The Vasodilating Effect and Angiotensin Converting Enzyme Inhibition Activity of Three Dietary Flavonols: Comparison between Myricetin, Quercetin and Morin, in vitro

Shih-Ying CHEN1,#, Chin-Chen CHU2,#, Chung-Lin JIANG1, Pin-Der DUH3,*

1Department of Health and Nutrition, Chia Nan University of Pharmacy and Science, Tainan, Taiwan
2Department of Anesthesiology, Chi-Mei Medical Center, Tainan, Taiwan
3Department of Food Science and Technology, Chia Nan University of Pharmacy and Science, Tainan, Taiwan

#These authors contributed equally to this work.
*Corresponding author: ipdduh@mail.cnu.edu.tw

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Abstract The aim of this study is to compare the vasodilating effects and angiotensin converting enzyme inhibition activity of three dietary flavonols, myricetin, quercetin and morin, in vitro. The three flavonols ranging from 0 to 200 μM show no cytotoxicity to small vessel endothelial cells (SVEC). For vasodilative assay, of the three flavonols, myricetin showed the greatest induction in the levels of nitric oxide (NO) and prostacyclin (PGI2), and the expression of cyclooxygenase (COX-2). Myricetin, quercetin and morin enhanced the expression of eNOS, but no significant differences (p > 0.05) were found between three flavonols. Myricetin, quercetin and morin showed marked inhibitory effect on reactive oxygen species (ROS) production in H2O2-induced SVEC cells. In addition, myricetin and quercetin showed relatively higher inhibitory activity on ACE activity than morin. Taken together, of the three flavonols, myricetin with the substitution by more hydroxyl groups in the flavonol structure enhanced vasodilating activity. Enzyme kinetic analysis showed myricetin is a non-competitive inhibitor of ACE. These structure-function relationships facilitate the design of new antihypertensive drugs based on flavonols.

Keywords: myricetin, quercetin, morin, angiotensin converting enzyme, vasodilative activity

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1. Introduction

Hypertension is a risk factor that can increase the chance of developing heart disease, a stroke, and related complications. In general, the higher the blood pressure, the greater the healthy risk. It is estimated about one-quarter of the adult population suffers from hypertension [1]. Thus, the development of novel agents and strategies for lowering high blood pressure is an important global medical issue. In addition, medication can effectively lower blood pressure, however, synthetic antihypertensive drugs sometimes bring serious adverse side effects [2]. Therefore, the search for natural antihypertensive drugs as alternative to synthetic ones is of great interest for researchers.

Vasorelaxation is a major mechanism of ameliorating hypertension. The mechanisms of vasorelaxation are characterized as endothelium-dependent or endothelium-independent [3]. The vasoactive substances, including nitric oxide (NO), prostacyclin (PGI2) and endothelium-derived hyperpolarizing factor derived from endothelium, play an important role in ameliorating hypertension [4]. Apart from these, angiotensin I-converting enzyme cleaves angiotensin I to angiotensin II, a powerful vasoconstrictor. Moreover, ACE inactivates bradykinin, a vasodilatory peptide. Hence, ACE has been measured as a major factor in hypertension. Consequently, ACE inhibitors have been widely developed to prevent angiotensin II production and are considered a useful therapeutic approach in clinical application [5]. Flavonoids are found in over 6000 plants, where they are involved in many biological activities [6]. Flavonols are a class of flavonoid compounds found in many plants. Many studies showed, in addition to their antioxidant activity, phenolic compounds enhance the production of vasodilating factors such as NO and are found to be effective ACE inhibitors both in vitro and in vivo [7,8]. Moreover, epidemiological evidence suggests flavonol-rich foods may protect against coronary disease, stroke, lung cancer, and stomach cancer [9]. For example,
myricetin is more powerful antioxidants than traditional vitamins and has antihypertensive action in the fructose model. [10,11] Quercetin, found in apples, berries, and onions, reduces blood pressure in hypertensive rodents [12]. Morin, originally isolated from members of the Moraceae family that can be extracted from leaves, fruits, stems and branches of numerous plants, exerts antihypertensive and other biological effects, by modulating the activity of many enzymes [13] Although myricetin, quercetin and morin have been confirmed to have a blood-pressure-lowering effect in vivo [1], not much is known about the comparison between these three dietary flavonols in vasodilating effect and inhibition of ACE activity. The objective of this study was, therefore, to determine the effect of myricetin, quercetin and morin, on vasodilating effects and inhibition of ACE activity. The enzyme kinetic parameters were also determined.

2. Materials and Methods

2.1. Chemicals and Sample Preparation

Flavonol compounds, purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA), were dissolved in 0.1% dimethylsulphoxide (DMSO); 2 mM of phenolic compounds were serially diluted with sterile phosphate dimethylsulphoxide (DMSO); 2 mM of phenolic compounds were added at final concentrations ranging from 0.5 to 200 μM. After 24 h incubation, the medium was collected from each well and cleared by centrifugation (3,000 ×g; 10 min; 4°C). The levels of 6-keto-PGF1α were determined using specific ELISA kits according to the manufacturer’s instructions (Cayman chemical No. 515211) [16].

2.2. Cell Culture and Cell Viability

SVECs endothelial cells obtained from Taiwan Bioresource Collection and Research Center (Hsinchu, Taiwan) were grown in DMEM containing 10% FBS, 1% antibiotic-antimycotic (100X) and fetal bovine serum (FBS) were both purchased from Gibco Company (NY, USA).

2.3. Determination of NO Production

Cells were plated in their growth medium at a density of 1 × 10^5 cells/well in 96 flat bottomed well plates. After 24 h plating, phenolic compounds were added at final concentrations ranging from 0.5 to 200 μM. After 24 h incubation, the medium was replaced with fresh medium. After preincubation for 15 min with various test compounds of 0.5 to 200 μM and then treated with H2O2 of 1 mM for 15 min, and 50 μM 2’, 7’-dichlorodihydrofluorescein diacetate (DCHF-DA) in a light-protected humidified chamber 37°C for 1.5 h, total of incubation for 2 h. Then washed, and overlaid with sterile PBS with strictly dark conditions to avoid any nonspecific artifacts. The fluorescence intensity was measured at 480 nm excitation and 530 nm in a FLX800 Fluorescence Microplate Reader (BioTeK, Winooski, VT, USA) [17].

2.4. Determination of PGI2 Formation

Culture media were saved for the determination of 6-keto-PGF1α, the stable metabolite of PGI2. Cells were plated in their growth medium at a density of 2.5 × 10^5 cells/well in 24 flat bottomed well plates. After 24 h plating, phenolic compounds were added at final concentrations ranging from 0.5 to 200 μM. After 24 h incubation, the medium was collected from each well and measured at 480 nm excitat ion and 530 nm in a FLx800 Fluorescence Microplate Reader (BioTeK, Winooski, VT, USA) [15].

2.5. Intracellular ROS Measurement

Cells were plated in the growth medium at a density of 3.5 × 10^5 cells/well in 6 flat bottomed well plates incubation for 24 h. Then washed, replaced with fresh medium. After preincubation for 15 min with various test compounds of 0.5 to 200 μM and then treated with H2O2 of 1 mM for 15 min, and 50 μM 2’, 7’-dichlorodihydrofluorescein diacetate (DCHF-DA) in a light-protected humidified chamber 37°C for 1.5 h, total of incubation for 2 h. Then washed, and overlaid with sterile PBS with strictly dark conditions to avoid any nonspecific artifacts. The fluorescence intensity was measured at 480 nm excitation and 530 nm in a FLX800 Fluorescence Microplate Reader (BioTeK, Winooski, VT, USA) [17].

2.6. Western Blot

Cells were collected by scraping with ice-cold PBS containing PMSF, and were lysed in the ice-cold lysis buffer. The protein concentration of the cytoplasmic lysates was determined using Bradford method. Cell lysates from each sample (20 μg) was separated by 10% sodium dodecyl sulfatepolyacrylamide gel (SDS-PAGE) and transferred onto a cellulose nitrate strips (NC) membrane (Sartorius Stedim). To block non-specific protein binding, NC membranes were incubated in 5% BSA overnight at 4°C. Subsequently, the membranes were washed three times with Tris-buffered saline containing 0.1% Tween 20 (TBST) for 10 min. Membranes were incubated with anti-endothelial nitric oxide oxide synthase (anti-eNOS) (1:2000; Cell Signaling Technology Inc.), anti-p-eNOS (1:1000; Cell Signaling Technology Inc.), anti-cyclooxygenase-2 (anti-COX-2) (1:2500; Cell Signaling Technology Inc.) and anti-β-actin (1:10000; Cell Signaling Technology Inc) primary antibodies with overnight at 4°C. After incubation with the corresponding secondary antibodies, the membranes were analyzed with a chemiluminescence reaction kit (Thermos, Rockford, IL, USA). The relative intensities of the bands were visualized and analyzed with S/N 012407-002 Image J Software (UVP, upland, CA, USA).

2.7. Determination of Kinetic Parameters of ACE Inhibition

The enzyme reaction carried out with the presence of 50 mU/mL ACE, 1-5 mM HHL, phosphate saline buffer
(pH 8.3) and different concentrations of test compounds. Reaction mixtures were incubated at 37°C using a shaker oven for 1 h. NaOH (0.5 M) was added to stop the enzyme activity. The formation of hippuric acid by the cleavage of HHL at the presence of ACE was determined using a fluorescence method by pyridine with benzenesulfonyl chloride (Py-BSC). The reaction solution in the optical density was measured at a wavelength of 405 nm [18]. Kinetic parameters were determined according to Michaelis Menten kinetic model. \[ V = \frac{V_{\text{max}} \times [S]}{K_m + [S]} \] where \( V \): Reaction rate, \( V_{\text{max}} \): Maximum reaction rate, \( K_m \): Michaelis constant, \( S \): Substrate concentration. The reaction rate (formation of hippuric acid) was plotted against the different substrate concentrations (1 - 5 mM HHL) and 50, 250, 500 μM phenolic compounds to obtain the saturation curves. Lineweaver-Burk plots were derived using the saturation curves to determine the type of inhibition. \[ \frac{1}{V} = \frac{K_m}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}} \] Kinetic parameters \([K_m, V_{\text{max}}, K_i](\text{dissociating constant})\) were calculated using Sigma Plot 12 (Systat software, Inc., Chicago, IL, USA) software. Dissociating constant was determined using the following equation. \[ m_i = m(1+[I])/K_i \] where \( m_i \): Slope from linear plot from inhibited reaction, \( m \): Slope from linear plot from non-inhibited reaction, \([I]\): Concentration of inhibitor, \( K_i \): Dissociating constant of the inhibitor.

2.8. Statistical Analysis

All statistical calculations were done with the statistical software program SPSS (ver. 12.0) (SPSS Inc., Chicago, IL, USA). The results were shown as mean±SD. Statistical analysis was done using one-way analysis of variance. A \( p < 0.05 \) was considered as statistically significant, and Duncan's test was used as a post-hoc test.

3. Results and Discussion

To investigate the effects of different dietary flavonols on induction of NO and PGI\(_2\), flavonol compounds including morin, myricetin and quercetin were selected by structure as tested compounds in this study (Figure 1) [19]. The effects of the three flavonols on induction of NO and PGI\(_2\) \textit{in vitro}, were investigated using an endothelial cell line in SVEC cells. If the cytotoxic effect occurred in a test treated with samples, the results of the biological effect of the samples would be affected and interfered. Therefore, the influence of the selected flavonol compounds on cell viability of SVEC cells was determined using the MTT assay after 24-h incubation. According to the results, the SVEC cell survival in the presence of the selected flavonols ranging from 0 to 200 μM was >85%, suggested that different flavonol compounds with the tested concentrations showed no cytotoxicity to SVEC cells.

The ability of three selected dietary flavonols to induce NO production in SVEC cells was measured. The nitrite levels in the culture medium, as an index of NO production, were determined. The inducing effect of myricetin, quercetin and morin at 200 μM on NO production is shown in Figure 2 A. Clearly, among the three flavonols, myricetin exhibited a remarkable activity on inducing NO production in SVEC cells, followed by morin and quercetin. NO, a freely diffusible gas, exerts many physiological functions by acting as an intracellular and intercellular messenger and is a key component in endothelium-dependent relaxation [20]. In other words, NO deficiency can cause hypertension. According to the data shown in Figure 2 A, phenolic compounds supplementation, especially myricetin, morin and quercetin can boost the production of NO, improve the function of blood vessels and lower blood pressure [20].

To further evaluate the mechanism of the generation of NO induced by the three flavonols. The effects of the selected three flavonol compounds on eNOS activity were determined. Figure 2 B shows myricetin, morin and quercetin at 200 μM showed a significantly increased \( p \)-eNOS/eNOS ratio compared to the control, suggested that the three flavonols can induce eNOS activity. NO is formed in the endothelium by the activation of eNOS using L-arginine as a substrate. Then, NO diffused out of the endothelium; some enters the underlying vascular smooth muscle cells where it activates soluble guanylyl cyclase (sGC). Next, sGC catalyzes the conversion of guanosine triphosphate (GTP) to guanosin 3’, 5’-cyclic monophosphate (cGMP). cGMP-activated cGMP-dependent protein kinase (PKG) induces vascular relaxation with variable signaling pathways [21]. In this study, we speculate these flavonols could cause activation of eNOS and therefore lead to increased release of NO.

Figure 1. Structure of dietary three flavonol compounds, myricetin, quercetin and morin (Pubchem, 2017)
Figure 2. Effect of dietary three flavonol compounds, myricetin, quercetin and morin (200 μM) on (A) NO production and (B) eNOS protein expression in SVEC cells for 24 hrs. Experiments were done in triplicates. Values are expressed as the means ± SD. Mean values with different subscripts were significantly different (p < 0.05) using Duncan's test.

Figure 3. Effect of dietary three flavonol compounds, myricetin, quercetin and morin (200 μM) on (A) PGI₂ production and (B) COX-2 protein expression in SVEC cells for 24 hrs. Experiments were done in triplicates. Values are expressed as the means ± SD. Mean values with different subscripts were significantly different (p < 0.05) using Duncan's test.

Figure 3A shows the effect of the three flavonol compounds on PGI₂ generation in SVEC cells. The addition of myricetin, quercetin and morin at 200 μM to medium prior to incubation of culture medium resulted in increased PGI₂ concentration. Of the three compounds, myricetin showed the highest induction of PGI₂ production, where the PGI₂ concentration was increased to 590% of control. In addition, quercetin showed significant induction of PGI₂ production compared to the control. However, morin enhanced PGI₂ production, there are no significant differences between morin and the control. In an attempt to understand the mechanism underlying inducing effects of the three flavonol compounds on flavonol-induced PGI₂ concentration, we examined the effect of three flavonol compounds on the activation of COX-2 (Figure 3B). Myricetin, and quercetin at 200 μM produced a significant increase in COX-2 activity in SVEC cells compared to the control. There are no significant differences between morin and control in increase in COX-2 activity (p > 0.05). This observation suggested that the myricetin and quercetin positively up-regulate COX-2 activity in SVEC cells. COX-2 is the product of an immediate early gene and is rapidly expressed with exposure of cells to mitogenic stimuli, hormones, and inflammatory mediators [22]. As a rule, COX-2 converts arachidonic acid (AA) to prostaglandin H₂ (PGH₂), which acts upon by discrete PG synthases to give rise to different PGs species. Consequently, the greater the COX-2 induction, the higher the number of PG species. PGI₂ is a member of PGs, which are a family of intercellular and intracellular messengers derived from AA. With regard to the PGI₂ property, PGI₂ evokes the following cellular responses, including smooth muscle relaxation, decreased fibroblast and smooth muscle proliferation, diminished collagen deposition, and decreased leukocyte function [22]. A growing large body of studies suggests that overexpression of COX-2 is strongly related to pathophysiological diseases [23]. Some studies showed phenolic compounds exhibits an inhibitory effect on LPS-induced COX-2 in RAW 264.7 cells [23]. Hence, the inhibitors of COX-2 are widely recognized as offering the promise of treatment of inflammatory conditions [24]. However, up-regulation of COX-2 in the endothelium will result in a drop in blood pressure.
addition, some vasodilators can activate COX, which is responsible for the biosynthesis of PGI₂ from AA [20]. After PGI₂ is liberated from endothelial cells, it binds to the prostacyclin receptor, a G-protein-coupled receptor in the vascular smooth muscle cell (VSMC), and then induces vasodilation using stimulating adenylate cyclase (AC), increasing the activity of cAMP-dependent protein kinase (PKA), and the subsequent phosphorylation of the K⁺ channel by PKA indirectly leads to a reduced [Ca²⁺]; in VSMC and thus reducing vascular tone [20]. In other words, PGI₂ is a strong hypotensive agent and believed to be responsible for vasodilatation activity [25]. The data in Figure 3B show myricetin and quercetin positively up-regulated COX-2 activity, suggesting myricetin and quercetin are COX-2 activators in SVEC cells. Interestingly, the PGI₂ generation increased in SVEC cells after incubating with myricetin and quercetin (Figure 2A). Hence, the induction of PGI₂ generation and COX-2 activity by the flavonol compounds facilitates the reduction of blood pressure.

With the physiological metabolism, an excessive level of ROS may decrease the intracellular antioxidant capacity and then produce oxidative stress. However, an increase in ROS generation in the human body can be suppressed by phytochemicals and antioxidants [26]. Therefore, to further understand whether these flavonol compounds scavenging intracellular ROS generation in SVEC cells, the DCFH-DA model was used. As seen in Figure 4, the DCF fluorescence intensity increased remarkably when the cells were treated with 1 mM H₂O₂, suggested that H₂O₂ has a strong inducing effect on ROS generation. However, the three flavonol compounds at a concentration of 200 μM significantly inhibited ROS generation, and there was no significant difference between three flavonol compounds (p > 0.05). This observation showed the three phenolic compounds act as a scavenger of ROS that is caused by H₂O₂ in the SVEC cells. The role of ROS in the pathogenesis of many human diseases is becoming increasingly recognized. Under pathological conditions, increased ROS bioactivity leads to endothelial dysfunction, increased contractively, VSMC growth, monocyte invasion, lipid peroxidation, inflammation, and increased deposition of extracellular matrix proteins. These factors contribute to hypertensive vascular damage [27]. In addition, increased ROS is associated with decreased NO synthesis or increased NO degradation because of its interaction with O₂⁻ to form ONOO⁻ [28]. Hence, ROS plays a pathophysiological role in the development of hypertension [29]. ROS is not implicated in the early stages of human hypertension, but could be more important in severe hypertension [27,30]. Therefore, increased antioxidant activity and levels of ROS scavengers may contribute to reducing oxidative stress thereby improving endothelial function. In this study, myricetin, quercetin and morin showed a marked scavenging effect on ROS generation in H₂O₂-induced SVEC cells. Clearly, the three flavonol compounds show significant antioxidant activity and remarkable ROS scavenging ability.

Figure 4. Effect of dietary three flavonol compounds, myricetin, quercetin and morin (200 μM) on intracellular ROS inhibition in SVEC cells for 2 hrs. Experiments were done in triplicates. Values are expressed as the means ± SD.

Figure 5. The inhibitory kinetics of myricetin on ACE activity was analyzed using a Lineweaver-Burk plot. (A) Purified lung ACE was incubated at 37°C for 30 to 40 min in the myricetin with different concentrations of HHL. (B) Secondary plots of Kₘ/V (slopes) versus myricetin concentrations to estimate Kᵢ of myricetin non-competitive inhibition. Experiments were done in triplicates. Values are expressed as the means ± SD.
To further understand the additional mechanisms of antihypertensive activity, in addition to the vasodilating factors, such as induction of NO and PGI2, and antioxidant activity, the effects of the three flavonol compounds on in vitro ACE inhibitory activity were evaluated. Myricetin, quercetin and morin inhibited ACE activity in a dose-dependent manner (Table 1). The 71.1%, 71.9% and 25.2% inhibition rates for myricetin, quercetin and morin were obtained at a concentration of 250 μM, respectively. These results suggested that the selected flavonol compounds are likely to affect blood pressure with inhibition of ACE activity. ACE, a zinc metalloprotease, is an important enzyme which is widely distributed throughout the body. The mechanisms of elevating blood pressure by ACE involve two pathways: ACE converts angiotensin I to angiotensin II, which induces the release of aldosterone that cause the retention of sodium ions by the kidney and elevates blood volume, thus increasing blood pressure, and the inactivation of the vasodilator bradykinin, which is conductive to lowering blood pressure [31]. In other words, ACE activity leads to an increase in blood pressure by producing the vasoconstrictor peptide bradykinin [32]. Therefore, the inhibition of ACE contributes to reducing both the formation of angiotensin II and the destruction of bradykinin. According to the Table 1, the selected flavonol compounds inhibited ACE activity, in vitro, in a dose-dependent manner. The characteristics of selected flavonol compounds proved in this study may be potential for candidates as functional and/or pharmaceutical compounds that can be used against hypertension. However, this suggestion requires further study in vivo. According to the data obtained, of the three flavonols, myricetin tended to show significant inhibitory action on ACE activity, in addition to induction of NO and PGI2. Therefore, the inhibitory kinetics of myricetin on ACE activity was analyzed by a Lineweaver-Burk plot, as shown in Figure 5. The Lineweaver-Burk plot, with an increasing concentration of myricetin, showed a noncompetitive type of inhibition with a Kj value of 232.8 μM, respectively. This observation suggested that myricetin did not compete with the substrate for binding the active site of ACE, rather myricetin bind to a site other than the active site on the ACE, thereby retarding the conversion of substrate to product [33].

Table 1. Dietary three flavonol compounds, myricetin, quercetin and morin on inhibition rate of ACE in vitro

| Concentration (μM) | Morin         | Quercetin    | Myricetin    |
|-------------------|---------------|--------------|--------------|
| 25                | 11.9 ± 2.7   | 18.9 ± 4.1   | 23.6 ± 0.1  |
| 50                | 18.6 ± 2.0   | 34.5 ± 3.5   | 43.3 ± 5.4  |
| 100               | 22.8 ± 5.0   | 62.8 ± 3.4   | 61.3 ± 4.8  |
| 250               | 24.6 ± 0.6   | 71.9 ± 0.2   | 71.1 ± 7.6  |
| 500               | 35.5 ± 6.3   | 78.2 ± 3.1   | 77.4 ± 9.6  |

Experiments were done in triplicates. Values are expressed as the means ± SD. Mean with different lower case subscripts in each column significantly different (p < 0.05) using Duncan’s test.

Phenolic compounds were found to have special biological activities and therefore have gained much attention. Many studies reported the biological activity of flavonols derived from its structure [34]. In regard to the bioactivity of flavonoids, the flavonoids have a wide range of structures differing in substitution patterns to the A-, B-, and C-ring which might affect their bioactivity [1]. On the basis of the polyhydroxy configuration on the benzene rings of the flavonol compound, the B ring is considered to be more redox-reactive than A ring [35]. In addition, structural changes in the B ring of flavonoids are considered to impact the antioxidant activity [36]. In addition, the number and positions of hydroxyl groups or other hydrogen-donating groups in the phenolic molecular structures and the glycosylation of the phenolic aglycones influenced their antioxidant activity. Flavonols possessing multiple antioxidant activities for scavenging reactive oxygen and nitrogen may be a result of the presence of the number of free hydroxyl groups in the B-ring of the flavonol molecule, a C2-C3 double bond in C-ring, or the presence of a 3-hydroxyl group [37]. In other words, flavonol with bioactivity depend on the flavonol molecular structure and particularly hydroxyl group substitution pattern [38]. The number of hydroxyl groups for myricetin, quercetin and morin are 6, 5 and 5, respectively. The results obtained showed myricetin showed higher inducing effects than quercetin and morin on NO production and PGI2 levels, suggesting the greater the number of hydroxyl groups, the more inducing effects there are in NO production and PGI2 levels.

With regard to ACE inhibition of flavonol compounds, the data obtained from Table 1, myricetin and quercetin at 250 μM showed > 71% inhibition of ACE activity, and there are no significant difference between myricetin and quercetin. In addition, quercetin showed higher inhibition of ACE activity than morin, suggesting that quercetin with hydroxyl group in 3’- and 4’-position is superior to morin with hydroxyl group in 1’- and 3’-position. That is to say, the ortho-dihydroxy structure in the B ring of the flavonol molecules led to potent ACE activity inhibition of flavonol. This suggests the number and position of the hydroxyl groups may increase or decrease the inhibitory effect of ACE activity. These observations agree with the reports of Al Shukor et al. [1], who noted the presence of hydroxyl groups, which can act as hydrogen-bond acceptors or donors, seems to increase the potency to inhibit ACE activity. In addition, the flavonols included in this study may have different molecular interactions with the amino acids at the active site of ACE, giving rise to a stable complex between the flavonol compound molecule and ACE [1].

4. Conclusion

In summary, this study provides a comparative analysis of vasodilative effect and inhibition of ACE activity of the three flavonol compounds. Myricetin, quercetin and morin positively induced NO and PGI2 production, by inhibition of ACE activity and removing ROS generation to make them available for modulating vasodilation in SVEC cells. Of the three flavonol compounds, myricetin exhibited the greatest vasodilating effects in terms of inducing NO production and PGI2 levels. The structure-activity relationship showed the number and position of hydroxyl groups of flavonol compounds were found to play an important role in the observed vasodilating effect and
ACE activity inhibition of the flavonol compounds. However, further animal and clinical studies are needed to confirm the results.

Conflict of Interest

The authors declare no conflict of interest.

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