties. Additionally, the effect of *O.* compactum was assessed on the contraction and relaxation responses of aortic rings to phenylephrine (PE), acetylcholine (ACh), and sodium nitroprusside (SNP). Results show that pre-incubation with *O. compactum* increases cardiomyocyte unloaded cell shortening, while L-type Ca^{2+}-influx seems to decrease. Contraction of aortic rings in response to PE increases and the endothelium-dependent relaxation in response to ACh significantly decreases after pre-incubation with *O. compactum*. Finally, pre-incubation with *O. compactum* could not prevent the decreased unloaded cell shortening caused by HMW-AGEs. Our study demonstrates that *O. compactum* increases cardiomyocyte unloaded cell shortening and contributes to arterial stiffness, promoting hypertension.

INTRODUCTION

Cardiovascular diseases (CVD) are the number one cause of death globally, with a predicted number of 17.9 million deaths each year (1, 2). CVD is a group of disorders of the heart and blood vessels like *e.g.* coronary artery disease and cardiomyopathy (1, 3). These pathologies often result in the end-stage of heart failure, which can be described as the inability of the heart to meet the energy demand of the body (2, 4). The most important risk factors for developing CVD include an unhealthy diet, physical inactivity, tobacco and alcohol use, hypertension, and obesity (1, 5). Growing evidence suggests that advanced glycation end-products (AGEs) contribute to the development and progression of CVD (3, 6).

AGEs are molecules that are formed by the glycation of proteins and lipids after they have been exposed to reducing sugars, such as glucose (7). Additionally, the formation of AGEs is promoted by elevated levels of oxidative stress (8). Over the past decades, the consumption of processed foods, fats, and sugars has dramatically increased, which is associated with increased exposure to AGEs (7,

9). Consequently, the levels of AGEs are increased in patients with obesity and diabetes leading to comorbidities like CVD (10, 11). Besides the exogenous sources, AGEs are also formed *in vivo* and accumulate inside the body as a natural process that occurs with aging (6-8, 11). AGEs mediate their effects through cross-linking to intra- and extracellular proteins and/or by binding to their cell-surface receptor RAGE, inducing multiple intracellular signalling pathways (11). Based on their molecular weight (MW), AGEs can be categorized into two classes: low-molecular-weight AGEs (LMW-AGEs, MW < 12 kDa) and high-molecular-weight AGEs (HMW-AGEs, MW > 12 kDa) (12). LMW-AGEs circulate as free proteins, while HMW-AGEs are considered to be protein-bound (8, 12, 13). Most studies focus on the effects of LMW-AGEs. However, the importance of HMW-AGEs in the development and progression of CVD is rising (8). Recent studies performed by our research group have shown that acute exposure to HMW-AGEs decreases cardiomyocyte unloaded cell shortening capacity (14). In addition, chronic exposure to HMW-AGEs induces cardiac dysfunction and leads to altered contraction and relaxation responses of aortic rings in healthy rats (3, 6).

Over three-quarters of deaths due to CVD occur in low- and middle-income countries (1). This number is linked to the fact that people in low- and middle-income countries do not have the benefit of integrated primary health care programs for the early detection and treatment of CVD compared to people living in high-income countries (1, 2). As a result, people in low- and middle-income countries with CVD are detected late and often die at a younger age of CVD (1). As an ancient tradition, plant extracts are commonly used by the population in low- and middle-income countries for their medicinal purposes (15). One of the plant extracts that is commonly used among the Moroccan population is plant extracts from *Origanum compactum* (*O. compactum*) (16). Plants from the genus *Origanum* are aromatic plants that are used all over the world for their aromatic properties, and as a culinary herb (16, 17). *O. compactum* is a plant that is endemic to Morocco and southern Spain (16, 17). Previous studies investigating *O. compactum* found that it contains antifungal, antibacterial, and antioxidant properties (16-18). Therefore, *O. compactum* is widely used by the Moroccan population as a medicine in the form of infusions or decoctions to treat multiple diseases such as gastrointestinal diseases, infections, diabetes, and hypertension (16, 17). The therapeutic applications of *O. compactum* depend on the part of the plant that is used. For instance, the flowering tops are mainly used to treat digestive diseases, while the leaves of *O. compactum* are used against diabetes and to treat hypertension (16). Despite the frequent use of *O. compactum* by the Moroccan population, no study was found that investigated the effects of *O. compactum* on the cardiovascular system.

Therefore, the purpose of this study is to investigate the acute effects of plant extracts of *O. compactum* on the cardiovascular system *in vitro*, and whether its antioxidant properties can prevent the detrimental effects of HMW-AGEs. We analysed the effect of *O. compactum* on the contractile and electrophysiological properties of cardiomyocytes. Furthermore, we evaluated the effects of *O. compactum* on the relaxation and contraction responses in aortic rings. Finally, we investigated whether *O. compactum* could restore the unloaded cell shortening capacity in cardiomyocytes exposed to HMW-AGEs.

EXPERIMENTAL PROCEDURES

Animal model – Healthy male Sprague-Dawley rats (Charles River Laboratories, France) were used. All animal experiments were performed according to the EU Directive 2010/63/EU for animal testing and were approved by the local ethical committee (Ethical Committee for Animal Experimentation, UHasselt, Belgium, ID 202072K). Rats were group-housed in standard cages at the conventional animal facility of UHasselt. All rats were maintained in controlled environmental conditions and were provided a standard pellet diet and water *ad libitum*. Rats were handled daily to reduce stress.

Plant extracts of Origanum compactum – Dry extracts from leaves of *O. compactum* were prepared in Morocco via decoction. In short, the leaves of *O. compactum* were boiled in distilled water (0.1 g/mL) for 15 minutes. The solution was filtered, and the obtained filtrate was dried. The dried *O. compactum* was then dissolved and used for the following experiments.

Formation and characterization of HMW-AGEs – HMW-AGEs were prepared as previously

described (6). Briefly, fatty acid-free and low endotoxin bovine serum albumin (BSA; 7 mg/mL) was incubated with glycolaldehyde dimers (90 mM; Sigma-Aldrich, Diegem, Belgium) in phosphate-buffered saline (PBS) for five days at 37°C. Unreacted glycolaldehyde was removed by dialysis against PBS using a cut-off value of 3.5 kDa. Finally, the solution was concentrated using Amicon Ultra Centrifugal Filter Units with Ultracel-50 membrane (Millipore, Brussel, Belgium) to obtain glycated BSA of high-molecular-weight.

Cardiomyocyte isolation – Rats were injected with heparin (1000 u/kg) and Dolethal (150 mg/kg). When no reflexes were visible, hearts were removed to isolate single adult cardiomyocytes from the left ventricle by enzymatic dissociation through retrograde perfusion of the aorta (19). The hearts were perfused with normal Tyrode solution (NT; 137 mM NaCl, 5.4 mM KCl, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 11.8 mM Hepes, 20 mM taurine and 10 mM glucose; pH 7.4). Hereafter, hearts were placed on a Langendorff set-up where they were first perfused with a Ca^{2+}-free solution (130 mM NaCl, 5.4 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 6 mM Hepes and 10 mM glucose; pH 7.2) at 37°C, followed by an enzyme solution consisting of Ca^{2+}-free solution with Collagenase type II (1 g/L; Worthington, Lakewood, NJ, USA) and Protease type XIV (0.06 g/L; Sigma, Diegem, Belgium) and finally by a low-Ca^{2+} solution (Ca^{2+}-free solution with 20 mM taurine and 0.1 mM $CaCl_2$). On the Langendorff set-up, the hearts were continuously provided with oxygen. Finally, the digested left ventricle was minced and filtered with a mesh of 300 µm to obtain a suspension of single cardiomyocytes. The cardiomyocytes were either used for unloaded cell shortening or electrophysiological measurements. Experiments were performed at room temperature within 6 hours after isolation.

Unloaded cell shortening measurements – The isolated cardiomyocytes were rested for approximately one hour before starting the experiment while gradually increasing Ca^{2+}-concentration in the solution. The cardiomyocytes were placed into a perfusion chamber with NT. Unloaded cell shorting of intact cardiomyocytes was measured with a video edge detector (Crescent Electronics, London, UK). Field stimulation was done with pulses of constant voltage using platinum electrodes at a frequency of 1 Hz. Unloaded cell shortening was normalized to diastolic cell length (L/L_0). Time to peak contraction (TTP) and time to half-relaxation (RT_{50}) were measured to assess the kinetics of cell shortening. Unloaded cell shortening was measured before and after application of HMW-AGEs (200 µg/mL) for 7 minutes. The same experiment was performed in cardiomyocytes after pre-incubation for 30 minutes with *O. compactum* (1, 5, 10 or 100 µg/mL).

Electrophysiological measurements – Patch pipettes (resistance: 2 – 4 MΩ) were filled with a pipette solution (120 mM KAsp, 20 mM KCl, 10 mM Hepes, 5 mM MgATP and 10 mM NaCl; pH 7.2) and NT was used as external solution. L-type Ca^{2+} current (I_{caL}) was measured during whole-cell patch-clamp and was normalized to cell capacitance, a measure of cell surface. Tail current of the sodium-calcium exchanger (NCX) was measured upon repolarization. The current-voltage relationship of I_{CaL} was measured during 10 mV depolarizing steps from -40 mV to +60 mV. Cardiomyocytes were either used as control or pre-incubated for 30 minutes with *O. compactum* (100 µg/mL).

Aortic vasomotor function – The descending thoracic aorta was isolated from sacrificed rats and placed into ice-cold Krebs solution (118.3 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 0.026 mM EDTA, 5.5 mM glucose). The aorta was cleaned of perivascular fat and connective tissue and cut into aortic rings of 4-5 mm in length. The aortic rings were fixed on a rod and placed into a tissue bath containing Krebs solution (37°C and continuously oxygenated) and connected to an isometric force transducer (MLT 050/A, AD Instruments) and a data acquisition system (PowerLab 4/25 T, AD Instruments). A passive tension of 0.8 mV was applied, and the rings were equilibrated for one hour. During this time, aortic rings were washed with fresh Krebs solution every 20 minutes. To assess the relaxation responses, aortic rings were precontracted with a single dose of phenylephrine (PE; 10^{-7} mol/L). When a stable plateau phase was reached, cumulative doses of acetylcholine (ACh; final bath concentrations: 10^{-10} to 10^{-5} mol/L) or sodium nitroprusside (SNP; final bath concentrations: 10^{-11} to 10^{-6} mol/L) were added every 4 minutes to determine dose-response curves of the endothelium-dependent or endothelium-

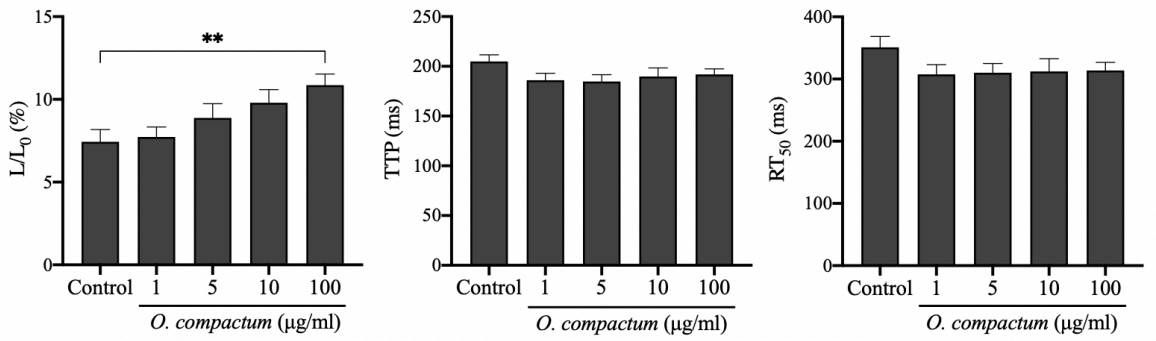

Fig. 1 – Cardiomyocyte unloaded cell shortening capacity increases after pre-incubation with *O. compactum*. Cardiomyocytes were isolated from left ventricles of healthy Sprague-Dawley rats (n = 12) by enzymatic dissociation through retrograde perfusion of the aorta. Unloaded cell shortening normalized to cell length (L/L_0; left), time to peak contraction (TTP; middle) and half relaxation time (RT_{50}; right) of control cardiomyocytes (n_{cells} = 24) and cardiomyocytes pre-incubated for 30 minutes with *O. compactum* (n_{cells} (1 µg/mL) = 19, n_{cells} (5 µg/mL) = 22, n_{cells} (10 µg/mL) = 22, n_{cells} (100 µg/mL) = 18) was measured. Data are presented as mean ± SEM. ** $P < 0.01$.

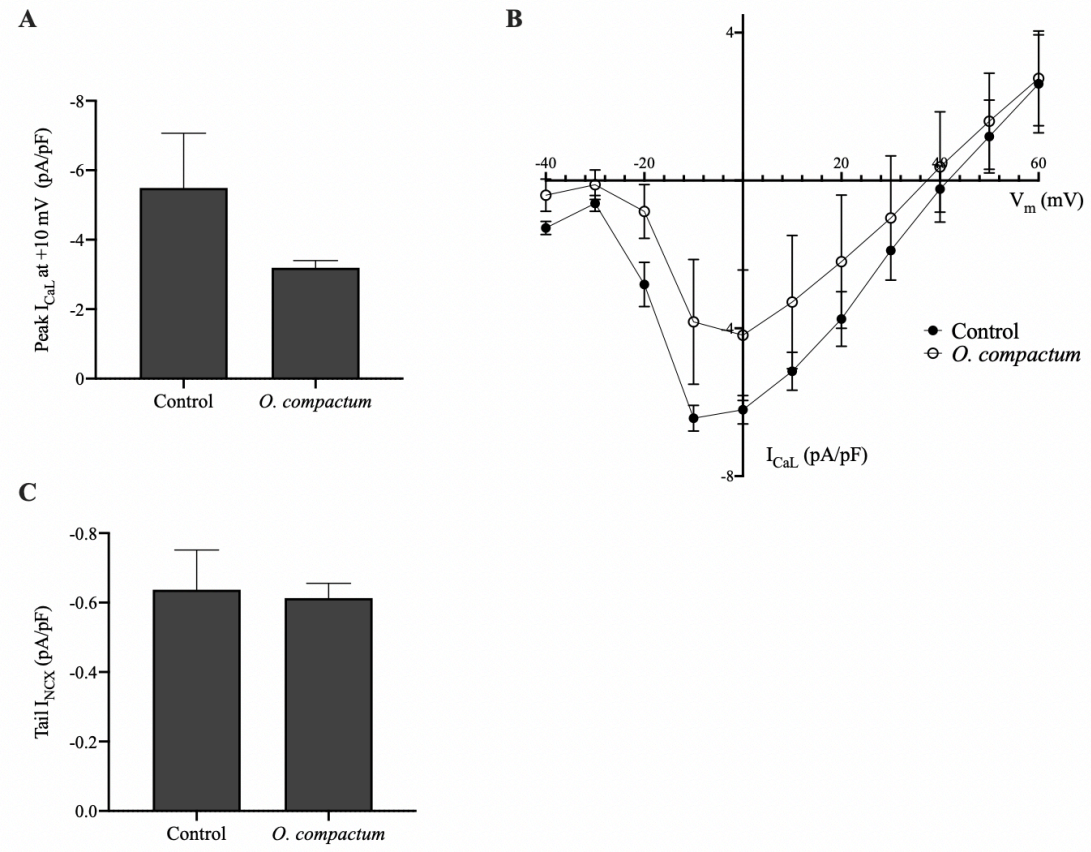

Fig. 2 – L-type Ca^{2+}-influx tends to decrease in cardiomyocytes pre-incubated with *O. compactum*. Cardiomyocytes were isolated from left ventricles of healthy Sprague-Dawley rats (n = 8) by enzymatic dissociation via retrograde perfusion of the aorta. Whole-cell patch-clamp was performed to measure Ca^{2+}-influx in control cardiomyocytes and after pre-incubation with *O. compactum* (100 µg/mL) for 30 minutes. (A) Peak I_{CaL} at +10 mV was measured and normalized to cell capacitance expressed in picoamperes per picofarad (pA/pF) (n_{cells} (control) = 6, n_{cells} (*O. compactum*) = 7). (B) Current-voltage relationship was assessed for I_{CaL} during 10 mV depolarizing steps from -40 mV to +60 mV (n_{cells} (control) = 3, n_{cells} (*O. compactum*) = 4). (C) Tail current of NCX (I_{NCX}) was measured upon repolarization (n_{cells} (control) = 6, n_{cells} (*O. compactum*) = 7). Data are presented as mean ± SEM. NCX, sodium-calcium exchanger.

independent relaxation, respectively. Contraction responses were measured in response to cumulative doses of PE (final bath concentrations: 10^{-10} to 10^{-5} mol/L) every 4 minutes. Between each experiment, aortic rings were washed three times for 5 minutes with fresh Krebs solution (37°C). Relaxation and contraction responses of aortic rings were also measured after pre-incubation for 30 minutes with *O. compactum* (1, 5, 10 or 100 µg/mL).

Statistical analysis – Statistical analysis was performed using GraphPad Prism (GraphPad software, version 9.0.2, San Diego, CA, USA). Cardiomyocyte cell shortening data were analysed using one-way ANOVA followed by Dunnett's multiple comparison test or using the nonparametric Kruskal-Wallis test followed by Dunn's multiple comparison test. The Mann-Whitney test was used to compare between two groups. A two-way ANOVA followed by Bonferroni post hoc test was performed to analyse the current-voltage relationship of I_{CaL}. The dose-response curves were fitted by nonlinear regression and were analysed using two-way ANOVA followed by Dunnett's multiple comparison test. All data were presented as mean ± SEM and $P < 0.05$ was considered statistically significant.

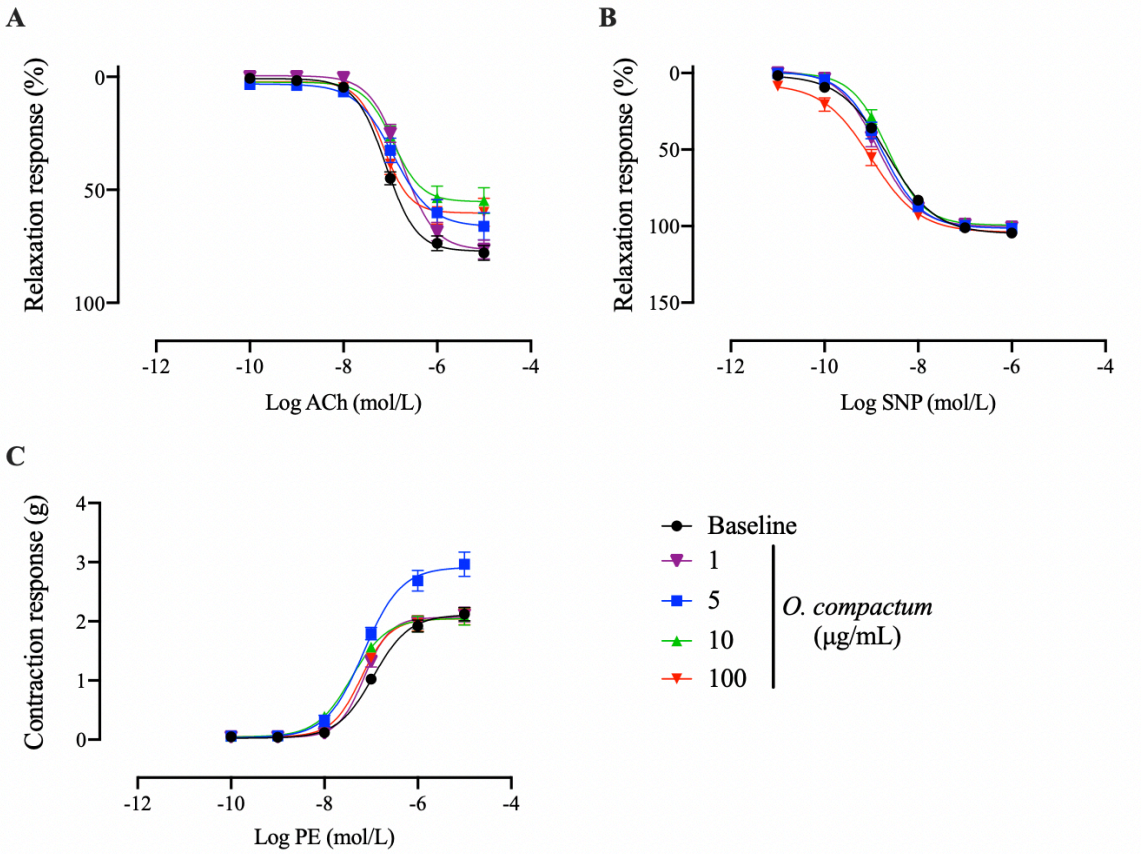

Fig. 3 – Relaxation and contraction of aortic rings in response to ACh, SNP and PE. The descending thoracic aorta was isolated from healthy Sprague-Dawley rats (n = 19) and cut into aortic rings. Relaxation and contraction responses were measured at baseline and after pre-incubation with *O. compactum* for 30 minutes. (A) Relaxation was measured in response to cumulative doses of ACh (final bath concentrations: 10^{-10} to 10^{-5} mol/L; n_{rings} (baseline) = 97, n_{rings} (1 µg/mL) = 26, n_{rings} (5 µg/mL) = 21, n_{rings} (10 µg/mL) = 22, n_{rings} (100 µg/mL) = 28). (B) Relaxation was measured in response to cumulative doses of SNP (final bath concentrations: 10^{-11} to 10^{-6} mol/L; n_{rings} (baseline) = 54, n_{rings} (1 µg/mL) = 25, n_{rings} (5 µg/mL) = 20, n_{rings} (10 µg/mL) = 21, n_{rings} (100 µg/mL) = 19). The relaxation response is expressed as % of PE-induced precontraction (10^{-7} mol/L). (C) Contraction was measured in response to cumulative doses of PE (final bath concentrations: 10^{-10} to 10^{-5} mol/L; n_{rings} (baseline) = 66, n_{rings} (1 µg/mL) = 26, n_{rings} (5 µg/mL) = 22, n_{rings} (10 µg/mL) = 23, n_{rings} (100 µg/mL) = 27). Contraction response is expressed in grams (g) of tension. Data are presented as mean ± SEM. ACh, acetylcholine; SNP, sodium nitroprusside; PE, phenylephrine.

RESULTS

O. compactum increases cardiomyocyte unloaded cell shortening – Cardiomyocytes were isolated to investigate the acute effects of *O. compactum* on unloaded cell shortening *in vitro*. Unloaded cell shortening was measured in control cardiomyocytes and in cardiomyocytes pre-incubated for 30 minutes with *O. compactum* (1, 5, 10 or 100 µg/mL). Pre-incubation with *O. compactum* (100 µg/mL) significantly increases unloaded cell shortening compared to baseline (Fig. 1; 7.99 ± 0.75 % at baseline *vs* 11.92 ± 0.83 % after pre-incubation, P = 0.0056). No significant differences were measured in time to peak (TTP) and half relaxation time (RT_{50}) after pre-incubation with *O. compactum* (Fig. 1).

O. compactum decreases L-type Ca^{2+}-influx in cardiomyocytes – Cardiomyocytes were isolated to measure the L-type Ca^{2+} current (I_{CaL}). Results show that L-type Ca^{2+}-influx tends to decrease after pre-incubation with 100 µg/mL of *O. compactum* (Fig. 2A; -5.59 ± 1.58 pA/pF at baseline *vs* -3.2 ± 0.21 pA/pF after pre-incubation, p = 0.23). Furthermore, I_{CaL} tends to be decreased in multiple depolarizing steps (Fig. 2B). Finally, the tail current of NCX (I_{NCX}) upon repolarization remained unchanged after pre-incubation with *O. compactum* (Fig. 2C).

O. compactum alters relaxation and contraction responses of aortic rings – The descending thoracic aorta was isolated and cut into aortic rings to assess relaxation and contraction responses. Responses were measured at baseline and after pre-incubation for 30 minutes with *O. compactum* (1, 5, 10 or 100 µg/mL). After precontraction with a single dose of PE (10^{-7} mol/L), relaxation responses to cumulative doses to ACh and SNP were assessed. The relaxation response to ACh was reduced in aortic rings pre-incubated with *O. compactum*, demonstrated by a rightward shift in the dose-response curves (Fig. 3A). In addition, a leftward shift can be observed in the lower concentrations of the dose-response curve of SNP after pre-incubation with 100 µg/mL of *O. compactum* (Fig. 3B). The E_{max} relaxation was significantly decreased after pre-incubation with 10 and 100 µg/mL of *O. compactum* compared to baseline in response to ACh (Table 1). In contrast to ACh, the E_{max} relaxation remained unchanged in response to SNP (Table 1). The contraction response was evaluated by applying cumulative doses of PE to the aortic rings. A leftward shift can be observed in all groups that were pre-incubated

Table 1 – Pharmacological responses of aortic rings to PE, ACh and SNP. The descending thoracic aorta was isolated from healthy Sprague-Dawley rats (n = 19) and cut into aortic rings. Relaxation and contraction responses were measured at baseline and after pre-incubation for 30 minutes with *O. compactum* (1, 5, 10 or 100 µg/mL). The negative logarithm or the half maximal effective dose ($-LogEC_{50}$) was derived from dose-response curves fitted in Labchart software. Maximal relaxation (E_{max} relaxation) is expressed as % of PE-induced precontraction. Maximal contraction (E_{max} contraction) is expressed in grams (g) of tension. Data are presented as mean ± SEM. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001 *vs* baseline.

		O. compactum			
	Baseline	**1 µg/mL**	**5 µg/mL**	**10 µg/mL**	**100 µg/mL**
ACh					
$-LogEC_{50}$ (mol/L)	6.98 ± 0.03	6.68 ± 0.05***	6.86 ± 0.11	6.93 ± 0.1	7.15 ± 0.11
E_{max} contraction (g)	77.93 ± 3.2	76.35 ± 3.98	66.13 ± 5.91	54.74±5.6****	59.91 ± 6.09***
SNP					
$-LogEC_{50}$ (mol/L)	8.72 ± 1.19	8.85 ± 1.77	8.78 ± 2.2	8.66 ± 1.94	9.02 ± 2.07*
E_{max} relaxation (%)	104.47 ± 1.11	100.77 ± 0.83	101.56 ± 0.66	99.71 ± 0.94	103.32 ± 1.12
PE					
$-LogEC_{50}$ (mol/L)	7.01 ± 0.05	7.14 ± 0.04	7.24 ± 0.08*	7.52±0.07****	7.26 ± 0.06**
E_{max} contraction (g)	2.12 ± 0.11	2.12 ± 0.12	2.96 ± 0.2****	2.08 ± 0.15	2.08 ± 0.14

ACh, acetylcholine; SNP, sodium nitroprusside; PE, phenylephrine.

with *O. compactum* compared to baseline (Fig. 3C). However, the E_{max} contraction was only significantly increased after pre-incubation with 5 µg/mL of *O. compactum*, and -LogEC50 was increased after pre-incubation with 5, 10 and 100 µg/mL of *O. compactum* (Table 1).

O. compactum cannot prevent the decreased unloaded cell shortening induced by HMW-AGEs – Cardiomyocytes were isolated to investigate whether pre-incubation with *O. compactum* could prevent the detrimental effects of HMW-AGEs. Cells were either used as control or pre-incubated with *O. compactum*. Unloaded cell shortening was measured at baseline and after application of HMW-AGEs (200 µg/mL) for 7 minutes. We found that HMW-AGEs decreases unloaded cell shortening in control cardiomyocytes (Fig. 4; 7.99 ± 0.75 % at baseline *vs* 5.09 ± 0.48 % after exposure to HMW-AGEs, p = 0.0029). Additionally, HMW-AGEs decreases unloaded cell shortening in cardiomyocytes pre-incubated with 100 µg/mL of *O. compactum* (Fig. 4; 11.91 ± 0.83 % at baseline *vs* 6.59 ± 0.55 % after exposure to HMW-AGEs, p < 0.0001). Finally, pre-incubation with *O. compactum* did not prevent the decreased unloaded cell shortening induced by HMW-AGEs (Fig. 4).

DISCUSSION

In our study, we investigated the acute effects of *O. compactum* on the cardiovascular system, and whether its antioxidant properties could be used to prevent the effects of HMW-AGEs. We found that pre-incubation with *O. compactum* increases unloaded cell shortening in cardiomyocytes. Furthermore, pre-incubation with *O. compactum* increases contraction and decreases endothelium-dependent relaxation responses in aortic rings. Finally, pre-incubation with *O. compactum* could not prevent the decreased unloaded cell shortening induced by HMW-AGEs.

Pre-incubation with O. compactum increases unloaded cell shortening capacity of cardiomyocytes – Intracellular Ca^{2+} cycling plays a crucial role in regulating the contraction and relaxation responses in cardiomyocytes (20). Multiple ion channels and transporters are responsible for maintaining the Ca^{2+} homeostasis, contributing to the proper functioning of the heart (20). In our study, we found that pre-incubation with *O. compactum* increases unloaded cell shortening in cardiomyocytes. In contrast to this, we did not observe an increase in Ca^{2+}-influx via the L-type Ca^{2+} channels. Instead, Ca^{2+}-influx tends to decrease after pre-incubation with *O. compactum*. One of the possible explanations could be that there is an increased activity of the reverse mode of the sodium-calcium exchanger (NCX) (21). NCX is one of the main Ca^{2+} extrusion mechanisms in cardiomyocytes (21-23). In its forward mode, NCX uses the electrochemical gradient of Na^+ to remove Ca^{2+} from the cytosol (21, 23). Conversely, NCX exports Ca^{2+} into the cytosol when in reverse mode (21, 23). In this way, increased activity of the reverse mode of NCX

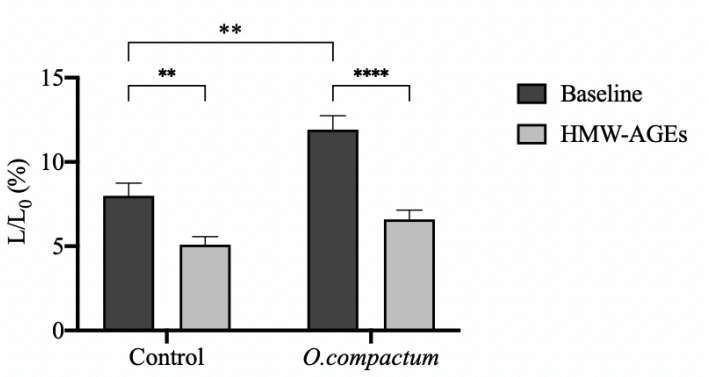

Fig. 4 – Pre-incubation with *O. compactum* cannot prevent the decreased unloaded cell shortening caused by HMW-AGEs. Cardiomyocytes were isolated from healthy Sprague-Dawley rats (n = 12) by enzymatic dissociation via retrograde perfusion of the aorta. Unloaded cell shortening normalized to cell length (L/L_0) was measured in control cardiomyocytes (n_{cells} = 24) and cardiomyocytes pre-incubated for 30 minutes with *O. compactum* (100 µg/mL; n_{cells} = 18). Unloaded cell shortening was measured at baseline and after application of HMW-AGEs (200 µg/mL) for 7 minutes. Data are presented as mean ± SEM. **P < 0.01, **** P < 0.0001.

could lead to increased intracellular Ca^{2+} levels responsible for the increased unloaded cell shortening seen in our results. In our study, we did not measure the activity of the reverse mode of NCX. However, we did measure the tail current of NCX upon repolarization which remained unchanged in cardiomyocytes after pre-incubation with *O. compactum*, meaning there is no changed activity of the forward mode of NCX. Another possibility is an altered activity of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump. The SERCA pump is responsible for pumping Ca^{2+} from the cytosol into the sarcoplasmic reticulum (SR) (21, 24). When the activity of the pump is decreased, elevated levels of Ca^{2+} will remain in the cytosol which could lead to the observed increased contraction after pre-incubation with *O. compactum* (21). However, a decrease in SERCA function would also lead to prolonged relaxation of the cardiomyocytes, which we did not observe in our results (21). Next, increased sensitivity of the myofilaments to Ca^{2+} could lead to an increased contraction without the increased L-type Ca^{2+}-influx (25). Last, the baseline level of Ca^{2+} could be elevated in cardiomyocytes pre-incubated with *O. compactum* compared to the baseline Ca^{2+} level in control cardiomyocytes. Additional experiments should be performed to unravel the mechanism responsible for the increased unloaded cell shortening observed in cardiomyocytes pre-incubated with *O. compactum*. These experiments should focus on measuring 1) the activity of the reverse mode of NCX, 2) the activity of the SERCA pump, 3) myofilament sensitivity, and 4) baseline Ca^{2+} levels in cardiomyocytes.

O. compactum contributes to arterial stiffness – Hypertension is an important risk factor for developing CVD and ultimately heart failure (5). Arterial stiffness, caused by structural and functional changes of the artery, contributes to hypertension (26). The structural changes include the fragmentation of elastin, an increased amount of collagen, and arterial calcification (26). Besides the structural changes, endothelium dysfunction contributes to vascular stiffness through a changed sensitivity to and/or an imbalance in vasoconstrictors and vasodilators (26). In our study, we found that pre-incubation with *O. compactum* increased PE-induced contraction in aortic rings. In addition, the endothelium-dependent relaxation in response to ACh was decreased and the endothelium-independent relaxation in response to SNP remained unchanged after pre-incubation with *O. compactum*. These results indicate that *O. compactum* impairs the endothelial function but does not change the vascular smooth muscle cell responsiveness. Previous studies have shown that an altered vascular reactivity contributes to hypertension (27-29). Therefore, our results indicate that *O. compactum* leads to increased arterial stiffness, observed as increased PE-induced contraction and decreased ACh-induced relaxation, and thereby contributes to hypertension.

Can O. compactum prevent HMW-AGEs-induced cardiac dysfunction? – HMW-AGEs are formed during increased levels of oxidative stress and are thought to contribute to the development and progression of CVD (6, 8). Additionally, the interaction between AGEs and their receptor RAGE activates nicotinamide adenine diphosphate (NADPH) oxidase, which produces reactive oxygen species (ROS) (8). Therefore, AGEs are considered to contribute to higher levels of oxidative stress in the heart, which in turn will lead to the accelerated formation of AGEs (8). Due to its antioxidant properties, *O. compactum* is widely used by the Moroccan population to treat diseases like diabetes and hypertension (16, 18). In this context, we wanted to investigate whether pre-incubation with *O. compactum* could prevent the detrimental effects induced by HMW-AGEs. Our results show that acute exposure to HMW-AGEs decreases unloaded cell shortening in cardiomyocytes, confirming the results previously found by our research group (14). In addition, we found that unloaded cell shortening is also decreased in cardiomyocytes pre-incubated with *O. compactum* after exposure to HMW-AGEs. These results indicate that pre-incubation with *O. compactum* cannot prevent the decreased unloaded cell shortening induced by HMW-AGEs.

CONCLUSION

Despite the lack of scientific knowledge, *O. compactum* is widely used by the Moroccan population to treat variable diseases. This study is the first one to investigate the effect of *O. compactum* on the cardiovascular system. We found that pre-incubation with *O. compactum* increases cardiomyocyte unloaded cell shortening caused by a disturbed Ca^{2+} homeostasis and

contributes to increased arterial stiffness, observed by an increased contraction response to PE and decreased endothelium-dependent relaxation response to ACh in aortic rings. In conclusion, these findings implicate that *O. compactum* negatively affects the cardiovascular system and therefore should not be used as a treatment for diabetes and hypertension.

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