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Article

Cardioprotective effects of grapefruit IntegroPectin extracted *via* hydrodynamic cavitation from by-products of *Citrus* fruits industry: role of mitochondrial potassium channels

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Abstract: *Citrus* flavonoids are well-known for beneficial effects at the cardiovascular and cardio-metabolic level, but often the encouraging *in vitro* results are not confirmed by *in vivo* approaches; also clinical trials are inconsistent. The limited bioavailability of them can be, at least in part, the reason of these discrepancies. Therefore many efforts were performed towards the improvement of their bioavailability. Hydrodynamic cavitation methods were successfully applied to the extraction of byproducts of the *Citrus* fruits industry, showing high process yields and affording stable phytocomplexes, known as IntegroPectin, endowed with great amounts of bioactive compounds and high water solubility. Cardioprotective effects of grapefruit IntegroPectin were evaluated by an *ex vivo* ischemia/reperfusion protocol. A further pharmacological characterization was carried out to assess the involvement of mitochondrial potassium channels. Grapefruit IntegroPectin, where naringin represented 98% of flavonoids, showed anti-ischemic cardioprotective activity, better than pure naringenin (the bioactive aglycone of naringin). On cardiac isolated mitochondria, this extract confirmed that naringenin/naringin were involved in the activation of mitochondrial potassium channels. The hydrodynamic cavitation-based extraction confirmed a valuable opportunity for the exploitation of *Citrus* fruits waste, with the end product presenting high levels of *Citrus* flavonoids and an improved bioaccessibility that enhances its nutraceutical and economic value.

Keywords: *Citrus* flavonoids; naringin; naringenin; pectin; byproducts; anti-ischemic myocardial protection; hydrodynamic cavitation.

1. Introduction

Citrus flavonoids are well-known for beneficial effects at the cardiovascular and cardio-metabolic level [1]. Recently, a daily hesperidin supplementation was shown to improve the blood pressure in pre- and stage 1 phase hypertensive patients [2]. Likewise, *Citrus* flavonoids naringin and naringenin, the latter being the aglycone of naringin, are promising nutraceuticals in the strategy addressed to the management of cardiovascular complications, improving the systolic pressure levels and the metabolic profile [3]. *In vitro* and *in vivo* evidence suggests that multiple pathways may be involved in these effects, including the positive modulation of Sirtuin 1 (SIRT1) enzyme pathway [4-6]. However, other putative mechanisms have been recognized in these biological actions, such as the stimulation of potassium channels, located both on sarcolemmal and on inner membrane of cardiac mitochondria and the increase of bioavailability of nitric oxide, contributing to the preservation of endothelial barrier at vascular level [7-10]. Of note, two of the most studied *Citrus* flavonoids, hesperetin and naringenin, were shown to be endowed with cardioprotective effects. For example, hesperetin protects the heart against the toxicity

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induced by doxorubicin treatment [11], while naringenin exerts protection against ischemia/reperfusion injury, both in young-adult and in 12-month aged rats, through the activation of mitochondrial calcium-activated potassium channels (mitoBK) [7, 12]. Recently, naringin also demonstrated to be an activator of BK channels [13, 14].

Despite numerous pre-clinical studies suggest the *Citrus* flavonoids nutraceutical value in the maintenance of cardiovascular and metabolic homeostasis, often clinical trials show inconsistencies, probably due to the poor systemic bioavailability of *Citrus* flavonoids. Then, many efforts have been performed in order to improve their pharmacokinetic profile, mainly attempting to get secondary metabolites more soluble and more accessible at the intestinal level, via structural transformation (i.e. glycosylation) and pharmacological technologies [15].

In the last few years, hydrodynamic cavitation (HC) technology is successful applied to the extraction of waste streams of the *Citrus* fruits industry [16]. This technique surprisingly leads to extracts rich in stable phyto-complexes comprising pectin, flavonoids and volatiles. These complexes are known as "IntegroPectin", which can be extracted from the water phase by means of a standard drying technique, e.g. freeze-drying [17, 18]. The innovative HC technique allows to achieve high value-added products from waste streams and byproducts, including from *Citrus* fruits, complying with the principles of circular economy as well as with the principles of green extraction of natural products [19].

Grapefruit IntegroPectin showed remarkable water solubility, which was directly related to the cavitation-based extraction process, as well as *in vitro* bactericidal activity against both Gram-negative and Gram-positive bacteria, much higher than commercial *Citrus* pectins [20]. Even more important for the purposes of this article, a previous study carried out on a human neuroblastoma cells line exposed on oxidative stress demonstrated that grapefruit IntegroPectin preserved mitochondrial membrane potential and cell morphology and played a powerful antiproliferative activity. Interestingly, similar commercial *Citrus* pectins showed a lower protective efficacy, if compared to IntegroPectin. The authors hypothesized that terpenes and flavonoids may be adsorbed on the pectic surface rich of RG-I "hairy" regions, containing galactose and arabinose units, making the biologically active secondary metabolites more bio-accessible [21].

In this study we investigated, for the first time, the *in vivo* efficacy of grapefruit IntegroPectin in a model of myocardial ischemia/reperfusion injury.

2. Materials and Methods

2.1 Production of grapefruit IntegroPectin

The grapefruit IntegroPectin was isolated *via* freeze-drying of the water phase extract upon hydrodynamic cavitation of waste peels of organic pink grapefruit kindly donated by OPAC Campisi (Siracusa, Italy). The details of the hydrodynamic cavitation-based extractor, comprising a closed hydraulic circuit with a centrifugal pump and a Venturi-shaped reactor, with electricity as the only energy source, were described in a previous study about the extraction of waste orange peel [16]. The details of the specific process for the extraction of waste grapefruit peels were as follows: fresh biomass in the amount of 34 kg, mixed with 120 L of water, with no other additives; process time of 1 hour, carried out at atmospheric pressure; free heating from 7.5°C to 38°C; overall consumed energy at the level of 0.2 kWh per kg of fresh waste grapefruit peel.

2.2 Animal experimentation and data analysis

Male Wistar rats of about 12-15 weeks and weight between 300 and 400 g were used. The animals were housed in cages, with freedom of movement, supplied with water and food, and exposed to light/dark cycles of 12 hours each, at 22 °C. The study was carried out in line with EU legislation (EEC Directive 63/2010) and Italian legislation (Legislative Decree No. 26/2014) about animal testing.

2.2.1 Ischemia/reperfusion on Langendorff isolated and perfused heart

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The animals were treated, by intraperitoneal injection (i.p.) of naringenin (100 mg/Kg), vehicle (dimethyl sulfoxide, DMSO, 1 mL/Kg) or grapefruit IntegroPectin (45 mg/kg; 135 mg/kg; 450 mg/kg), 2 hours before heart removal.

The animals were subjected to an i.p. injection of heparin (2500 I.U.) (Sigma-Adrich), and to a subsequent one of Pentothal Sodium (100 mg/kg) (MSD Italia) to induce a deep anesthesia. Each animal was sacrificed, and the explanted heart was immersed in a Krebs solution ($NaHCO_3$ 25.0 mM, Glucose 11.7 mM, NaCl 117.9 mM, KCl 4.8 mM, $MgSO_4$ 2.5 mM, $CaCl_2$ 2.2 mM, KH_2PO_4 1.2 mM; clioxicarb: 95% O_2 and 5% CO_2; pH 7.4; 4°C). Then, the heart was set up on a Langendorff apparatus and perfused with Krebs saline solution at 37 °C and constant pressure of 70-80 mmHg, through a peristaltic pump (Peristar, 2Biological Instruments). A latex balloon filled with bidistilled water at a pressure of 5-10 mmHg was placed inside the left ventricle through the mitral valve to monitor functional parameters of the heart. The ischemia/reperfusion protocol consisted of 30 minutes stabilization time, 30 minutes of global ischemia and 120 minutes of reperfusion. At the end of reperfusion time, the heart was removed, dried, weighed, then the left ventricle was isolated. This was sliced into cross sections and immersed in a 1% w/v solution of 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma-Aldrich) dissolved in PBS (pH 7.4) (Sigma-Aldrich) for 20 minutes at 37 °C, in the dark. Finally, the slices were fixed in a 10% v/v aqueous formaldehyde solution. As a result, it gives vital areas a red color.

The latex balloon was connected to a pressure transducer (Bentley Trantec, mod. 800) and a data acquisition system (Biopac Systems Inc. California, USA). Recorded parameters were left ventricular pressure (LVDP), heart rate (HR), and contraction/relaxation time (dp/dt). The rate-pressure product (RPP) was calculated as the product of the first two parameters. The effects of the different doses of grapefruit IntegroPectin on cardiac functional parameters (RPP, dp/dt) were expressed in % of the basal values measured at the end of the stabilization period.

The cardiac areas involved in ischemia/reperfusion damage, necrotic or apoptotic, remain white or pale pink. Ischemic area was calculated as % of left ventricle total area (Ai/Avs %) using the software GIMP (release 2.10.32).

All data were obtained from 6 different animals and graphed using the GraphPad Prism 8.0 program and expressed as mean ± standard error of mean (SEM). Statistical analyzes were performed with the t-student test, with $p < 0.05$ considered as an indicator of significant difference.

2.2.2 Mitochondrial isolation protocol

The animals were sacrificed after Isoflurane anesthesia (R584S Small Animal Aesthesia Machine, Shenzhen RWD Life Science and Technology Co., Ltd. San Diego, Ca, USA). Then heart was removed and immediately placed in isolation buffer (STE: Sucrose 250 mM, Tris 5 mM, EGTA 1mM; pH 7.4; 4 °C) constantly kept ice cold.

The heart was cleaned and finely chopped. Heart fragments were suspended in 10 mL of STE and homogenized using Ultra-Turrax homogenizer (IKA, T-18 Basic, IKA-Werke GmbH & Co., Staufen, Germany). The suspension obtained was subjected to centrifugations to isolate the mitochondrial component taking care to keep it refrigerated during each step to preserve mitochondrial integrity and functionality [7, 22]. Mitochondrial proteins were determined using Bradford assay.

2.2.3 Cardiac Mitochondrial Membrane Potential

The membrane potential ($\Delta\Psi$) of isolated mitochondria was determined by a potentiometric method using the liposoluble cation tetraphenylphosphonium (TPP^+) and a selective electrode coupled with a reference one (WPI-World Precision Instruments, Sarasota, Florida, USA), connected with a data acquisition software (Biopac Inc., California, USA). The electrodes were calibrated with known concentrations of TPP^+Cl^- before each experiment.

The isolated mitochondria (1 mg of protein/mL) were kept on suspension with gentle and constant shaking in the incubation medium (IM: KCl 120 mM, K_2HPO_4 5 mM, Hepes 10 mM, succinic acid 10 mM, $MgCl_2$ 2 mM, EGTA 1 mM; pH 7,4) with the addition of

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TPP^+Cl^- (30 μM). Grapefruit IntegroPectin was tested at concentration levels between 0.01 and 0.3 mg/mL, naringenin between 1-30 μM. The effects of the corresponding vehicle (DMSO 0,1%) were also evaluated. ΔΨ was calculated using the following fitted Nernst equation, as previously described [7, 21]:

$$\Delta\psi = 60log\frac{V_0\frac{[TPP^+]_0}{[TPP^+]_t} - V_t - K_0P}{V_mP + K_iP} \quad (1)$$

ΔΨ was expressed as millivolt (mV) changes from baseline levels. Mitochondria showing a starting value > -170 mV were discarded, being poor energized. All results were obtained from 6 different animals. Statistical analyses were performed with the t-student test using the GraphPad Prism 8.0 program, with $p < 0.05$ considered as an indicator of significant difference.

2.2.4 Mitochondrial changes in calcium-uptake

Mitochondrial calcium uptake was measured with a Ca^{2+} sensitive mini electrode (Tip_Ca, WPI, FL, USA) coupled to a reference one (WPI, FL, US), using a data acquisition software (Biopac Systems Inc., California, USA). Calibration curves were generated before each experiment using known concentrations of $CaCl_2$. The electrodes were placed in IM with the addition of $CaCl_2$ solution (100 μM) and grapefruit IntegroPectin at the concentration levels of 0.03, 0.1 and 0.3 mg/mL or naringenin 30 and 100 μM or vehicle (1% DMSO). Then, mitochondria (1 mg protein/mL) were added under gentle stirring and the changes in calcium uptake were evaluated recording mV variation.

Decreasing mV levels in medium concentration of calcium was linked to its accumulation in the mitochondrial matrix. Each result was obtained with mitochondria isolated from the heart of 6 different animals. All data were expressed as mean ± SEM and were analyzed by GraphPad Prism 8.0. The data were statistically analyzed using the t-student test, with $p < 0.05$ considered as an indicator of significant difference.

3. Results

3.1 Cardioprotective effects of grapefruit IntegroPectin in ex vivo model of cardiac ischemia/reperfusion injury

An ischemia/reperfusion episode produced a marked damage to the isolated hearts of vehicle-treated rats. In this regard, a decrease of the functional parameters of myocardial contractile function (RPP) and myocardial performance (dP/dt) were observed during the reperfusion time. At the end, the levels of RPP and dP/dt were 14 ± 5% and 21 ± 9%, respectively, of the levels observed in the pre-ischemic period, as shown in Figure 1(a,b). Figure 1(c) shows that, consistently with the functional status, also the morphometric parameter revealed a marked damage, indeed the size of the ischemic area measured using formazan salt was equal to 36 ± 3% compared to left ventricle area.

Based on the concentration level of the flavonoids in grapefruit IntegroPectin [17], we decided to treat the rats with three different doses of grapefruit IntegroPectin (45, 135 and 450 mg/kg), in order to ensure an amount of naringin (representing 98% of total flavonoids) equal to 3, 10 and 30 mg/kg, respectively. Interestingly, in these experimental conditions, grapefruit IntegroPectin achieved a dose-dependent cardioprotection, reaching at the end of the reperfusion period a significant effect with the dose of 135 mg/kg (corresponding to 10 mg/kg of naringin). In particular, hearts deriving from grapefruit IntegroPectin 135 mg/kg treatment showed at the 120th minute of reperfusion the RPP level of 34 ± 10%, the dP/dt level of 42 ± 9% and Ai/ALV level of 24 ± 3%, meant as percentages of the respective levels observed in the pre-ischemic period, as shown in Figure 1 (a-c)).

The cardioprotection efficacy shown by grapefruit IntegroPectin was almost superimposable to the efficacy of the naringenin administered at the higher dose of 100 mg/kg that, accordingly with previous studies [7], played protective effects against ischemia/reperfusion injury. In particular, hearts of animals treated with naringenin showed at the 120th minute of reperfusion, RPP level of 42 ± 9%, dP/dt level of 38 ± 15% and Ai/ ALV

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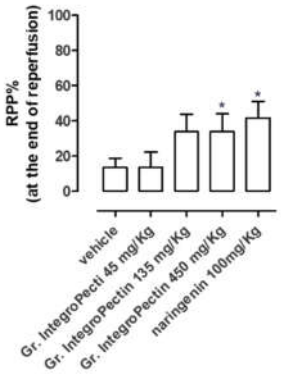
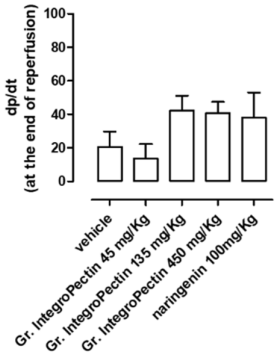
level of 22± 2%, again meant as percentages of the respective levels observed in the pre-ischemic period, as shown in Figure 1 (a-c). Overall, the experimental results clearly demonstrated the dose-dependent cardioprotective activity of grapefruit IntegroPectin against ischemia/reperfusion injury, showing an efficacy comparable to the purified naringenin but with 10 times lower dose. Moreover, it was reported in the literature that also naringin possesses effective cardioprotection against several types of myocardial insults, yet at higher dose (in the range between 20-100 mg/Kg) compared to that found in grapefruit IntegroPectin [23-28].



(**a**)



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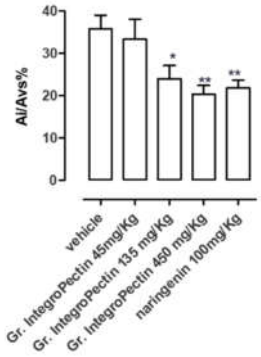

(**c**)

Figure 1. The histograms represent the functional and morphological changes induced by treatment of the animals with grapefruit IntegroPectin, naringenin or with vehicle before the ischemia/reperfusion episode: (**a**) Changes in RPP% at the end of reperfusion; (**b**) Changes in dP/dt% at the end of reperfusion; (**c**) Changes in the percentage of ischemic area vs left ventricle area (AI/ALV%). The vertical bars symbolize the standard errors (n = 6). Asterisks show a statistically significant difference from the value observed in the hearts of vehicle-treated animals (* $p < 0.05$; ** $p<0.01$).

3.2 Mitochondriotropic effects of grapefruit IntegroPectin on cardiac isolated mitochondria

Naringenin is endowed with cardioprotective effects at least in part based on its capacity to stimulate mitoBK channels [7, 12]. In this regard, usually an activator of mitochondrial potassium (mitoK) channels is able to promote a mild depolarization of mitochondrial membrane potential just to contain the accumulation of calcium ions into the matrix, thus reducing apoptosis and preserving the cell viability. Accordingly, the addition of grapefruit IntegroPectin in isolated cardiac mitochondria, at the concentration levels of 0.01, 0.03, 0.1 and 0.3 mg/mL, corresponding to about 1, 3, 10 and 30 micromolar of naringin (the glycoside-derivative of naringenin), showed a concentration-dependent de-

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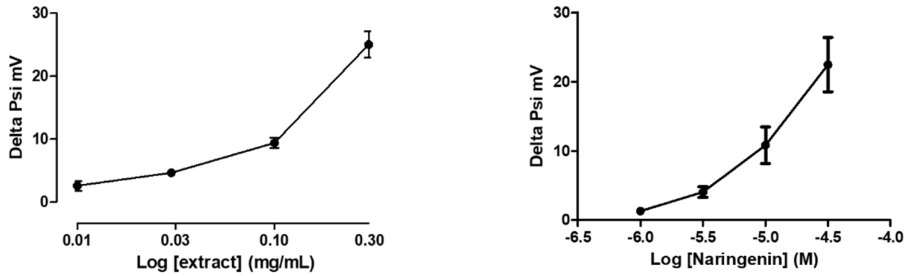
polarization, likewise to the *Citrus* flavonoid naringenin, as shown in Figure 2(a,b)). Consistently, Figure 3 shows that the addition of grapefruit IntegroPectin reduced concentration-dependently the uptake of calcium into the mitochondrial matrix, suggesting that the activation of mitoK channels might be responsible for the cardioprotection shown by the extract.



(**a**) (**b**)

Figure 2. Changes in Delta Psi value (mV) following the addition, into mitochondrial suspension buffer, of concentrations cumulatively increasing of: (**a**) Grapefruit IntegroPectin; (**b**) Naringenin. The vertical bars symbolize the standard errors (n = 6).

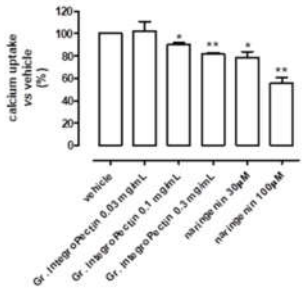


Figure 3. Changes in calcium uptake following the addition of increasing concentrations of grapefruit IntegroPectin or naringenin in the suspension mitochondrial buffer. The vertical bars symbolize the standard errors (n = 6). Asterisks show a statistically significant difference from the value observed in the hearts of vehicle-treated animals (* $p < 0.05$; ** $p<0.01$).

4. Discussion

Naringin and its aglycone naringenin have shown several beneficial health effects, including cardiovascular, metabolic and, recently, anti-SARS CoV-2 effects [29], but their absorption, distribution, metabolism and excretion, particularly in the presence of food matrix, impact on their bioavailability, which in turn affects the bioactivities of these flavonoids *in vivo* [30]. As mentioned in Section 1, often the encouraging *in vitro* results are not confirmed in *in vivo* approaches and clinical trials are inconsistent. The limited bioavailability of *Citrus* flavonoids can be at least in part the reason of these discrepancies. Indeed, *Citrus* flavonoids show poor water solubility, their bioavailability is low and undergo an extensive metabolism [30]. These pharmacokinetic complications limit their translational value and the possibility to use them as nutraceuticals in human. In order to improve this aspect, great efforts are underway. One of the most promising paths is the use of delivery systems, such as nanotechnologies, that may allow controlled release and also targeting to specific organs or tissues. Concerning naringenin and its glycosidic form naringin, the most used nanocarriers are lipid-based, polymeric and nanoemulsions. These particles can encapsulate naringenin or naringin inside their structures and release it *in vivo* [31]. Another relevant aspect in the *Citrus* fruits industry is represented by the

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amount of waste [32]. Indeed, mesocarp and endocarp are by-products, though containing high levels of bioactive constituents, especially *Citrus* flavonoids, such as naringin [33]; therefore, another field of research is addressed towards the recovery of waste products.

In this context, HC may be considered as an innovative green technique allowing a convenient exploitation of waste streams from the *Citrus* fruits industry, complying with the principles of bioeconomy [16]. This technique affords end products (*Citrus* fruits IntegroPectins) with higher water solubility, in which a great amount of bioactive compounds is adsorbed at the surface of pectin. Indeed, high levels of naringin (about 73 mg/g of extract) and other characteristic flavanones as well as volatile compounds (such as limonene and alpha-linalool) emerged from phytochemical analysis [16, 34].

Other commercial *Citrus* pectins have been characterized from the phytochemical and pharmacological point of view, however they did not contain relevant levels of polyphenols and their interest is mainly linked to the polysaccharide portion [19, 35]. Conversely, recent studies demonstrated that cavitation-derived IntegroPectins have more pronounced efficacy if compared to the commercial products [20]. Indeed, this HC-derived pectin not only contain plentiful flavonoids and terpenes, but it also features a unique molecular structure with very low degree of esterification and little degradation of the highly bioactive "hairy" RG-I chains [20]. This ensures immediate dissolution of the low-methoxyl grapefruit IntegroPectin in water at room temperature, whereas commercial citrus pectins (a high-methoxyl pectin virtually devoid of RG-I regions) require prolonged heating.

In order to explore this new product from a pharmacodynamic and pharmacokinetic point of view, we carried out an *in vivo* treatment through intraperitoneal injection and, 2 hours later, the heart was explanted and set up on Langendorff apparatus to proceed with ischemia/reperfusion protocol. Through this type of administration, bioactive compounds don't need to be adsorbed at intestinal level, however they must pass in the blood and be distributed at the organs and tissues, in order to play the pharmacological action; among which the liver, recognized as a main district responsible for the metabolism of flavonoids. Therefore, we can discuss, even if not exhaustively, about a putative improvement of the bioavailability. Moreover, we chose to evaluate the anti-ischemic cardioprotection, that has been previously demonstrated for naringin and naringenin [7, 12, 24-28].

Interestingly, our results suggest that grapefruit IntegroPectin is endowed with an anti-ischemic cardioprotective activity, exceeding the pure bioactive flavanone naringenin on a dose-dependent basis and leading us to conclude that this extract might have a more favorable pharmacokinetics at systemic level. A deeper insight into the mechanism of action in cardiac isolated mitochondria confirmed that the flavonoid portion, most likely naringenin/naringin, is the main actor of this promising pharmacological profile, being involved in the activation of mitoK channels.

5. Conclusions

This study further confirms that the emerging extraction technique based on HC processes offers a valuable opportunity for the convenient exploitation of waste *Citrus* fruits, in particular from grapefruit, with the end product presenting high concentration levels of *Citrus* flavonoids and an improved bioaccessibility that enhances its nutraceutical and, consequently, economic value. The performed *in vivo* experiments showed that grapefruit IntegroPectin was endowed with an effective anti-ischemic cardioprotective activity, exceeding that of pure naringenin on a dose-dependent basis, as well as that such activity was mediated by the activation of mitochondrial potassium channels.

Future experiments will be focused on the evaluation of oral bioavailability of flavonoids present in grapefruit IntegroPectin, aimed at investigating its actual translational nutraceutical value.

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Lorenzo Flori, Lorenzo Albanese and Federica Zabini; Project administration, Francesco Meneguzzo and Lara Testai; Supervision, Francesco Meneguzzo, Mario Pagliaro and Lara Testai; Writing – original draft, Francesco Meneguzzo and Lara Testai; Writing – review & editing, Vincenzo Calderone, Mario Pagliaro, Rosaria Ciriminna and Federica Zabini.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Testai, L., Calderone, V. Nutraceutical Value of Citrus Flavanones and Their Implications in Cardiovascular Disease. *Nutrients* **2017**, *9*, 502. doi:10.3390/nu9050502.
2. Pla-Pagà, L., Pedret, A., Valls, R.M., Calderón-Pérez, L., Llauradó, E., Companys, J., Martín-Luján, F., Moragas, A., Canela, N., Puiggròs, F., Caimari, A., Del Bas, J.M., Arola, L., Solà, R., Mayneris-Perxachs, J. Effects of Hesperidin Consumption on the Cardiovascular System in Pre- and Stage 1 Hypertensive Subjects: Targeted and Non-Targeted Metabolomic Approaches (CITRUS Study). *Mol Nutr Food Res* **2021**, *65*, e2001175. doi:10.1002/mnfr.202001175.
3. Naeini, F., Namkhah, Z., Tutunchi, H., Rezayat, S.M., Mansouri, S., Yaseri, M., Hosseinzadeh-Attar, M.J. Effects of naringenin supplementation on cardiovascular risk factors in overweight/obese patients with nonalcoholic fatty liver disease: a pilot double-blind, placebo-controlled, randomized clinical trial. *Eur J Gastroenterol Hepatol* **2022**, *34*, 345-353. doi:10.1097/MEG.0000000000002323.
4. Liu, P., Li, J., Liu, M., Zhang, M., Xue, Y., Zhang, Y., Han, X., Jing, X., Chu, L. Hesperetin modulates the Sirt1/Nrf2 signaling pathway in counteracting myocardial ischemia through suppression of oxidative stress, inflammation, and apoptosis. *Biomed Pharmacother* **2021**, *139*, 111552. doi:10.1016/j.biopha.2021.111552.
5. Li, H., Liu, L., Cao, Z., Li, W., Liu, R., Chen, Y., Li, C., Song, Y., Liu, G., Hu, J., Liu, Z., Lu, C., Liu, Y. Naringenin ameliorates homocysteine induced endothelial damage via the AMPKα/Sirt1 pathway. *J Adv Res* **2021**, *34*, 137-147. doi 10.1016/j.jare.2021.01.009.
6. Testai, L., Piragine, E., Piano, I., Flori, L., Da Pozzo, E., Miragliotta, V., Pirone, A., Citi, V., Di Cesare Mannelli, L., Brogi, S., Carpi, S., Martelli, A., Nieri, P., Martini, C., Ghelardini, C., Gargini, C., Calderone, V. The Citrus Flavonoid Naringenin Protects the Myocardium from Ageing-Dependent Dysfunction: Potential Role of SIRT1. *Oxid Med Cell Longev* **2020**, *2020*, 4650207. doi:10.1155/2020/4650207. .
7. Testai, L., Martelli, A., Marino, A., D'Antongiovanni, V., Ciregia, F., Giusti, L., Lucacchini, A., Chericoni, S., Breschi, M.C., Calderone, V. The activation of mitochondrial BK potassium channels contributes to the protective effects of naringenin against myocardial ischemia/reperfusion injury. *Biochem Pharmacol* **2013**, *85*, 1634-43. doi:10.1016/j.bcp.2013.03.018.
8. Saponara, S., Testai, L., Iozzi, D., Martinotti, E., Martelli, A., Chericoni, S., Sgaragli, G., Fusi, F., Calderone, V. (+/-)-Naringenin as large conductance Ca(2+)-activated K+ (BKCa) channel opener in vascular smooth muscle cells. *Br J Pharmacol* **2006**, *149*, 1013-21. doi:10.1038/sj.bjp.0706951.
9. Liu, Y., Niu, L., Cui, L., Hou, X., Li, J., Zhang, X., Zhang, M. Hesperetin inhibits rat coronary constriction by inhibiting Ca(2+) influx and enhancing voltage-gated K(+) channel currents of the myocytes. *Eur J Pharmacol* **2014**, *735*, 193-201. doi:10.1016/j.ejphar.2014.03.057.
10. Morand, C., Dubray, C., Milenkovic, D., Lioger, D., Martin, J.F., Scalbert, A., Mazur, A. Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. *Am J Clin Nutr* **2011**, *93*, 73-80. doi:10.3945/ajcn.110.004945.
11. Trivedi, P.P., Kushwaha, S., Tripathi, D.N., Jena, G.B. Cardioprotective effects of hesperetin against doxorubicin-induced oxidative stress and DNA damage in rat. *Cardiovasc Toxicol* **2011**, *11*, 215-25. doi:10.1007/s12012-011-9114-2.
12. Testai, L., Da Pozzo, E., Piano, I., Pistelli, L., Gargini, C., Breschi, M.C., Braca, A., Martini, C., Martelli, A., Calderone, V. The Citrus Flavanone Naringenin Produces Cardioprotective Effects in Hearts from 1 Year Old Rat, through Activation of mitoBK Channels. *Front Pharmacol* **2017**, *8*, 71. doi:10.3389/fphar.2017.00071.
13. Shi, R., Xu, J.W., Xiao, Z.T., Chen, R.F., Zhang, Y.L., Lin, J.B., Cheng, K.L., Wei, G.Y., Li, P.B., Zhou, W.L., Su, W.W. Naringin and Naringenin Relax Rat Tracheal Smooth by Regulating BK_{Ca} Activation. *J Med Food* **2019**, *22*, 963-970. doi:10.1089/jmf.2018.4364.
14. Fusi, F., Trezza, A., Tramaglino, M., Sgaragli, G., Saponara, S., Spiga, O. The beneficial health effects of flavonoids on the cardiovascular system: Focus on K^+ channels. *Pharmacol Res* **2020**, *152*, 104625. doi:10.1016/j.phrs.2019.104625.
15. Zhao, J., Yang, J., Xie, Y. Improvement strategies for the oral bioavailability of poorly water-soluble flavonoids: An overview. *Int J Pharm*. **2019**, *570*, 118642. doi:10.1016/j.ijpharm.2019.118642.

16. Meneguzzo, F., Brunetti, C., Fidalgo, A., Ciriminna, R., Delisi, R., Albanese, L., Zabini, F., Gori, A., dos Santos Nascimento, L.B., De Carlo, A., Ferrini, F., Ilharco, L.M., Pagliaro, M. Real-Scale Integral Valorization of Waste Orange Peel via Hydrodynamic Cavitation. *Processes* **2019**, *7*, 581. doi.org/10.3390/pr7090581.
17. Scurria, A., Sciortino, M., Presentato, A., Lino, C., Piacenza, E., Albanese, L., Zabini, F., Meneguzzo, F., Nuzzo, D., Pagliaro, M., Chillura Martino, D.F., Alduina, R., Avellone, G., Ciriminna, R. Volatile Compounds of Lemon and Grapefruit IntegroPectin. *Molecules* **2020**, *26*, 51. doi:10.3390/molecules26010051.
18. Scurria, A., Sciortino, M., Garcia, A.R., Pagliaro, M., Avellone, G., Fidalgo, A., Albanese, L., Meneguzzo, F., Ciriminna, R., Ilharco, L.M. Red Orange and Bitter Orange IntegroPectin: Structure and Main Functional Compounds. *Molecules* **2022**, *27*, 3243. doi:10.3390/molecules27103243.
19. Meneguzzo, F.; Albanese, L.; Zabini, F. Hydrodynamic Cavitation in Beer and Other Beverage Processing. In *Innovative Food Processing Technologies*; Knoerzer, K., Muthukumarappan, K., Eds.; Elsevier: Amsterdam, Netherlands, 2021; pp. 369–394. doi:10.1016/b978-0-08-100596-5.23022-9
20. Presentato, A., Piacenza, E., Scurria, A., Albanese, L., Zabini, F., Meneguzzo, F., Nuzzo, D., Pagliaro, M., Martino, D.C.. Alduina, R. A New Water-Soluble Bactericidal Agent for the Treatment of Infections Caused by Gram-Positive and Gram-Negative Bacterial Strains. Antibiotics **2020**, *9*, 586. doi:10.3390/antibiotics9090586.
21. Nuzzo, D., Scordino, M., Scurria, A., Giardina, C., Giordano, F., Meneguzzo, F., Mudò, G., Pagliaro, M., Picone, P., Attanzio, A., Raimondo, S., Ciriminna, R., Di Liberto, V. Protective, Antioxidant and Antiproliferative Activity of Grapefruit IntegroPectin on SH-SY5Y Cells. *Int J Mol Sci* **2021**, *22*, 9368. doi:10.3390/ijms22179368.
22. Calderone, V., Testai, L., Martelli, A., Rapposelli, S., Digiacomo, M., Balsamo, A., Breschi, M.C. Anti-ischemic properties of a new spiro-cyclic benzopyran activator of the cardiac mito-KATP channel. *Biochem Pharmacol* **2010**, *79*, 39-47. doi:10.1016/j.bcp.2009.07.017.
23. Heidary Moghaddam, R., Samimi, Z., Moradi, S.Z., Little, P.J., Xu, S., Farzaei, M.H. Naringenin and naringin in cardiovascular disease prevention: A preclinical review. *Eur J Pharmacol* **2020**, *887*, 17353. doi:10.1016/j.ejphar.2020.173535.
24. Rajadurai, M., Prince, P.S. Preventive effect of naringin on isoproterenol- induced cardiotoxicity in Wistar rats: an in vivo and in vitro study. *Toxicology* **2007**, *232,* 216-25. doi:10.1016/j.tox.2007.01.006.
25. You, Q., Wu, Z., Wu, B., Liu, C., Huang, R., Yang, L., Guo, R., Wu, K., Chen, J. Naringin protects cardiomyocytes against hyperglycemia-induced injuries in vitro and in vivo. *J Endocrinol* **2016**, *230*, 197-214. doi:10.1530/JOE-16-0004.
26. Rani, N., Bharti, S., Manchanda, M., Nag, T.C., Ray, R., Chauhan, S.S., Kumari, S., Arya, D.S. Regulation of heat shock proteins 27 and 70, p-Akt/p-eNOS and MAPKs by Naringin Dampens myocardial injury and dysfunction in vivo after ischemia/reperfusion. *PLoS One* **2013**, *8,*: e82577. doi:10.1371/journal.pone.0082577.
27. Liu, W., Cheng, L., Li, X., Zhao, L., Hu, X., Ma, Z. Short-term pretreatment of naringin isolated from Citrus wilsonii Tanaka attenuates rat myocardial ischemia/reperfusion injury. *Naunyn Schmiedebergs Arch Pharmacol* **2022**. doi:10.1007/s00210-022-02255-x.
28. Li, F., Zhan, Z., Qian, J., Cao, C., Yao, W., Wang, N. Naringin attenuates rat myocardial ischemia/reperfusion injury via PI3K/Akt pathway-mediated inhibition of apoptosis, oxidative stress and autophagy. *Exp Ther Med* **2021**, *22*, 811. doi:10.3892/etm.2021.10243tj Antibacterial. *ChemMedChem* **2020**, *15*, 2228–2235. doi:10.1002/cmdc.202000518.