

Chapter 12

Polyphenols and Their Components in Experimental Allergic Asthma

M. Joskova, V. Sadlonova, G. Nosalova, E. Novakova, and S. Franova

Abstract The aim of the study was to investigate the potential anti-inflammatory effects in experimental allergic asthma of natural polyphenolic compounds or their single major components. The experiment was performed after 21-days sensitization of guinea pigs with ovalbumin suspension. Changes in airway reactivity after the long-term treatment with the polyphenolic compounds Provinol and Flavin-7 and their single major components quercetin and resveratrol during were assessed using a whole body plethysmography. Reactivity of tracheal smooth muscle was studied *in vitro* in response to cumulative doses of the bronchoconstrictive mediators histamine and acetylcholine. Furthermore, concentrations of the inflammatory cytokines IL-4 and IL-5 were measured in bronchoalveolar lavage fluid. The results demonstrate significant anti-inflammatory effects of Provinol and Flavin-7 exerted in the airways. In contrast, chronic treatment with quercetin and resveratrol, single components of the two polyphenols, did not show such activity. We conclude that polyphenolic compounds are more effective in the anti-inflammatory effects in the airways than their separate components.

Keywords Allergic inflammation • Airway hyperreactivity • Asthma • Polyphenols • Quercetin • Resveratrol • Inflammatory cytokines

12.1 Introduction

Allergic asthma is a chronic inflammatory disorder of airways in which many inflammatory cells, airways structural cellular elements, and inflammatory mediators play a role in the induction of airways hyperreactivity and bronchoconstriction. The early allergic response occurring within seconds to minutes of allergen exposure is manifested with bronchoconstriction, edema in the airways and mucin hypersecretion induced by inflammatory mediators of mast cells (histamine, prostaglandin D₂,

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and leukotriene C4). The late-phase allergic reaction related to activation of inflammatory cells (mast cells, T cells, eosinophils, and neutrophils), inflammatory cell recruitment in the lungs, airway hyper-reactivity, and production of IgE might occur a few hours later (Galli et al. 2008). Th1/Th2 imbalance with a predominance of Th2 inflammatory cytokines such as IL-4, IL-5, IL-9, and IL-13 is a consequence of allergen challenge. In the presence of IL-4 and IL-13, and co-stimulatory molecules (CD40 and CD80 or CD86) B lymphocytes undergo a class switch to produce IgE antibodies (Lebman and Coffman 1988; Van der Pouw Kraan et al. 1998). Higher expression of IL-5 is associated with eosinophil-mediated inflammation in the airways (Shi et al. 1998). IL-9 enhances proliferation of mast cells (Temann et al. 1998).

Recently, there has been a great deal of interest in polyphenols due to their beneficial pulmonary actions both in animals and humans. These natural substances are present in all plants. They are generally divided into two main categories: flavonoids (anthocyanins and anthoxanthins) and non-flavonoids like stilbenes, phenolic acids, and tannins. Polyphenolic natural products exhibit a wide range of biological and pharmacological properties. On the basis of many experimental studies, it has been accepted that polyphenols have anti-inflammatory, bronchodilator, antioxidant, and expectorant effects in the airways (Lee et al. 2009; Gilani et al. 2006; Brozmanova et al. 2007; Moon et al. 2008; Park et al. 2009). They are able to suppress allergen-induced inflammatory cell infiltration during the late-phase of allergic reaction, to reduce levels of inflammatory cytokines IL-4, IL-5 and IL-13 in plasma and bronchoalveolar lavage fluid (BALF), to decrease serum IgE levels, and to inhibit histamine release from mast cells during both early and late phase of allergic reaction, which all results in their anti-inflammatory effects in airways (Jung et al. 2007; Hurst et al. 2010; Iwamura et al. 2010).

The aim of our study was to assess changes in respiratory defence reflexes during experimental allergic inflammation in the airways in response to treatment with selected polyphenols. The study was designed to evaluate the potential anti-inflammatory effects of polyphenols by the use of *in vivo* and *in vitro* methods; the latter analyzing the inflammatory cytokines IL-4 and IL-5 in BALF. In the study we used the phenolic compounds Provinol and Flavin-7, as well as their separable major components quercetin and resveratrol.

Provinol as an alcohol-free red wine extract which contains more than 95% of polyphenols. We took interest in this compound due to its reported vasorelaxant effect on arterial smooth muscle (Andriambeloson et al. 1997). Flavin-7, in turn, is able to relax the tracheal smooth muscle of healthy animals and possesses antioxidant properties in airways (Brozmanova et al. 2007; Szentmiklósi 2003). We were also interested in the antiasthmatic effects of the flavonol quercetin and the stilbene resveratrol which belong to the main polyphenolic components of Provinol and Flavin-7.

12.2 Methods

12.2.1 Experimental Protocol

The study protocol was approved by a local Ethics Committee of Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia. Male Trix guinea pigs were used in our experiment (250–300 g). They were randomly divided into three main groups each consisting of ten animals: control ($n=10$) – treated for 21 days only with saline, sensitized with the allergen ovalbumin (OVA) for 21 days, and POVA, FOVA, KOVA or ROVA – groups of guinea pigs sensitized for 21 days with OVA and simultaneously treated with Provinol (20 mg kg⁻¹ per day); Flavin-7 (2 ml kg⁻¹ per day); quercetin (20 mg kg⁻¹ per day), or resveratrol (10 mg kg⁻¹ per day), respectively. Polyphenols were administered for 21 days daily orally simultaneously with sensitization of guinea pigs. Evaluating the chronic anti-inflammatory effect of polyphenols was performed on the 21st day of animal sensitization.

12.2.2 Materials

Provinol (dry powder of red wine polyphenolic compounds) was provided by D. Ageron (Société Francaise de Distillerie, Vallont Pont d'Arc, France). Provinol is a mixture of proanthocyanidins, total anthocyanins, free anthocyanins, catechins, phenolic acids and flavonols. Flavin-7 is a concentrated fruit extract (red grape, blackberry, black cherry, black currant, elderberry, blackthorn, and plum) riched on bioflavonoids and stilbene resveratrol. Flavin-7 was a gift from Vita Crystal Slovakia (Slovakia). Quercetin, resveratrol, ovalbumin (egg albumin grade V), histamine hydrochloride, acetylcholine and other chemicals were purchased from Sigma Aldrich (Taufkirchen, Germany) and aluminium hydroxide from AFT (Bratislava, Slovakia). RayBio® mouse IL-4 and IL-5 ELISA kits were purchased from BioTech X-ray (St.Louis, MO).

The composition of Provinol was (in mg g⁻¹ dry powder): proanthocyanins 480, total anthocyanins 61, free anthocyanins 19, catechin 38, hydroxycinnamic acid 18, and flavonols 14. The composition of Flavin-7 was: total polyphenols 220 mg of which 170 mg flavonoids (myricetin, quercetin, kaempferol, isorhamnetine, (+)-catechin, (-)-epicatechin, malvidin-3-glucoside, chrysin, galangin, apigenin, fisetin and luteolin) and resveratrol 0.17 mg in 10 ml of this product.

12.2.3 Sensitization of Guinea Pigs

The intermittent exposure to allergen (suspension of 5 mg ovalbumin and 100 mg aluminium hydroxide) took 21 days. The suspension (0.5 ml) was injected both subcutaneously and intraperitoneally on the 1st day, subsequently either subcutaneously or intraperitoneally again during 2 weeks. 1% ovalbumin was nebulized by guinea pigs for 1–3 min last week.

12.2.4 Assessment of Airways Reactivity

Changes in specific airway resistance of sensitized guinea pigs were induced by inhaling histamine (10⁻⁴ mol l⁻¹) for 2 min during *in vivo* conditions. A non-invasive technique for the measurement of these changes was conducted in a whole-body plethysmography after the long-term administration of polyphenols (Provinol, Flavin-7, quercetin, and resveratrol).

Changes in force of tracheal smooth muscle contraction evoked by cumulative doses (10⁻⁸–10⁻³ mol l⁻¹) of histamine and acetylcholine were analyzed during *in vitro* conditions. These bronchoconstrictor mediators were added into the organ bath with Krebs-Henseleit solution (in mmol l⁻¹: NaCl 110.0; KCl 4.80; CaCl₂ 2.35; MgSO₄ 1.20; KH₂PO₄ 1.20; NaHCO₃ 25.0; glucose 10.0). The amplitude of tracheal smooth muscle contraction (mN) after the long-term administration of polyphenols to sensitized animals was monitored.

12.2.5 Assessment of Airways Inflammation

Levels of inflammatory cytokines IL-4 and IL-5 were analyzed in BALF of the left lung. The lavage was performed twice with sterile isotonic saline solution (37°C; 10 ml kg⁻¹) using a tracheal cannula. The supernatant of BALF after centrifugation (1,500 rpm for 10 min) was used for immunological analysis of cytokine concentrations using commercially available ELISA kits (RayBiotech, Inc). The minimum detection levels for each cytokine was <5 pg ml⁻¹.

12.2.6 Statistical Data Analysis

The values for bronchial hyperreactivity and inflammatory cytokines in BALF were expressed as means $\pm$ SE. ANOVA was performed to test for any differences in the mean values between groups. $p < 0.05$ was considered to be statistically significant.

12.3 Results

On the 21st day of animal sensitization, the experiments focused on the assessment of airway reactivity and allergic inflammation and the effects on them of the long-term administration of the antioxidants. Allergen-induced bronchial hyperreactivity resulted in an increase in specific airways resistance in response to histamine *in vivo* and in amplitude of tracheal smooth muscle contraction in response to both histamine and acetylcholine *in vitro*. There were also significant elevations in the inflammatory cytokines IL-4 and IL-5 in BALF.

The polyphenolic compounds Provinol and Flavin-7 significantly reduced both airway reactivity (Fig. 12.1a) and tracheal smooth muscle contraction evoked by both histamine and acetylcholine (Fig. 12.2a). The single components of these compounds quercetin and resveratrol had only mild, insignificant bronchodilator effects (Fig. 12.1b). Incubation of tracheal smooth muscle with quercetin reduced the amplitude of contraction in response to histamine and to higher concentrations of acetylcholine (10^{-5} – 10^{-3} mol l $^{-1}$). Resveratrol failed to reduce contraction of tracheal smooth muscle provoked by both histamine and acetylcholine in sensitized guinea pigs (Fig. 12.2b).

Likewise, the polyphenolic Provinol and Flavin-7 effectively reduced the levels of IL-4 and IL-5 in BALF of sensitized animals, while quercetin and resveratrol failed to cause appreciable changes in these inflammatory cytokines (Fig. 12.3).

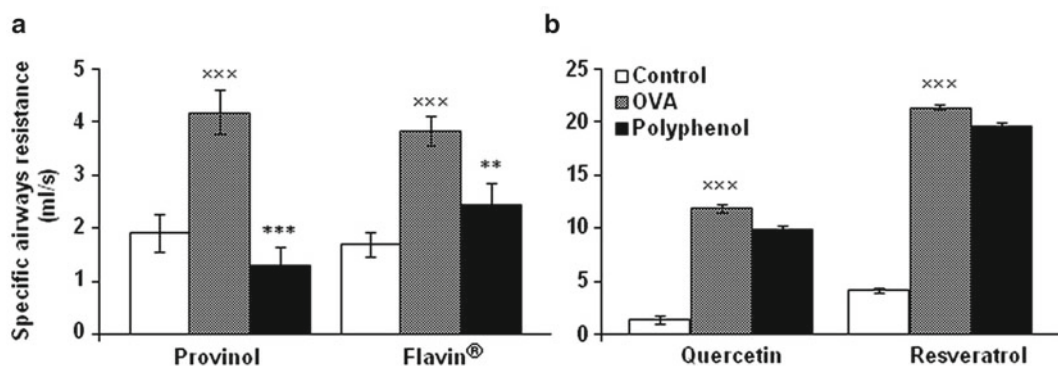


Fig. 12.1 Specific airway resistance after chronic administration of polyphenols in guinea pigs. (a) Provinol and Flavin-7 and (b) quercetin and resveratrol. Control – non-sensitized guinea pigs treated for 21 days with saline; OVA – guinea pigs sensitized for 21 days with ovalbumin (OVA); Polyphenol – guinea pigs sensitized with OVA for 21 days and simultaneously treated with Provinol (20 mg kg $^{-1}$ per day) and Flavin-7 (2 ml kg $^{-1}$ per day) or quercetin (20 mg kg $^{-1}$ per day) and resveratrol (10 mg kg $^{-1}$ per day). Data are means $\pm$ SE, $n = 10$; *** $p < 0.001$ compared with the control group; ** $p < 0.01$ and *** $p < 0.001$ compared with the OVA group

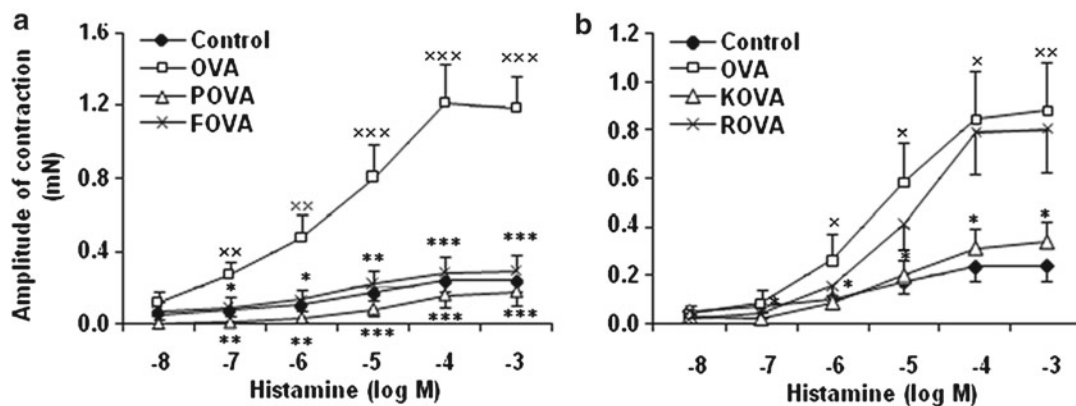


Fig. 12.2 Amplitude of tracheal smooth muscle contraction evoked by histamine 10^{-8} – 10^{-3} mol l^{-1} after chronic administration of (a) Provinoxil and Flavin-7 and (b) quercetin and resveratrol. Control – non-sensitized guinea pigs treated for 21 days with saline; OVA – guinea pigs sensitized for 21 days with ovalbumin (OVA); POVA – guinea pigs sensitized with OVA for 21 days and simultaneously treated with Provinoxil (20 mg kg^{-1} per day); FOVA – guinea pigs sensitized OVA for 21 days and simultaneously treated with Flavin-7 (2 ml kg^{-1} per day); KOVA – guinea pigs sensitized with OVA for 21 days and simultaneously treated with quercetin (20 mg kg^{-1} per day), and ROVA – guinea pigs sensitized with OVA for 21 days and simultaneously treated with resveratrol (10 mg kg^{-1} per day). Data are means $\pm$ SE; $n=10$; * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ compared with the control group; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with the OVA group

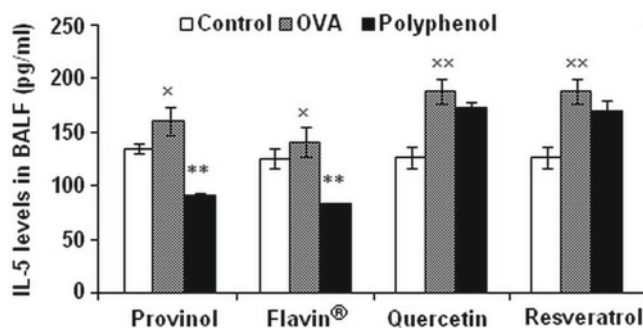


Fig. 12.3 Inflammatory cytokine IL-5 in bronchoalveolar lavage fluid (BALF) after chronic administration of polyphenols: Provinoxil, Flavin-7, quercetin, and resveratrol. Control – guinea pigs treated for 21 days with saline; OVA – guinea pigs sensitized for 21 days with ovalbumin; polyphenol – guinea pigs sensitized for 21 days with OVA and simultaneously treated with Provinoxil (20 mg kg^{-1} per day), Flavin-7 (2 ml kg^{-1} per day), quercetin (20 mg kg^{-1} per day), or resveratrol (10 mg kg^{-1} per day). Data are means $\pm$ SE; $n=10$; * $p<0.05$ and ** $p<0.01$ compared with the control group; ** $p<0.01$ compared with the OVA group

12.4 Discussion

Treatment strategy of bronchial asthma is aimed at reducing airways hyperresponsiveness and allergic inflammation, the main features of asthma. Natural substances are less harmful to human health and have fewer unwanted effects compared with bronchodilators or corticosteroids. Recently, such compounds have become research objects in inflammatory disorders.

In the present study we assessed the effects of selected polyphenols on airway hyperresponsiveness and inflammation in experimentally evoked allergic asthma. The polyphenolic compounds Provinol and Flavin-7 effectively reduced bronchial hyperreactivity and contractile responses of tracheal smooth muscle to bronchoconstrictive mediators. Zenebe et al. (2003) have found similar biological effects of Provinol on vascular smooth muscle. This polyphenolic compound of red wine produces endothelium-dependent relaxation as a result of enhanced nitric oxide (NO) synthesis. In our previous study, we have also shown that the relaxant effect of Provinol in the airways is partially mediated through NO metabolism (Joskova et al. 2009). With regard to Flavin-7, Szentmiklósi (2003) has reported the compound's bronchodilator activity in healthy animals. On the other hand, the bronchodilating and anti-inflammatory effects of quercetin and resveratrol, single components of the two polyphenols outlined above were not confirmed in the present study. The latter negative result is divergent with a number of experimental studies which have shown potential benefits of quercetin in regard to the limitation of allergic inflammation in the airways. In those studies, however, quercetin was administered for a much shorter time or in much higher doses (Nanua et al. 2006; Jung et al. 2007; Moon et al. 2008; Park et al. 2009; Rogerio et al. 2010). Quercetin and its metabolites preferentially accumulate in the rat lungs (de Boer et al. 2005), which is in accord with its local protective action against experimentally-induced lung fibrosis in rats (El-Sayed and Rizk 2009). The present study failed to confirm the beneficial effect of quercetin in the bronchial allergic condition. One reason of the negative finding could be difficult or reduced solubility of quercetin (Ferry et al. 1996; Kim et al. 2009). Concerning resveratrol, the lack of an effect could be due to too small a dose we employed, as doses three times higher apparently are able to exert an effect on the airways (Lee et al. 2009). In the present study we chose to use a moderate dose of resveratrol, although higher doses might be justified in view of its oxidative degradation *in vitro* in the presence of bicarbonate ions (Yang et al. 2010).

Allergen administration induces early and late allergic reactions in which many inflammatory cytokines play a role. IL-4 mediates induction of the IgE isotype switch and its secretion by B-lymphocytes (Lebman and Coffman 1988), expression of vascular cell adhesion molecule-1 (VCAM-1) on vascular endothelium (Iademarco et al. 1995), migration of eosinophils through endothelium, mucus induction, and differentiation of T helper type 2 lymphocytes and their recruitment to the lungs (Cohn et al. 1997). IL-5 promotes activation of B-lymphocytes resulting in enhanced serum antibody levels and eosinophil number in the blood and their infiltration into the lungs (Shi et al. 1998). In the present study we confirmed that both polyphenols Provinol and Flavin-7, but not their separable components quercetin and resveratrol, reduced the levels of inflammatory cytokines IL-4 and IL-5 in BALF of sensitized guinea pigs. In contrast, some other studies have found that the separable components of polyphenols may suppress allergic inflammation in airways due to a reduction in proinflammatory cytokines (Park et al. 2007, 2009). It is therefore a reasonable assumption that the final effect of polyphenolic compounds is a result of mutual interactions among their separable components. The latter components if applied alone may require much higher doses for the biological effect to show up. That assumption is in line with the studies in which the antioxidant effects of a mixture of flavonoids were produced (Kühnau 1976), while flavonoid monomers did not induce the biological response (Fitzpatrick et al. 1993).

In conclusion, the results of our experiments point to the appreciable anti-inflammatory effects of the polyphenolic compounds Provinol and Flavin-7 in experimental asthma. Such compounds are more effective than their separable components. Natural polyphenols may become useful ancillary therapy in the management of asthma.

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References

- Andriambeloson, E., Kleschyov, A. L., Muller, B., Beretz, A., Stoclet, J. C., & Andriantsitohaina, R. (1997). Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. *British Journal of Pharmacology*, 120(6), 1053–1058.
- Brozmanova, M., Bartos, V., Plank, L., Plevkova, J., & Tatar, M. (2007). Dietary intake of flavonoids and hyperoxia-induced oxidative stress related cough in guinea pigs. *Bratislavské Lekárske Listy*, 108(12), 489–494.
- Cohn, L., Homer, R. J., Marinov, A., Rankin, J., & Bottomly, K. (1997). Induction of airway mucus production by T helper 2 (Th2) cells: A critical role for IL-4 in cell recruitment but not mucus production. *The Journal of Experimental Medicine*, 186(10), 1737–1747.
- De Boer, V. C., Dihal, A. A., van der Woude, H., Arts, I. C., Wolffram, S., Alink, G. M., Rietjens, I. M., Keijer, J., & Hollman, P. C. (2005). Tissue distribution of quercetin in rats and pigs. *Journal of Nutrition*, 135(7), 1718–1725.
- El-Sayed, N. S., & Rizk, Sh. M. (2009). The protective effect of quercetin, green tea or malt extracts against experimentally-induced lung fibrosis in rats. *African Journal of Pharmacy and Pharmacology*, 3(5), 191–201.
- Ferry, D. R., Smith, A., Malkhandi, J., Fyfe, D. W., deTakats, P. G., Anderson, D., Baker, J., & Kerr, D. J. (1996). Phase I clinical trial of the flavonoid quercetin: Pharmacokinetics and evidence for *in vivo* tyrosine kinase inhibition. *Clinical Cancer Research*, 2(4), 659–668.
- Fitzpatrick, D., Hirschfield, S. L., & Coffey, R. G. (1993). Endothelium-dependent relaxing activity of wine and other grape products. *American Journal of Physiology*, 265, 774–778.
- Galli, S. J., Tsai, M., & Piliponsky, A. M. (2008). The development of allergic inflammation. *Nature*, 454(7203), 445–454.
- Gilani, A. H., Khan, A. U., Ghayur, M. N., Ali, S. F., & Herzig, J. W. (2006). Antispasmodic effects of Rooibos tea (*Aspalathus linearis*) is mediated predominantly through K⁺ channel activation. *Basic & Clinical Pharmacology & Toxicology*, 99(5), 365–373.
- Hurst, S. M., McGhie, T. K., Cooney, J. M., Jensen, D. J., Gould, E. M., Lyall, K. A., & Hurst, R. D. (2010). Blackcurrant proanthocyanidins augment IFN- γ induced suppression of IL-4 stimulated CCL26 secretion in alveolar epithelial cells. *Molecular Nutrition & Food Research*, 54, 159–170.
- Iademarco, M. F., Barks, J. L., & Dean, D. C. (1995). Regulation of vascular cell adhesion molecule-1 expression by IL-4 and TNF- α in cultured endothelial cells. *Journal of Clinical Investigation*, 95(1), 264–271.
- Iwamura, Ch, Shinoda, K., Yoshimura, M., Watanabe, Y., Obata, A., & Nakayama, T. (2010). Naringenin chalcone suppresses allergic asthma by inhibiting the type-2 function of CD4 T cells. *Allergy International*, 59(1), 67–73.
- Joskova, M., Franova, S., Nosalova, G., Pechanova, O., Prisenznakova, L., & Sutovska, M. (2009). A beneficial influence of provinol on the reduction of allergen induced hyperreactivity in guinea pigs. *Bratislavské Lekárske Listy*, 110(8), 454–458.
- Jung, Ch. H., Lee, J. Y., Cho, C. H., & Kim, C. J. (2007). Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin. *Archives of Pharmacol Research*, 30(12), 1599–1607.
- Kim, M. K., Park, K. S., Yeo, W. S., Choo, H., & Chong, Y. (2009). In vitro solubility, stability and permeability of novel quercetin-amino acid conjugates. *Bioorganic & Medicinal Chemistry*, 17(3), 1164–1171.
- Kühnau, J. (1976). The flavonoids: A class of semi-essential food components: Their role in human nutrition. *World Review of Nutrition and Dietetics*, 24, 117–191.
- Lebman, D. A., & Coffman, R. L. (1988). Interleukin-4 causes isotype switching to IgE in T cell-stimulated clonal B cell cultures. *The Journal of Experimental Medicine*, 168(3), 853–862.
- Lee, M., Kim, S., Kwon, O. K., Oh, S. R., Lee, H. K., & Ahn, K. (2009). Anti-inflammatory and anti-asthmatic effects of resveratrol, a polyphenolic stilbene, in a mouse model of allergic asthma. *International Immunopharmacology*, 9(4), 418–424.
- Moon, H., Choi, H. H., Lee, J. Y., Moon, H. J., Sim, S. S., & Kim, C. J. (2008). Quercetin inhalation inhibits the asthmatic responses by exposure to aerosolized-ovalbumin in conscious guinea-pigs. *Archives of Pharmacol Research*, 31(6), 771–778.
- Nanua, S., Zick, S. M., Andrade, J. E., Sajjan, U. S., Burgess, J. R., Lukacs, N. W., & Hershenson, M. B. (2006). Quercetin blocks airway epithelial cell chemokine expression. *American Journal of Respiratory Cell and Molecular Biology*, 35(5), 602–610.
- Park, S. J., Shin, W. H., Seo, J. W., & Kim, E. J. (2007). Anthocyanins inhibit airway inflammation and hyperresponsiveness in a murine asthma model. *Food and Chemical Toxicology*, 45(8), 1459–1467.
- Park, H. J., Lee, C. M., Jung, I. D., Lee, J. S., Jeong, Y. I., Chang, J. H., Chun, S. H., Kim, M. J., Choi, I. W., Ahn, S. C., Shin, Y. K., Yeom, S. R., & Park, Y. M. (2009). Quercetin regulates Th1/Th2 balance in a murine model of asthma. *International Immunopharmacology*, 9(3), 261–267.
- Rogério, A. P., Dora, C. L., Andrade, E. L., Chaves, J. S., Silva, L. F., Lemos-Senna, E., & Calixto, J. B. (2010). Anti-inflammatory effect of quercetin-loaded microemulsion in the airways allergic inflammatory model in mice. *Pharmacological Research*, 61(4), 288–297.

- Shi, H. Z., Xiao, C. Q., Zhong, D., Qin, S. M., Liu, Y., Liang, G. R., Xu, H., Chen, Y. Q., Long, X. M., & Xie, Z. F. (1998). Effect of inhaled interleukin-5 on airway hyperreactivity and eosinophilia in asthmatics. *American Journal of Respiratory and Critical Care Medicine*, 157(1), 204–209.
- Szentmiklósi, A. J. (2003). Study on cardiovascular and bronchial effects of Flavin-7 in isolated tissue. *Internal Issue of Crystal Institute Ltd.*, pp. 1–79.
- Temann, U. A., Geba, G. P., Rankin, J. A., & Flavell, R. A. (1998). Expression of interleukin-9 in the lungs of transgenic mice causes airway inflammation, mast cell hyperplasia, and bronchial hyperresponsiveness. *The Journal of Experimental Medicine*, 188(7), 1307–1320.
- Van der Pouw Kraan, T. C., Van der Zee, J. S., Boeijs, L. C., De Groot, E. R., Stapel, S. O., & Aarden, L. A. (1998). The role of IL-13 in IgE synthesis by allergic asthma patients. *Clinical and Experimental Immunology*, 111(1), 129–135.
- Yang, N. Ch., Lee, C. H., & Song, T. Y. (2010). Evaluation of resveratrol oxidation *in vitro* and the crucial role of bicarbonate ions. *Bioscience, Biotechnology, and Biochemistry*, 74(1), 63–68.
- Zenebe, W., Pechánová, O., & Andriantsitohaina, R. (2003). Red wine polyphenols induce vasorelaxation by increased nitric oxide bioactivity. *Physiological Research*, 52(4), 425–432.