Journal of Pharmacy & Pharmacognosy Research
ISSN: 0719-4250
editor@jppres.com
Asociación de Académicos de Ciencias Farmacéuticas de Antofagasta
Chile

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Journal of Pharmacy & Pharmacognosy Research,
vol. 6, núm. 3, 2018, Mayo-Junio, pp. 158-166
Asociación de Académicos de Ciencias Farmacéuticas de Antofagasta
Antofagasta, Chile

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ISSN 0719-4250
http://jppres.com/jppres

Original Article | Artículo Original

Direct actions of the flavonoids naringenin, quercetin and genistein on rat cardiac and vascular muscles

[Acciones directas de los flavonoides naringenina, quercetina y genisteína sobre los músculos cardiaco y vascular de rata]

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Abstract

Context: Flavonoids are natural polyphenolic compounds that are ubiquitous in plants. Numerous prospective epidemiological studies have found beneficial effects of flavonoid consumption on overall cardiovascular mortality. These effects have been attributed to nonspecific antioxidant, anti-atherosclerotic and antithrombotic properties of flavonoids. However, little is known about direct actions of flavonoids on cardiac and vascular smooth muscles.

Aims: To evaluate the effects of three flavonoids (naringenin, quercetin and genistein) on electrical and contractile activities of isolated rat hearts and on contraction of rat abdominal aortic rings.

Methods: Surface electrogram (ECG) and force of contraction (FC) were recorded in isolated rat hearts. The ventricular fibrillation threshold was evaluated with the flavonoids. The effects on aortic contraction were assessed in rat abdominal aortic rings pre-contracted with 60 mM of KCl.

Results: Genistein tended to prolong the QT interval while naringenin tended to decrease it. The effects of all flavonoids on QRS and RR interval were mild and not statistically significant. Genistein and naringenin produced a negative inotropic effect in isolated rat hearts with an IC_{50} of 13.8 µM and 33.8 µM, respectively. Quercetin at low concentrations had a positive inotropic effect; at high concentrations it exerted a negative inotropic effect. Naringenin and quercetin increased the threshold for ventricular fibrillation, whereas genistein decreased it. Naringenin, quercetin and genistein induced concentration-dependent relaxation in endothelium-denuded rat aortic rings.

Conclusions: These flavonoids have direct actions on rat cardiac and vascular smooth muscles.

Keywords: cardiovascular; flavonoid; genistein; naringenin; quercetin.

Resumen

Contexto: Los flavonoides son compuestos naturales polifenólicos omnipresentes en las plantas. Estudios epidemiológicos han mostrado beneficios con el consumo de flavonoides sobre la disminución de la mortalidad cardiovascular global. Esto se les atribuye principalmente por propiedades no específicas como antioxidantes, anti-ateroscleróticos y antitrombóticos. Sin embargo, se conoce poco acerca de las acciones cardiovasculares directas de los flavonoides.

Objetivos: Evaluar los efectos de tres flavonoides (naringenina, quercetina y genisteína) sobre las actividades eléctrica y contráctil de corazones aislados de rata y sobre la contracción de anillos de aorta abdominal de rata.

Métodos: El electrocardiograma y la fuerza de contracción se registraron en corazones aislados de rata. Se evaluó la variación del umbral de fibrilación ventricular con los flavonoides. Los efectos sobre la contracción aórtica fueron evaluados en anillos de aorta abdominal de rata pre-contraídos con 60 mM de KCl.

Resultados: La gensiteína tendió a prolongar el intervalo QT. Naringenina tendió a disminuirlo. Los efectos de los flavonoides sobre los intervalos QRS y RR, no tuvieron diferencias significativas. Genisteína y naringenina produjeron un efecto inotrópico negativo sobre los corazones aislados de rata con una IC_{50} de 13.8 µM y 33.8 µM, respectivamente. Quercetina a bajas concentraciones tuvo un efecto inotrópico positivo; a altas concentraciones tuvo un efecto inotrópico negativo. Naringenina y quercetina aumentaron el umbral de fibrilación, mientras que genisteína lo disminuyó. Naringenina, quercetina y genisteína relajaron los anillos de aorta abdominal de rata denudados de endotelio.

Conclusiones: Estos flavonoides tuvieron acciones directas sobre los músculos cardiaco y liso vascular.

Palabras Clave: cardiovascular; flavonoide; genisteína; naringenina; quercetina.

ARTICLE INFO
Received: October 30, 2017.
Received in revised form: January 26, 2018.
Accepted: February 12, 2018.
Available Online: February 21, 2018.
Declaration of interests: The authors declare no conflict of interest.
Funding: This work was supported by the Cuban Ministry of Public Health (Research Project N° 1301002).

INTRODUCTION

Flavonoids are bioactive polyphenolic compounds found in vegetable foods. They represent a diverse group of more than 5000 different biologically active compounds synthesized during plant metabolism, and several hundred are known to occur in commonly consumed foods (Cook and Samman, 1996). Structurally, flavonoids consist of two aromatic rings linked by a 3-carbon chain that forms an oxygenated heterocyclic ring. Differences in the generic structure of the heterocyclic ring, as well as the oxidation state and functional groups of the heterocyclic ring, classify flavonoids as flavonols, flavones, flavanones, flavan-3-ols (flavans), isoflavones and anthocyanins (Cook and Samman, 1996).

Epidemiologic studies suggest a role of flavonoids in the prevention of cardiovascular diseases. Several studies have shown that the consumption of flavonoid-rich foods tends to reduce the risk of cardiovascular diseases (Clark et al., 2015; Ivey et al., 2015; Liu et al., 2017).

Following consumption, flavonoids may contribute to a variety of beneficial biological activities in humans. Flavonoids improve endothelial function, they can influence blood pressure, oxidative damage, produce vascular relaxation, and reduce vascular peripheral resistance, reduce atherosclerotic plaque formation, inflammation, platelet function and thrombosis, blood lipids, and glucose metabolism (see for review Ivey et al., 2015; Rezende et al., 2016). These effects may help to explain the findings that flavonoids and flavonoid-rich foods exhibit cardioprotective properties.

These compounds generally show satisfactory bioavailability and, indeed, a good correlation between constant dietary intake of flavonoids and pharmacologically relevant plasma concentrations has been clearly demonstrated in humans (Cassidy and Minihane, 2017). However, little is known about direct actions of flavonoids on cardiac and vascular smooth muscles.

Many flavonoids such as naringenin, quercetin and genistein are sold as dietary supplements and have attracted interest from the pharmaceutical industry.

Quercetin is a flavonol and is one of the most widely distributed dietary flavonoids, found in fruits such as apples and in vegetables such as onions, as well as in tea and wine (see for review Clark et al., 2015). Quercetin has been shown to induce a dose-dependent reduction in blood pressure when given chronically in the most common rodent models of hypertension (see for review Pérez-Vizcaino et al., 2009). Reductions in systolic, diastolic and mean arterial pressures were observed in hypertensive patients after a high dose of quercetin treatment (Brüll et al., 2015).

Naringenin is a flavanone and is one of the major antioxidants present in citrus fruits (see for review Clark et al., 2015). Naringin, the glycoside of naringenin, is metabolized to its aglycone naringenin in humans. Naringin induced contraction of rat aortic rings and exerted a negative inotropic effect on isolated rat hearts (López-Medina et al., 2014). In mice hearts, naringin exerted a negative inotropic effect that could be explained by a decrease in sodium and calcium currents (Alvarez-Collazo et al., 2014). Since naringenin could be used as a dietary supplement, antioxidant, anti-inflammatory and even as a template to develop cardiovascular drugs, it is important to investigate its possible direct cardiovascular actions.

The isoflavone genistein is found in leguminous plants, especially soy beans (Sureda et al., 2017). Numerous studies have been focused on the promising effects of genistein as a hypotensive agent (Li et al., 2004; see for review Sureda et al., 2017). Genistein could act as a vasodilator, anti-thrombotic, and anti-atherosclerotic agent, exerting these effects through different mechanisms of action (see for review Sureda et al., 2017).

Further investigations of the cellular mechanisms are needed to investigate the effects of these flavonoids. The aim of the present investigation was to characterize the possible direct actions of the flavonoids naringenin, quercetin and genistein on electrical and contractile activities of rat isolated hearts and on the contraction of endothelium-deprived rat aortic rings. Furthermore, changes in threshold for ventricular fibrillation by these three flavonoids were also studied. Preliminary results were presented in "Proceedings of the 4th International Symposium on Pharmacology of Natural Products FAPRONATURA 2015" September 21st-

25th, 2015; Cuban Society of Pharmacology. Topes de Collantes, Sancti Spiritus, Cuba (Fleites et al., 2015).

MATERIAL AND METHODS

Animals

Male adult (7-8 weeks) Wistar rats were obtained from the National Center for Laboratory Animal Reproduction (CENPALAB; La Habana). Prior to experiment, animals were adapted for seven days to laboratory conditions (controlled temperature 25 ± 2°C, relative humidity 60 ± 10% and 12 h light/dark cycles). Tap water and standard diet for rodents supplied by CENPALAB were freely provided. All procedures were also conducted according to the European Commission guide-lines for the use and care of laboratory animals and approved by the Committee for Animal Care in Research of the Center (No. 08-2012, folio 73, book 01, 2012). The minimum number of animals and duration of observation required to obtain consistent data were employed.

Isolated hearts

As previously reported (Galán et al., 1998), under pentobarbital anaesthesia rat hearts were removed and placed in cold Tyrode (see below). Hearts were carefully dissected, mounted on a Langendorff column and perfused at constant flow (10 mL/min) with a Tyrode solution of the following composition (mmol/L): 140 NaCl, 2.5 KCl, $MgCl_2$, 2 $CaCl_2$, 10 Tris-hydroxymethylaminomethane, 10 Glucose (pH = 7.4, gassed with O_2; T = 35°C). A bipolar platinum recording electrode was placed on the ventricular epicardium to record the surface electrocardiogram (ECG). Another bipolar platinum electrode was placed near the atrioventricular ring and was connected to an electronic stimulator.

To record the force of contraction (FC), the cardiac apex was fixed to a force-displacement transducer with a surgical 6-0 silk thread. ECG and FC values were recorded at the spontaneous heart rate and at a fixed stimulus rate (400-ms RR interval).

The ventricular fibrillation threshold (VFT) was determined using a stimulation program that consisted of a train of twenty current pulses with 2 ms of duration (2 ms interval). Current intensity (mA) was measured with the stimulus-isolating unit. VFT was the current intensity at which at least 5 spontaneous arrhythmic complexes were observed after the end of the stimulus train.

Aortic rings

Abdominal aortic rings were dissected from rats under pentobarbital anaesthesia. Care was taken to mechanically remove the endothelium with the purpose of verifying the direct actions of the flavonoids on vascular smooth muscle. They were fixed to a force transducer and placed in bath chamber continuously perfused (10 mL/min) with the same Tyrode solution used for isolated hearts (pH = 7.4, gassed with O_2; T = 35°C) and stabilized, under a load of 0.5 g, for 30 min before the beginning of the experiment (Galán et al., 1998). Contraction was induced by replacing NaCl by KCl (60 mM). After an equilibration period of 30 min, the endothelium removal was confirmed by the administration of acetylcholine (10 µM) to KCl (60 mM)-precontracted vascular rings.

Flavonoids and chemicals

Flavonoids were prepared in ethanol as stock solutions and they were diluted in the bathing solution on the day of the experiment. All chemicals were from Sigma Aldrich (St. Louis, MO, USA).

Statistical analysis

Results are expressed as means and standard errors of means. Statistical significance was evaluated by means of Student's t test using the statistical software OriginPro 8 SRO v8.0724 (MA, USA). Differences were considered statistically significant for $p < 0.05$.

RESULTS

Effects of flavonoids on electrical and contractile activities of isolated rat hearts

Only genistein of the studied flavonoids prolonged the corrected QT (QTc) interval of surface electrocardiogram ($QTc = QT/\sqrt{RR}$) in all range of concentrations, even though it is statistically significant ($p < 0.05$) only at 10 µmol/L (from 88.1 ± 5.1 ms in control to 130.4 ± 16.5 ms). Naringenin decreased the QTc with statistical significance ($p < 0.05$) at 3,

100 y 300 µM (from 86.0 ± 3.0 ms in control to 72.5 ± 4.3 ms, 64.4 ± 3.4, and 57.0 ± 2.0 ms, respectively). QTc was not significantly affected by quercetin at concentrations from 1 to 10 µM. However, at 30 and 100 µM quercetin showed a tendency to increase QTc (from 89.4 ± 10 ms in control to 99.9 ± 10 and 94.5 ± 9 ms, respectively) but without statistical significance.

All flavonoids showed a tendency to increase QRS interval and the RR of surface electrocardiogram, but only in naringenin at 100 y 300 µM, this increase in QRS is statistically significant (the predrug control QRS was 7.2 ± 0.5 ms, at 100 y 300 µM it was increased to 10.8 ± 0.6 ms and 10.9 ± 1.2, respectively).

Quercetin and naringenin increased ventricular fibrillation threshold in 40 ± 8.2% and 16 ± 1.2%, respectively, after applying concentrations from 1 to 100 µM. However, genistein decreased it in 20 ± 0.8%. The changes in the ventricular fibrillation threshold were independent of concentration by the three flavonoids.

In the concentration range from 1 to 300 µM, naringenin and genistein significantly decreased the force of contraction (FC) in isolated rat hearts (Fig. 1A). Since RR interval was slightly changed by the three flavonoids, hearts were paced at 400-ms stimulus interval (slightly over the spontaneous RR interval under control condition; 410 ± 2 ms) in order to avoid any frequency-dependent change in FC. Experimental data were fitted to a Hill function (Fig. 1A) and the estimated IC_{50} for inhibition of contraction was 33.8 ± 9.5 µM for naringenin and 13.8 ± 2.5 µM for genistein. The action of naringenin and genistein on FC was not reversible upon washout with normal Tyrode solution.

Quercetin had a dual effect on cardiac contraction; at concentrations from 1 to 3 µM had positive inotropic effect (Fig. 1B). At 10 µM the effects were not statistically significant compared to control condition. However, at concentrations from 30 to 100 µM significantly decreased the FC in isolated rat hearts ($p < 0.05$; 94 ± 0.3 % maximal decrease at 100 µM) (Fig. 1B).

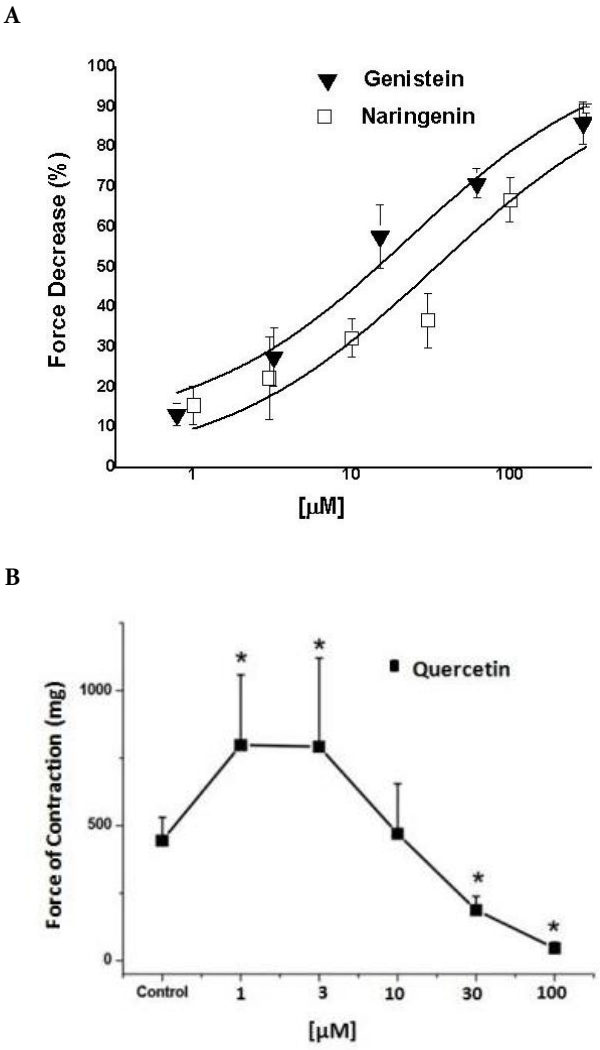

Figure 1. Effects of flavonoids (naringenin, genistein and quercetin) on contractile activity of isolated, Langendorff-perfused rat hearts.

A: Concentration-response curves for the inhibition of force of contraction by naringenin and genistein. Experimental data (n = 6 for each point) were fitted to a Hill function. **B:** Effect of quercetin on force of contraction recorded in isolated rat hearts. *p < 0.05 compared to control values.

Vasorelaxant effect of flavonoids on rat aortic rings

In endothelium-denuded rat aortic rings precontracted with KCl (60 mM), the three flavonoids (naringenin, quercetin and genistein) induced a

dose-dependently relaxing response (Fig. 2A-C). Concentration-response curve, based on the Hill function, was fitted to the experimental data obtained after applying naringenin concentrations from 1 to 100 µM; the results are presented in Fig. 2D. The IC_{50} value for naringenin estimated was IC_{50} = 30.07 ± 1.55 µM. Genistein and quercetin exerted a modest but concentration-dependent vasorelaxing effect. A maximal decrease in contraction force of 44.3 ± 11.2% for gensitein and 46.4 ± 5.5% for quercetin were attained at the highest concentrations used at 100 µM. The IC_{50} values for genistein and quercetin could not be calculated because these compounds showed an efficacy parameter lower than (or close to) 50%. Likewise, naringenin was more potent than quercetin and genistein for vasorelaxing effect.

DISCUSSION

The present results show that the three flavonoids: quercetin, naringenin and genistein, exert direct cardiovascular actions.

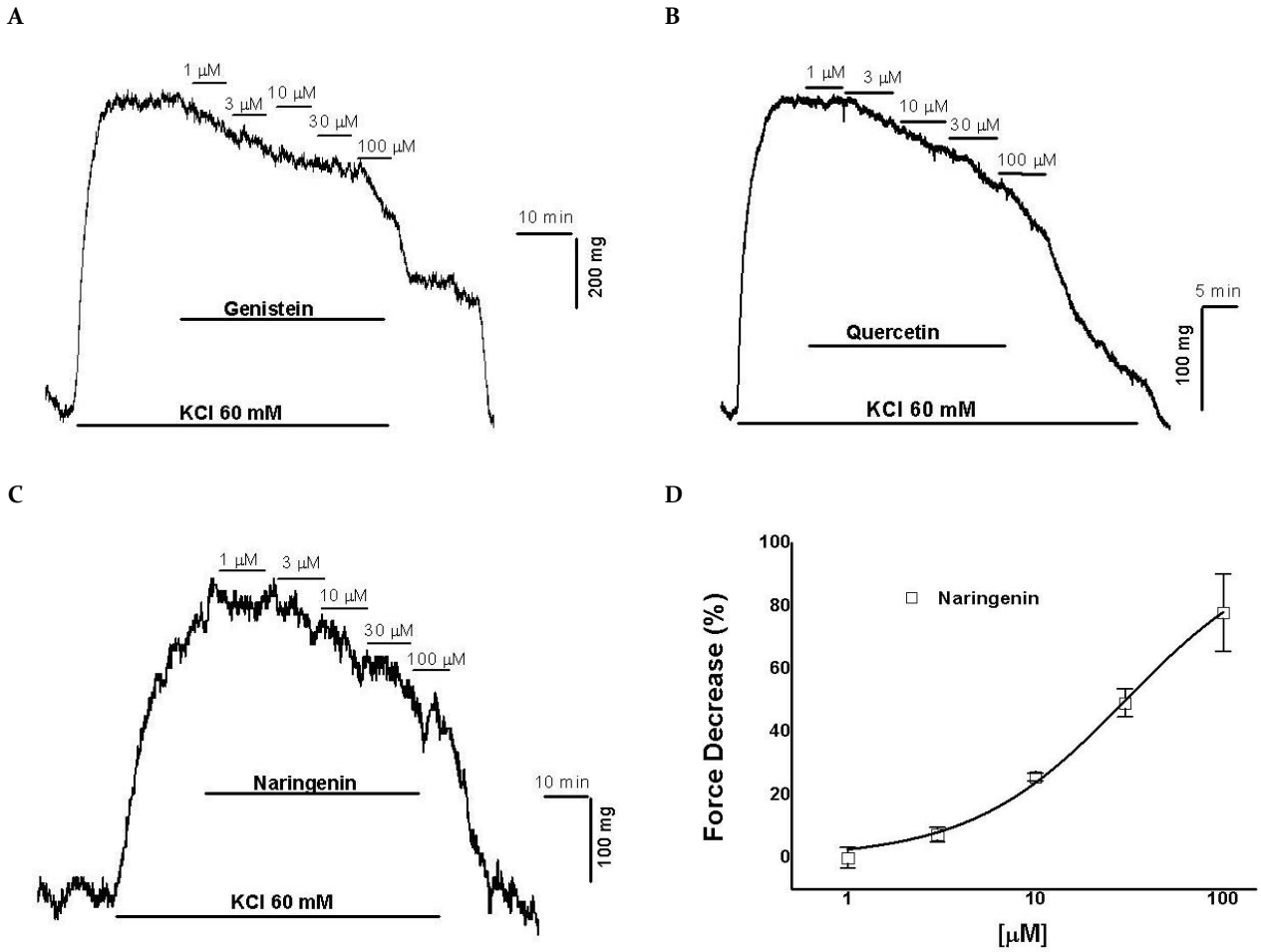

Figure 2. Effects of flavonoids (naringenin, genistein and quercetin) on rat aortic rings.

A-C: Examples of the vasorelaxing effect of genistein (**A**), quercetin (**B**) and naringenin (**C**) at different concentrations in a rat aortic ring pre-contracted with 60 mM KCl. **D**: Concentration-dependent vasorelaxing effect of naringenin (n = 6). The solid line represents the fit with a Hill function.

Only genistein of the studied flavonoids tended to prolong the QTc interval of surface electrocardiogram, but this increase was statistically significant only at 10 μM, concentration closely related to IC_{50} value for the decrease of FC in present study (13.8 ± 2.5 μM). Genistein also increased the QRS interval and the RR of surface electrocardiogram, but with no difference significant statistically. These results could be related with the fat that genistein as a tyrosyne kinase inhibitor, inhibits inwardly-rectifying Kir2.1 channels expressed in HEK 293 cells and it participates in the modulation of human cardiac excitability (Zhang et al., 2011).

Naringenin, like its glycoside naringin (López-Medina et al., 2014), showed a tendency to decrease QTc. At 100 y 300 μM, naringenin significantly prolonged QRS interval. The RR interval was not influenced by naringenin significantly. Grapefruit juice (it contains high amounts of naringenin) causes significant QT prolongation in healthy volunteers and naringenin has been identified as the most potent human ether-a-go-go-related gene (HERG) channel blocker among several dietary flavonoids (Scholz et al., 2005; Zitron et al., 2005; Piccirillo et al., 2008).

Drugs prolong the QT interval by blocking voltage-gated K^+ channels, especially the rapid component of the delayed rectifier potassium current I_{Kr}, expressed by HERG. However, rat hearts are believed to be less susceptible to these effects because of their dominant transient outward K^+ current (I_{to}), which is believed to override any effect on I_{Kr} (Gralinski, 2003). In the absence of information on this issue, and for the results reported perhaps genistein and naringenin acts on Na^+, K^+ and/or on Na-Ca exchanger.

On the other hand, quercetin, no modified QTc, QRS and RR significantly, but it showed a tendency to increase these intervals.

These results should be possible because these three flavonoids could exert multiple actions on different ionic channels that counterbalance to each other, resulting in an "apparent" absence of effects on cardiac surface electrogram. As a fat, all of three flavonoids modulate several ionic channels (Summanen et al., 2001; Wu et al., 2003; Scholz et al., 2005; Cogolludo et al., 2007; Zhao et al., 2008; Huang et al., 2009; Saponara et al., 2011; Zhang et al., 2011; Alvarez-Collazo et al., 2014; Hou et al., 2014; Fusi et al., 2016).

As shown in Results quercetin and naringenin increased the threshold for ventricular fibrillation. These findings indicate clear cut acute protection of the heart from ventricular fibrillation by quercetin and naringenin as well as their efficacy to facilitate sinus rhythm restoration. Protective effects on heart against ischemia-reperfusion injury by quercetin were founded by several authors (Barteková et al., 2010; Jin et al., 2012). Testai et al. (2013) reported that naringenin exhibit direct cardioprotective effects against the injury induced by drastic ischaemia/reperfusion in Langendorff-perfused rat heart.

Genistein, however, decreased the threshold for ventricular fibrillation. Indeed, the present findings are in agreement with previous data showing that genistein, by inhibiting tyrosine kinase attenuates or abolishes the cardioprotective effects of ischaemic preconditioning (Fatehi-Hassanabad and Parratt, 1997). Meanwhile, Deodato et al. (1999) suggest that genistein limits the inflammatory response and protects against myocardial ischaemia-reperfusion injury.

Naringenin and genistein exerted a negative inotropic action in rat isolated heart in concentration dependent way. Saponara et al. (2011) reported that naringenin and genistein reduced the current of the channel Cav1.2 in a concentration-dependent manner in rat tail artery. Like naringenin, naringin (the glycoside of naringenin) exerted a negative inotropic effect on isolated rat hearts (López-Medina et al., 2014). Similar results were seen in mice hearts, where naringin exerted a negative inotropic effect that could be explained by a decrease in sodium and calcium currents (Alvarez-Collazo et al., 2014).

Genistein was recently found to inhibit Cav3.1 currents in rat tail artery (Fusi et al., 2016). Moreover, genistein should be considered a promiscuous ion channel modulator of cardiovascular Cav1.2 channels, including direct interaction with the channel (Zhao et al., 2008) and in indirect manner, as an inhibitor of tyrosine kinase-mediated modulation of these channel (Schröder et al., 2004).

In present study, quercetin had dual effect on cardiac contraction. At smaller concentration (1 to 3 μM) quercetin had positive inotropic action, and at higher concentration (30 to 100 μM) quercetin exerted negative inotropic action. Summanen et al. (2001) demonstrated that quercetin increases calci-

um current in clonal rat pituitary GH4C1 cells. Several other groups provided direct electrophysiological evidence, in various cell types, that quercetin is a stimulator of calcium channels, e.g., Huang et al. (2009) in smooth muscle cells of the guinea-pig proximal colon, Wu et al. (2003) in GH3 cells, and Saponara et al. (2011) in rat tail artery. On the contrary, Hou et al. (2014) demonstrated that calcium channel block by quercetin (IC_{50} value of about 10 μM) along with potassium channel stimulation accounts, for its spasmolytic effects in rat coronary artery preparations.

Vasodilator effects were seen in the results with these three flavonoids in endothelium-denuded rat aortic rings precontracted with KCl (60 mM). Although most studies focused on the endothelium dependent vascular relaxation induced by flavonoids, there are several studies, which have demonstrated the direct effects of some flavonoids on vascular smooth muscle cells. Many of these compounds modulate ion channels such as Ca^{2+}, Cl^-, and K^+ causing relaxation through reduction of Ca^{2+} influx (see for review Rezende et al., 2016).

Li et al. (2004) showed that genistein could dose-dependently relax 40 mM KCl precontracted arterial strips of rabbit aortic artery, in endothelium independent manner; in the same way, genistein vasorelaxed in the present results.

It has been previously reported that naringin, the glycoside of naringenin, induced contraction of rat aortic rings (López-Medina et al., 2014). In some cases, flavonoid glycosides showed similar or different biological activity than their flavonoid aglycones, it is due to the different hydrophobicity and the specific membrane receptor for each flavonoid (Xiao, 2017). Cavia-Saiz et al. (2010) reported that naringenin showed higher antioxidant potential and hydroxyl/superoxide radical scavenger capacity than those of naringin. Moreover, naringenin exhibits a more significant protective effect against oxidative damage to lipids (Cavia-Saiz et al., 2010).

In agreement with the present results, Cogolludo et al. (2007) indicated that quercetin produced relaxant effects in rat coronary arteries; it may play a role in the antihypertensive effects of quercetin in rat models of hypertension and in the protective effects of quercetin in coronary heart disease as observed in epidemiological studies.

CONCLUSIONS

The present study showed that naringenin, quercetin, and genistein have direct actions on rat cardiac and vascular muscles. This suggests that the three flavonoids could exert multiple actions on different ionic channels of both muscles. Future studies are needed to clarify these findings. Consumption of diets rich in these flavonoids should account these effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This work was supported by the Cuban Ministry of Public Health (Research Project N° 1301002). The authors are grateful to Dr. Julio L. Alvarez for help with editing of the article.

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Author contribution:

Contribution	Galán-Martínez L	Herrera-Estrada I	Fleites-Vázquez A
Concepts or ideas	X	X	
Design	X		
Definition of intellectual content	X		
Literature search	X	X	
Experimental studies	X	X	X
Data acquisition	X	X	X
Data analysis	X	X	X
Statistical analysis	X	X	X
Manuscript preparation	X		
Manuscript editing	X		
Manuscript review	X	X	X

Citation Format: Galán-Martínez L, Herrera-Estrada I, Fleites-Vázquez A (2018) Direct actions of the flavonoids naringenin, quercetin and genistein on rat cardiac and vascular muscles. J Pharm Pharmacogn Res 6(3): 158–166.