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Vasorelaxan Effect and Potent Antioxidant Activity of Natural Flavones Isolated from *Lourteigia stoechadifolia* and *Ageratina stevioides*, Two Venezuelan Plants

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JMP, EM and EGDL designed the study and performed the vascular experiments. Authors MJM and MAS performed the antioxidant assays. Authors AA and JMAL collected the plant material, isolated and provided the investigated flavones. Authors EGDL and JLLP managed the literature searches and the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was undertaken to investigate the vasorelaxant and antioxidant effects of natural flavones, 5,3'-dihydroxy-6,7,4'-trimetoxyflavone (eupatorin) **(1)**, 5-hydroxy-6,7,3',4'-tetrametoxyflavone **(2),** and 5,4'-dihydroxy-7-metoxyflavone (genkwanin) **(3)**.
Study Design: Biomedical assays in isolated vascular tissue.
Place and Duration of Study: Department of Pharmacology, Faculty of Medicine, University of Panama, Panama Between March 2015 and September 2016.
Methodology: The relaxation responses to cumulative concentrations of flavones **1, 2** and **3** (10^{-6} - 10^{-4} M) were tested on aortic rings, with or without endothelium, precontracted with phenylephrine (10^{-6} M). In complementary experiments, the tissues were preincubated with N^{ω}-nitro-L-arginine methyl ester (L-NAME), methylene blue or indomethacin. In order to evaluate their antioxidant capacity, the effect of each compound on acetylcholine-response was studied in aortic pre-treated with high dose of lucigenin. Additionally, superoxide anion generation was measured in isolated aortic rings using the lucigenin-enhanced chemiluminescence method.
Results: Flavones **1**, **2** and **3** induced a significantly vasorelaxant effect above 80% in aortic with endothelium. Mechanical removal of endothelium significantly decreased vasorelaxation of flavones **1** and **3** (15.6±4.6 and 28.4±2.9%, respectively). The vasorelaxant effect of flavones **1** and **3** were almost abolished when tissue was incubated with L-NAME or methylene blue. In aortic rings pre-treated with high dose of lucigenin reduced the relaxation to acetylcholine (28.4%) and it was prevented by all flavones (**1**=80.5; **2**=81.4; and **3**=66.2%). Complementary, all flavones significantly reduced superoxide levels.
Conclusion: Flavones isolated from two tropical plants are able to induce vasorelaxation, improve the impaired response to acetylcholine induced by lucigenin and exhibit radical scavenging activity.

Keywords: Eupatorin; genkwanin; flavones; antioxidant; vasorelaxation; Lourteigia stoechadifolia; Ageratina stevioides.

1. INTRODUCTION

Flavonoids are a broad class of natural products that includes subgroups such as chalcones, flavones, flavonols, isoflavones, anthocyanidins, and others. These groups of natural compounds have been associated with a large spectrum of cardiovascular activity. In this sense, Duarte et al. [1] demonstrated that these natural products can reduce high blood pressure and improve vascular function in spontaneous hypertensive animals. Other studies proved that flavonoids are able to modulate the vascular tone and produce concentration-dependent relaxation responses on isolated aortic rings [2,3]. Indeed, many flavonoids show vasorelaxant properties, due to different, and often poorly defined, mechanisms of action. Among these properties, flavonoids have been shown to reduce vascular contractility by endothelium-dependent and independent mechanisms involving the inhibition of calcium movements through cell membranes [4]. In addition, the inhibitory effect of cyclic nucleotide phosphodiesterase was shown to be responsible for the aortic ring's vasorelaxation produced by some flavonoids [5]. Among the subgroups of flavonoids, flavonols and flavones have demonstrated to have the greatest vasorelaxant effect [6].

Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. In this sense, flavonols are known to exhibit a scavenger superoxide radicals (•O2–) activity, and their lipid oxidation inhibition has been extensively investigated in both chemical and *in vitro* systems [7]. It has also been described its ability to inhibit nitric oxide (NO) and inflammatory enzymes (collagenase, hyaluronidase, 15-lipoxygenase and cyclooxygenase) [8], effects which has been related to the antioxidant and anti-inflammatory activity.

Epidemiological studies have suggested that flavonoid consumption is associated with lower risk of death from cardiovascular disease [9]. In particular, McCullough et al. [10], suggest that most inverse associations appeared with intermediate intakes, indicating that even relatively small amounts of flavonoid-rich foods may be beneficial.

These findings have increased the interest in isolated new natural flavonoids and in

determining the mechanism of their potential cardioprotective effects [11]. Therefore, the aim of the present study is to investigate the vasorelaxant and antioxidant effects of three flavones (Fig. 1): 5,3'-dihydroxy-6,7,4'-trimetoxyflavone (eupatorin) (**1**) and 5-hydroxy-6,7,3',4'-tetrametoxyflavone (**2**) isolated from *Lourteigia stoechadifolia*, and 5,4'-dihydroxy-7-metoxyflavone (genkwanin) (**3**) isolated from *Ageratina stevioides*.

2. MATERIALS AND METHODS

2.1 Plant Material

Lourteigia stoechadifolia (L.*f.*) King & Robinson [family ASTERACEAE] was collected in Gavidia, Municipio Rangel, Estado Mérida (Venezuela) in August, 2003. *Ageratina stevioides* (Steyermark) King & Robinson [family ASTERACEAE] was collected in El Delgadito, Páramo de la Negra, Municipio Rivas Dávila, Estado Mérida (Venezuela), in September, 2004. Authentication of both species was performed by the engineer Juan A. Carmona (Herbarium MERF of Pharmacy Faculty, University of Los Andes) and voucher specimens (J. M. Amaro, N°1610 and J. M. Amaro, N°1621, respectively) were deposited into the Herbarium of the Faculty of Pharmacy in Merida, Venezuela.

2.2 Extraction, Isolation and Characterization of Flavones

Aerial parts of *Lourteigia stoechadifolia* were dried and ground. The plant material (5.7 Kg) was soaked with methanol at room temperature for 3 days [12], resulting in 800 g of a crude extract. This extract was chromatographed on a silica gel column, eluted with methanol and ethyl acetate mixtures of increasing polarity. Dried fractions (35 g) were eluted again with *n*-hexane/EtOAc (4:1), followed by a silica gel G preparative thin layer chromatography to obtain flavones **1** (55 mg) and **2** (35 mg).

The aerial parts of *Ageratina stevioides* (1.8 Kg) were extracted with ethanol in a soxhlet (Pyrex®) [13]. The solvent was removed under a vacuum below 45ºC to provide a residue (52 g) that was chromatographed on a silica gel column with *n*-hexane/EtOAc mixtures (19:1) to obtain the flavone **3** (42 mg).

The structures of these three compounds (Fig. 1) were deduced by a detailed analysis and a rigorous interpretation of their ^{1}H NMR spectroscopy data, and corroborated from an iterative search of their ^{13}C NMR chemical shift, carried out within our NAPROC-13 RMN spectroscopical database (http://c13.usal.es) [14]. Their physico-chemical properties have been published in the cited references.

2.3 Preparation of Rat Aortic Rings

Wistar male rats (180–200 g) were anesthetized with 40 mg/Kg sodium pentobarbital (i.p.) and exsanguinated. The thoracic aorta was immediately excised and placed in Krebs–Henseleit (KH) solution (in mM: NaCl, 115.5; KCl, 4.6; NaH_2PO_4, 1.3; $NaHCO_3$, 24; $CaCl_2$, 2.5; $MgSO_4$, 1.2; glucose, 11.1) at 4℃ and pH 7.4. After removal of adherent fat and connective tissue, the aortas were cut into rings (3 mm in length) that were placed between stainless steel hooks and set up in organ chambers filled with 5 mL of KH solution, aerated with carbogen (95% O_2 and 5% CO_2) and kept at 37ºC. One

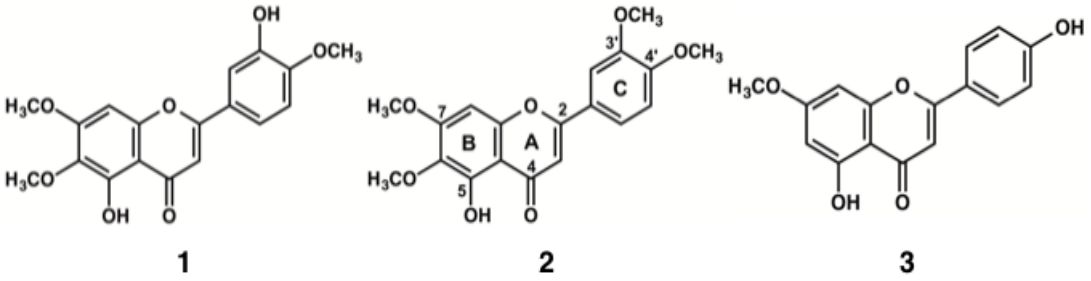

Fig. 1. Chemical structures of the flavones 5,3'-dihydroxy-6,7,4'-trimetoxyflavone (1), 5-hydroxy-6,7,3',4'-tetrametoxyflavone (2) and 5,4'-dihydroxy-7-metoxyflavone (3) tested in this study

of the hooks was fixed to the bath and the other connected to an isometric force transducer (model TRI-201, LETICA). Changes in tension were recorded continuously by a bio-signal recording system (PowerLab/400 AD Instruments). All rings were allowed to equilibrate for 1 hour at a resting tension of 2 g, replacing the bath solution every 20 min during this period.

In some of the aortic rings, the endothelium was mechanically removed according to the method described by Chen et al. [15].

2.4 Vasorelaxant Activity in Pre-contracted Rat Aortic Rings

After the equilibration period, the endothelial integrity was confirmed by eliciting a relaxation with acetylcholine (10^{-6} M) after contraction induced by phenylephrine (10^{-6} M). Only endothelium-intact rings exhibiting greater than 60% relaxation to acetylcholine were used for these experiments. A set of previous experiments indicated that the maximum contractile response produced by phenylephrine was attained within 15 minutes and was relatively well sustained over the subsequent 60 minutes. Under this condition, the relaxation responses to cumulative concentrations of flavones **1**, **2** and **3** (10^{-6}-10^{-4} M) were tested separately in aortic rings pre-contracted with phenylephrine (10^{-6} M). Quercetin (10^{-6}-10^{-4} M), a well know flavonoid with vasorelaxing activity, was used as positive control. Additionally, the removal of the endothelium was carried out to measure the vasorelaxant effect of flavones on aortic rings in absence of the endothelium.

All flavones tested were dissolved in dimethylsulfoxide (DMSO) to perform all the experiments. Control tissues were subjected to the same procedures simultaneously, but omitting the compounds and adding the vehicle (appropriate dilutions of DMSO, 0.001 - 0.1%).

Relaxations are expressed as the percentage inhibition of tension developed by phenylephrine.

2.5 Mechanism Involved in Vasorelaxant Effect of Flavones

The vasorelaxation of flavones **1** and **3**, was studied on isolated aortic rings with endothelium-intact pre-incubated for 30 min with L-NAME (10^{-4} M), an inhibitor of nitric oxide synthase; methylene blue (10^{-5} M), an inhibitor of guanylyl cyclase, or indomethacin (10^{-5} M), a cyclooxygenase inhibitor, in order to determine their mechanism. In another series of experiments, rat aortic rings were incubated with flavone **2** (10^{-4} M) for 30 min and the contractile response to norepinephrine (10^{-11} - 10^{-5} M) was evaluated.

2.6 Determination of Radical Scavenging Activity on Aortic Rings

The method to determine radical scavenging activity by flavones was previously described by Kassan et al. [16]. The scavenging activity of all flavones was evaluated in vessels with functional endothelium incubated with high concentration of lucigenin (250 μM) for 1 hour in the presence or absence of flavones **1**, **2** and **3** (10^{-5} M). In this protocol, a concentration-response curve to acetylcholine (10^{-8}-10^{-4} M) was evaluated in phenylephrine pre-contracted aortic rings. In parallel experiments, the effect of vehicle alone (DMSO, 0.001%) and quercetin (10^{-5} M) were also tested.

2.7 Effect of Flavones on Superoxide Anion Production

Isolated aortic ring segments were incubated in a HEPES buffer (in mM: NaCl, 119; HEPES, 20; $MgSO_4$, 1; KCl, 4.6; KH_2PO_4, 0.4; Na_2HPO_4, 0.15; $NaHCO_3$, 5; $CaCl_2$, 1.2; glucose, 5.5; pH 7.4) aerated with carbogen and maintained at 37℃ for 30 min. After the incubation period, the rings were transferred into tubes containing 1 mL of the HEPES buffer containing 5 μM lucigenin supplemented with the corresponding tested flavones **1**, **2**, **3** (10^{-5} M), quercetin (10^{-5} M) or DMSO. Under these conditions, superoxide levels were measured by chemiluminiscence using a luminometer (LUMAT LB-9507). Luminescence units were recorded every 30 s for 5 minutes. The relative values of superoxide anion production were expressed as relative luminescence units per min and mg of tissue (RLU/min/mg).

2.8 Drugs and Reagents

Phenylephrine hydrochloride, norepinephrine hydrochloride, acetylcholine chloride, *N*$^{\omega}$-nitro-L-arginine methyl ester (L-NAME), indomethacin, methylene blue, *N*,*N*-dimethyl-9,9'-biacridinium dinitrate (lucigenin) and dimethylsulfoxide (DMSO) of the highest grade were purchased

from Sigma. Drugs and Krebs solution were freshly prepared in bidestilate water.

2.9 Statistical Analysis

Data are expressed as means±standard error of the mean (S.E.M.). Concentration-response curves were fitted to the logistic equation and the concentration causing a 50% effect (EC_{50}) was calculated using the GraphPad Prism 5.0 software. To compare concentration-response curves, statistical analyses were performed according to the extra sum of squares F test principle. Other comparisons among groups were made using analysis of variance (ANOVA) followed by the post hoc Bonferroni's test. Statistically significant values were considered when $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Vascular Effects of Flavones

The vasorelaxation responses to increasing concentrations to flavones **1**, **2** and **3** on aortic rings with and without endothelium are shown in Fig. 2. The maximum response was similar for the three flavones, but for flavones **1** and **3** was dramatically reduced when the endothelium was removed (Table 1).

The present study was performed in order to investigate the vasodilatory properties of 5,3'-dihydroxy-6,7,4'-trimetoxyflavone (**1**); 5-hydroxy-6,7,3',4'-tetrametoxyflavone (**2**) and 5,4'-dihydroxy-7-metoxyflavone (**3**), and also to elucidate the pathway implicated in the vasodilator effect. Flavonoids are natural products that display a broad range of pharmacological actions including antioxidant and vasorelaxant effects [17,18,19].

In this sense, our results obtained with flavone **1** are consistent with the observations of Duarte et al. [1], who reported that flavonoids with an additional hydroxyl group at the 3' position of the C ring are potent vasodilators (Fig. 1). Woodman et al. [20] suggest that the hydroxyl group at this position may improve the ability of the other hydroxyl groups on the A and B rings to increase the vascular activity of the flavonols. Furthermore, these authors reported that the hydroxyl group at C-4' may be less important than the hydroxyl group at C-3'. However, flavone **3**, which has a hydroxyl group at C-4', exhibited a similar vasodilator activity that flavone **1**. It has been described that the presence of methoxyl groups at positions C-3' and/or C-4' could abolish the high vascular activity of the catechol group [19], however we noted that flavone **2** exhibits a strong vascular activity. Although studies on the structure-activity relationships of flavones are not conclusive as their structural diversity and different mechanism of actions, several structural features have been found to be involved in the endothelium-dependent vasodilator activity [21].

3.2 Mechanism Involved in Vaso-relaxation Effects Induced by Flavones

In complementary assays, we observed that vasorelaxant effect induced by flavone **1** was abolished in the presence of L-NAME and methylene blue, and partially reduced by indomethacin (Fig. 3A). Pre-incubation with indomethacin significantly reduced the vasodilatation of flavone **3**, and this effect was completely abolished when vascular tissues were pre-incubated with L-NAME (10^{-4} M) or methylene blue (Fig. 3B).

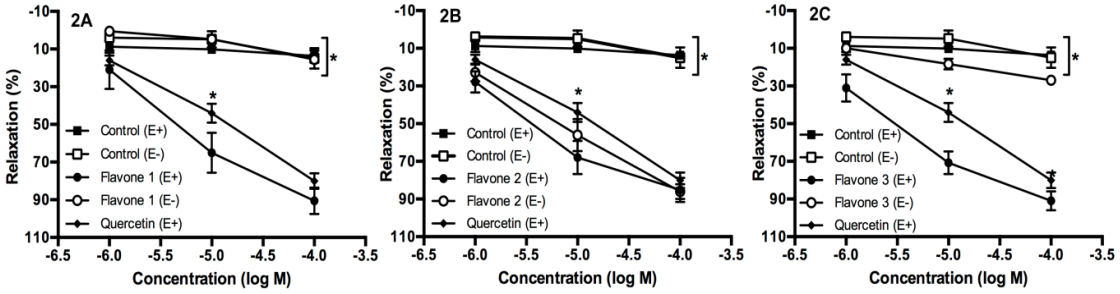

Fig. 2. Concentration–response curves to flavones 1 (A), 2 (B) and 3 (C) on rat aortic preparations with (E+) and without endothelium (E-) precontracted with phenylephrine

*DMSO was used in control rings and quercetin as positive control. Results are presented as mean±S.E.M., n=6, *p<0.05 compared with Control E+*

In order to determine whether flavone **2** could act on the α_1-adrenoceptors of vascular smooth muscle, cumulative concentration–response curves to noradrenaline were performed in the absence or presence of this compound (10^{-4} M) in rat thoracic aortic rings. Our results indicated that flavone **2** did not modify the maximal contraction of norepinephrine (E_{max} = 98.1±1.3% and 97.0±1.3%, respectively). Additionally, pD2 values showed not significantly difference in absence (8.2±0.1) or presence (7.9±0.1) of flavone **2**, as showed in Fig. 4.

Regulation of nitric oxide has been demonstrated as an important regulator of vascular functions by controlling the tone of blood vessels as well as the vascular mechanism of several natural products [22,23]. It is well known that a reduced production of nitric oxide by endothelial cells is associated with development of cardiovascular diseases such as hypertension and atherosclerosis [24]. These findings have increased interest in the investigation of how these products act on nitric oxide pathway. We observe that the removal of functional endothelium inhibited the relaxant response of flavones **1** and **3**, suggesting an endothelium-dependent effect. Vasorelaxation induced by both flavones was reduced after inhibition of nitric oxide synthase activity by L-NAME. Furthermore, inhibition of guanylyl ciclase by methylene blue, significantly reduced vasorelaxation induced by flavone **1**, and abolished the effect of flavone **3**. These results indicate that nitric oxide -cGMP pathway is involved in the relaxation induced by both flavones in endothelium-intact aorta.

In the other hand, we can observe that flavone **2** was the only compound able to induce relaxation in rat aortic rings through an endothelium-independent mechanism. Several mechanisms of action have been proposed to describe the endothelium-independent relaxation response, including adrenergic receptor interactions. In this sense, we observed that noradrenaline-contraction was not modified by flavone **2**, suggesting an effect independent of adrenoceptor α_1 antagonism. Previous data obtained by Chan et al. [25], suggest that the effect of some flavonoids on vascular contraction is not related to the antagonism of α_1 receptor, but involve an interference with the calcium mobilization. Therefore, flavone **2** could interfere in calcium signalling.

Table 1. EC_{50} values and maximal relaxation (E_{max}) of flavones 1, 2, and 3 on endothelium-intact (E+) and on endothelium-denuded (E-) thoracic aorta rings

Flavone	E_{max} (E+)	EC_{50} (M)	E_{max} (E-)	EC_{50} (M)
1	90.5 ± 4.0*	$7.1X10^{-6}$	15.5 ± 4.6	ND
2	87.3 ± 5.4*	$4.5X10^{-6}$	81.7 ± 4.0*	$6.6X10^{-6}$
3	90.9 ± 6.5*	$4.2X10^{-6}$	28.4 ± 2.8*	ND
Quercetin	80.5 ± 4.0*	$9.3X10^{-6}$	74.2± 5.6*	$7.8X10^{-5}$
Vehicle				
DMSO	7.7 ± 2.9	ND	8.4 ± 5.1	ND

*ND, not determined. Results are presented as mean ± S.E.M., n=6, *p<0.05 compared with DMSO (0,5%)*

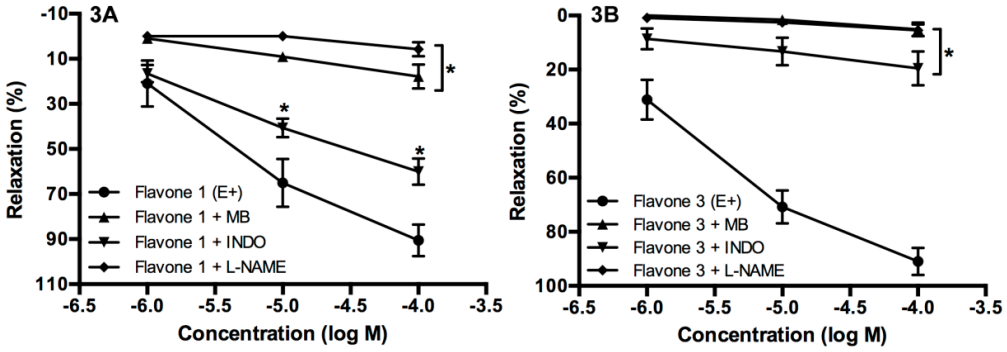

Fig. 3. Concentration–response curves flavones 1 (A) and 3 (B) on rat aortic preparations precontracted with phenylephrine in absence or presence of *L*-NAME, methylene blue (MB) or indomethacin (INDO)

*Results are presented as mean±S.E.M., n=6, *p<0.05 compared with Flavone (E+)*

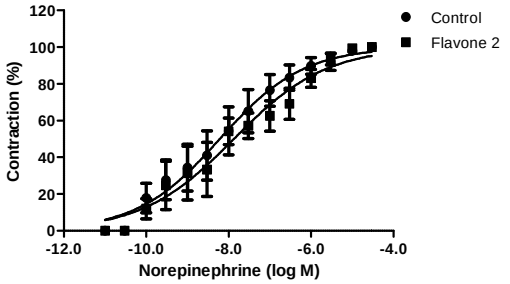

Fig. 4. Effect of flavone 2 (10^{-4} M) on the contraction induced by norepinephrine (10^{-11}-10^{-5} M) on aortic rings

Results are presented as mean±S.E.M., n=6

3.3 Determination of Radical Scavenging Activity in Aortic Rings

Aortic rings incubated with high dose of lucigenin (250 µM) impaired the relaxation to acetylcholine in comparison to control rings (28.4±3.7 vs 85.4±5.7%) (Fig. 5). Interestingly, under the same condition, vasorelaxant response to acetylcholine was preserved in the presence of flavones **1**, **2** and **3** (80.5±5.6, 81.4±6.9% and 65.7±7.7%). This scavenger activity was similar to quercetin (71.6±7.9%), a compound used as positive control. DMSO did not modify the effect of lucigenin (29.1±2.4 vs 28.4±3.7%).

3.4 Effect of Flavones on Superoxide Anion Production

The basal production of superoxide anion in aortic rings incubated with lucigenin was significantly higher than in absence of lucigenin (399.3±24.5 vs 110.6±16.4 RLU/min/mg) (Fig. 6). Luminescence signal observed in lucigenin control rings was decreased when tissues were incubated with flavones **1**, **2** and **3** (233.3±35.5, 205.6±26.1 and 259.7±28.7 RLU/min/mg) or quercetin (278.6±23.3 RLU/min/mg).

It is well known that increments in reactive oxygen species impair endothelial-dependent vasorelaxation, and it is enticing to consider that the beneficial cardiovascular effects of flavonoids may be related to their antioxidant ability [26]. In the present study, the possibility that the relaxant effect of flavones **1**, **2** and **3** resulted from prevention of nitric oxide neutralization through superoxide anion was investigated. Other authors have previously reported that high concentrations of lucigenin increased the production of reactive oxygen species in aortic rings, mainly superoxide anion, and under this experimental condition, endothelial-dependent vasorelaxation was reduced [16]. In fact, the current study shows that the incubation of aortic rings with lucigenin reduced maximal relaxation to acetylcholine. Under this condition, all of the three flavones significantly increased acetylcholine response, denoting improvements in endothelial function related to radical-scavenging properties. Antioxidant action was corroborated by a chemiluminiscence assay, where flavones demonstrated a pronounced antioxidant effect, which was even greater than quercetin with compound **2**.

The antioxidant activity of flavonoids depends upon the arrangement of functional groups around the nuclear structure. The C-ring hydroxyl groups arrangement is the most significant determinant of scavenging of reactive oxygen species (ROS) [27,28] and reactive nitrogen species (RNS) [29]. Compounds **1** and **3** retain hydroxyl groups at the 3' and 4' positions, respectively, which could explain their effect in the production of superoxide anion in aortic rings. It is known that B-ring substitution model affect little to the antioxidant activity, although a hydroxyl group at C-5 could contribute to the antioxidant effect which would explain the stronger peroxynitrite scavenging ability exhibits by genestein [30]. This fact could explain why our

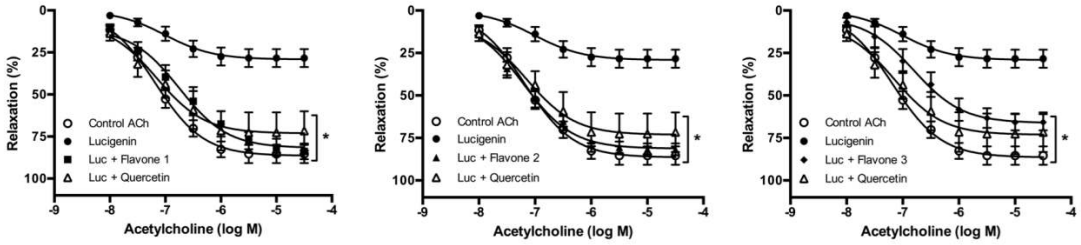

Fig. 5. Concentration–response curves of acetylcholine in aorta rings control, incubated whit lucigenin or lucigenin plus flavones 1, 2, 3 and quercetin

*Values are means±S.E.M. of n=8. *p<0.05 compared with Lucigenin group*

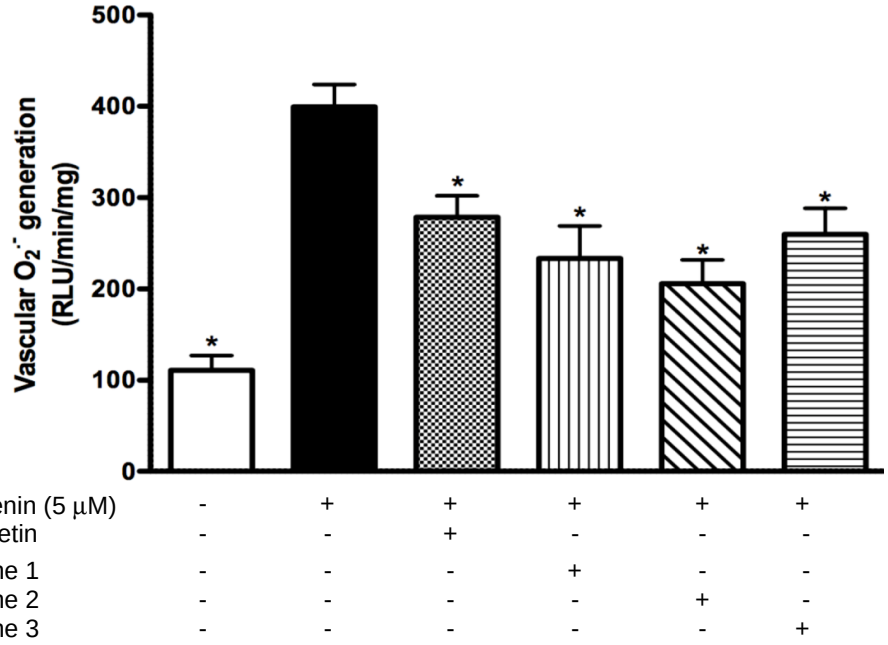

Fig. 6. Effect of quercetin and flavones 1, 2, 3 (10^{-4} M) on the vascular superoxide anion ($^{\bullet}O_2^-$) production in aorta rings

*Results are presented as mean±S.E.M., n=6, *p<0.05 compared with Lucigenin group*

compounds (**1**, **2** and **3**), with hydroxyl group in C-5, have antioxidant effect.

The presence of multiple methoxy groups in ring B also reverse the positive effect of a C-ring catechol. However, some authors have described that *O*-methylation enhances antioxidant activity in some microsomal systems through cooperative actions [31]. Our results are consistent with these findings, because the polymethoxylated compounds **1** and **2** possess a great scavenger ability using aortic rings. Their lipophilicity may contribute to total antioxidant activity as has been described [32]. Based on our results and bibliographic review data [33], it can be conclude that *O*-methylation can generate changes in the profile of antioxidant activity, which can depends on the method employed and radical evaluated.

In summary, a large number of evidence described that imbalance between the production of reactive oxygen species and the antioxidant defence mechanisms leading to oxidative stress could contribute to the etiology of atherosclerosis and hypertension [34]. However, many authors argue that the beneficial effects of flavonoids as cardioprotective agents are chiefly ascribed to their vasorelaxant and antioxidant properties [17,35]. Therefore, the development of flavones with the ability to decrease the concentration of reactive oxygen species can be of great value for the treatment of these cardiovascular diseases. Additionally, flavones assayed in our study improved endothelial function in lucigenin models, acting as scavengers for free radicals, as well as reported with other flavonoids, such as baicalein and catechin [36]. Endothelium-dependent effect might result from thus superoxide scavenging properties of 5,3'-dihydroxy-6,7,4'-trimetoxyflavone **(1)** and 5,4'-dihydroxy-7-metoxyflavone **(3)**, preventing the superoxide-induced nitric oxide degradation and thus prolonging its half-life. These actions have been described as synergic cardiovascular effects, because in vascular smooth muscle cells, nitric oxide can be transformed into peroxynitrite by combination with superoxide anion. Several authors suggest that increased concentration of peroxynitrite could decrease nitric oxide activity. In addition, other reactive oxygen species as superoxide anion generation can induce uncoupled endothelial nitric oxide synthase.

4. CONCLUSION

The present study suggests that flavones isolated from *Lourteigia stoechadifolia* and *Ageratina stevioides* could preserve the vascular

function through both vasorelaxant effect and scavenging free radicals. These results contribute to knowledge of the existing relationship between the structure and the vascular activity of flavones, and corroborate the cardiovascular effects of this type of compounds. These findings open the possibility that flavones may be selected as leader compounds for the development of therapeutic agents to be used to prevent and treat cardiovascular disease.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical committee of University of Panama approved the guidelines of the experimental procedure using the animals (VIP 01-07-00-07-2008-04). All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Duarte J, Pérez-Palencia R, Vargas F, Ocete MA, Pérez-Vizcaino F, Zarzuelo A, et al. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. Br J Pharmacol. 2001;133(1):117-24.
2. Ajay M, Gilani AU, Mustafa MR. Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. Life Sci. 2003;74(5):603-12.
3. Macedo CL, Vasconcelos LH, de Correia AC, Martins IR, de Lira DP, de OSBV, et al. Mechanisms underlying vasorelaxation induced in rat aorta by galetin 3,6-dimethyl ether, a flavonoid from *Piptadenia stipulacea* (Benth.) Ducke. Molecules. 2014;19(12):19678-95.
4. Cicala C, Morello S, Iorio C, Capasso R, Borrelli F, Mascolo N. Vascular effects of caffeic acid phenethyl ester (CAPE) on isolated rat thoracic aorta. Life Sci. 2003;73(1):73-80.
5. Orallo F, Camina M, Álvarez E, Basaran H, Lugnier C. Implication of cyclic nucleotide phosphodiesterase inhibition in the vasorelaxant activity of the citrus-fruits flavonoid (+/-)-naringenin. Planta Med. 2005;71(2):99-107.
6. Pietta PG. Flavonoids as antioxidants. J Nat Prod. 2000;63(7):1035-42.
7. Qin CX, Chen X, Hughes RA, Williams SJ, Woodman OL. Understanding the cardioprotective effects of flavonols: discovery of relaxant flavonols without antioxidant activity. J Med Chem. 2008;51(6):1874-84.
8. Lee JH, Kim GH. Evaluation of antioxidant and inhibitory activities for different subclasses flavonoids on enzymes for rheumatoid arthritis. J Food Sci. 2010;75(7):H212-7.
9. Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A, et al. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr. 2002;76(3):560-8.
10. McCullough ML, Peterson JJ, Patel R, Jacques PF, Shah R, Dwyer JT. Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. Am J Clin Nutr. 2012;95(2):454-64.
11. Huxley RR, Neil HA. The relation between dietary flavonol intake and coronary heart disease mortality: A meta-analysis of prospective cohort studies. Eur J Clin Nutr. 2003;57(8):904-8.
12. Olmedo D, Rodriguez N, Vasquez Y, Solis PN, Lopez-Perez JL, Feliciano AS, et al. A new coumarin from the fruits of *Coutarea hexandra*. Nat Prod Res. 2007;21(7):625-31.
13. San Feliciano A, Miguel del Corral JM, Lopez JL, Pascual Teresa Bd. Lignans from polar extracts of *Juniperus thurifera*. Phytochemistry. 1992;31(1):267-70.
14. López-Pérez JL, Therón R, Del Olmo E, Diaz D. NAPROC-13: A database for the dereplication of natural product mixtures in bioassay-guided protocols. Bioinformatics. 2007;23(23):3256-7.
15. Chen SJ, Wu CC, Yang SN, Lin CI, Yen MH. Abnormal activation of K(+) channels in aortic smooth muscle of rats with endotoxic shock: Electrophysiological and

functional evidence. Br J Pharmacol. 2000;131(2):213-22.
16. Kassan M, Montero MJ, Sevilla MA. *In vitro* antioxidant activity of pravastatin provides vascular protection. Eur J Pharmacol. 2010;630(1-3):107-11.
17. Zenebe W, Pechanova O, Bernatova I. Protective effects of red wine polyphenolic compounds on the cardiovascular system. Exp Clin Cardiol. 2001;6(3):153-8.
18. Woodman OL, Chan E. Vascular and anti-oxidant actions of flavonols and flavones. Clin Exp Pharmacol Physiol. 2004;31(11): 786-90.
19. Boydens C, Pauwels B, Decaluwe K, Brouckaert P, Van de Voorde J. Relaxant and antioxidant capacity of the red wine polyphenols, resveratrol and quercetin, on isolated mice corpora cavernosa. J Sex Med. 2015;12(2):303-12.
20. Woodman OL, Meeker WF, Boujaoude M. Vasorelaxant and antioxidant activity of flavonols and flavones: Structure-activity relationships. J Cardiovasc Pharmacol. 2005;46(3):302-9.
21. Xu YC, Leung SW, Yeung DK, Hu LH, Chen GH, Che CM, et al. Structure-activity relationships of flavonoids for vascular relaxation in porcine coronary artery. Phytochemistry. 2007;68(8):1179-88.
22. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol. 2012;10(1):4-18.
23. Bielli A, Scioli MG, Mazzaglia D, Doldo E, Orlandi A. Antioxidants and vascular health. Life Sci. 2015;143:209-16.
24. Spieker LE, Flammer AJ, Luscher TF. The vascular endothelium in hypertension. Handb Exp Pharmacol. 2006;176(Pt 2): 249-83.
25. Chan EC, Pannangpetch P, Woodman OL. Relaxation to flavones and flavonols in rat isolated thoracic aorta: Mechanism of action and structure-activity relationships. J Cardiovasc Pharmacol. 2000;35(2):326-33.
26. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997;2(4): 152-9.
27. Sekher Pannala A, Chan TS, O'Brien PJ, Rice-Evans CA. Flavonoid B-ring chemistry and antioxidant activity: Fast reaction kinetics. Biochem Biophys Res Commun. 2001;282(5):1161-8.
28. Burda S, Oleszek W. Antioxidant and antiradical activities of flavonoids. J Agric Food Chem. 2001;49(6):2774-9.
29. Haenen GR, Paquay JB, Korthouwer RE, Bast A. Peroxynitrite scavenging by flavonoids. Biochem Biophys Res Commun. 1997;236(3):591-3.
30. Lai HH, Yen GC. Inhibitory effect of isoflavones on peroxynitrite-mediated low-density lipoprotein oxidation. Biosci Biotechnol Biochem. 2002;66(1):22-8.
31. Cholbi MR, Paya M, Alcaraz MJ. Inhibitory effects of phenolic compounds on CCl4-induced microsomal lipid peroxidation. Experientia. 1991;47(2):195-9.
32. Arora A, Nair MG, Strasburg GM. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radic Biol Med. 1998;24(9):1355-63.
33. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relation-ships. J Nutr Biochem. 2002;13(10):572-84.
34. Heistad DD, Wakisaka Y, Miller J, Chu Y, Pena-Silva R. Novel aspects of oxidative stress in cardiovascular diseases. Circ J. 2009;73(2):201-7.
35. Lemos VS, Freitas MR, Muller B, Lino YD, Queiroga CE, Cortes SF. Dioclein, a new nitric oxide- and endothelium-dependent vasodilator flavonoid. Eur J Pharmacol. 1999;386(1):41-6.
36. Chang WT, Shao ZH, Yin JJ, Mehendale S, Wang CZ, Qin Y, et al. Comparative effects of flavonoids on oxidant scavenging and ischemia-reperfusion injury in cardiomyocytes. Eur J Pharmacol. 2007;566(1-3):58-66.

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