Nutritional Sciences 5(4), November, 2002, pp. 197-202

Free Radical Scavenging of Flavonoids and Their Effects on Erythrocyte Na Leak, Platelet Aggregation and TBARS Production

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We compared the radical scavenging activity of flavonoids and their antioxidant effects on erythrocyte Na leak, platelet aggregation and TBARS (thiobarbituric acid reactive substance) production, using Sprague Dawley rats. The concentrations of flavonoids needed for scavenging radicals by 50% (SC_{50}) in 0.1mM DPPH (2,2 Diphenyl 1-picryl hydrazyl) were: Quercetin, 7.4μM; Catechin, 10.6μM; Morin, 22μM; Hesperidin, 400μM; and Naringin, 3.95mM. Morin completed its antioxidant activity in 2 minutes, while catechin, hesperidin and naringin had slow but long lasting antioxidant activity. Whole blood platelet aggregation, when incubated with quercetin or catechin, was significantly decreased ($P<0.05$) compared with the control. Sodium leak in intact erythrocytes was significantly lower when incubated with quercetin, compared with other flavonoids ($P<0.05$). Morin, hesperidin and naringin somewhat increased Na leak in intact erythrocytes. Sodium leak in erythrocytes treated with phenazine methosulfate (PMS) was increased overall, but was not affected by flavonoids. Intracelluar Na and K were not affected by treatment with PMS. TBARS production in platelet rich plasma (PRP) was significantly lower ($P<0.05$) than the control when incubated with quercetin or hesperidin. PMS treatment caused an increase in TBARS production regardless of flavonoids. In the present study, antioxidant effects of flavonoids were not well correlated with their radical scavenging activities, although quercetin, which showed the strongest radical scavenging activity, had the greatest antioxidant effect.

Key words : flavonoids, DPPH test, erythrocyte Na-leak, platelet aggregation, TBARS

INTRODUCTION

Flavonoids, more than 4000 in number, are phenolic compounds widely distributed in plants. Recently, interest in food phenolics has been greatly increased because their antioxidant ability might have beneficial effects on human health. High intakes of foods rich in flavonoids such as wine, grape fruit juice and green tea might reduce the risk of cardiovascular diseases. Epidemiological studies indicate that consumption of flavonoid-rich foods is associated with lowered incidences of cardiovascular diseases.[1),2),3)] Dietary red wine has favorable effects on human plasma high-density lipoprotein and blood chemistry,[4)] and consumption of catechin and quercetin or drinking grape juice inhibit platelet aggregation in humans.[5),6)] There are also reports that catechin derived from tea has hypocholesterolemic and antihypertensive effects,[7)] and that tea flavonoids retard LDL oxidation.[8)] On the other hand, others report that consumption of green or black tea did not affect LDL oxidation in humans[9)] and that high intakes of blackcurrent and apple juices have a prooxidant activity on plasma protein.[10)] These contradictions may result from the differences in the dosage or the time period of consumption, and individual variations. It has been suggested that significant amounts of flavonoids can be absorbed in human subjects and can be bioavailable to act as antioxidants in vivo.[10)-13)] However, it is not clear if the habitual diet of the general population can provide sufficiently concentrated flavonoids to exert protective effects against free radicals. The physiological functions of flavonoids are based on their radical scavenging activity, preventing oxidative modification of biomembranes, lipoproteins, platelets and prostanoids,[14),15)] but it is currently unclear whether flavonoids really can act as an antioxidant in vivo. Since the beneficial effects of dietary flavonoids in humans are primarily related to their antioxidant ability, further studies are necessary to determine in which in vivo/in vitro situations flavonoids can act as antioxidants. Overall, more in vitro and in vivo studies are needed on exactly which flavonoids are beneficial and at what safe dosage, before recommendations can be made for therapeutic use.

We conducted an experiment to compare the DPPH (2,2 Diphenyl 1-picryl hydrazyl) free radical scavenging activity of flavonoids and their antioxidant effects by examining erythrocyte Na leak, platelet aggregation and TBARS production in an ex vivo system after incu-

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Accepted : October 18, 2002

bation with flavonoids. The scavenging activities of flavonoids can be evaluated in vitro by DPPH free radical assay.[16] DPPH is a stable nitrogen radical with a purple color of absorbance at 517nm, and this absorbance decreases upon reaction with antioxidants. We chose five major dietary flavonoids: the flavonols quercetin and morin (onion, red grape); the flavanol catechin (green and black tea); and the flavanones hesperidin and naringin (citrus fruits). We examined the antioxidant effects of these five flavonoids in order to provide data to support future clinical trials.

MATERIALS AND MATERIALS

1. Flavonoids and animal samples

DPPH (2,2-diphenyl-1-picrylhydrazyl) and the test flavonoids (quercetin, catechin, morin, hesperidin, naringin) were purchased from Sigma-Aldrich (St. Louis, MO). Male Sprague Dawley rats were fed ad libitum commercial pellets and were housed in groups of four in a plastic cage in a room maintained at 20-25℃ with a 12 hours dark-light cycle. At the age of 12-14 weeks, blood samples were obtained by cardiac puncture into vacuum tubes containing heparin, and platelet aggregation and erythrocyte Na leak tests were immediately performed.

2. DPPH radical scavenging test

Radical scavenging activities of flavonoids were evaluated by the DPPH method of Brand-Williams.[16] Radical scavenging activity is defined as the amount of antioxidant necessary to decrease the DPPH concentration by 50%. Flavonoids were first dissolved in DMSO (Dimethyl sulfoxide, Sigma-Aldrich) to a 50mM stock solution, then rediluted with methanol (Merck) to each working concentration. The DPPH concentration was measured by its absorbance of 517nm at 0 min through 10 min after addition of the flavonoid (in methanol) to 0.1mM DPPH (in methanol) to achieve 1.5㎖ of total volume. SC_{50} (50% scavenging activity) is defined as the concentration of the flavonoid at which absorbance is decreased by 50%, 10 minutes after the addition of the flavonoid.

3. Platelet aggregation

Platelet aggregation was measured using a Chronolog Whole Blood Aggregometor (model 500-Ca, Havertown, Pennsylvania, USA). The whole blood, incubated with 100μM of flavonoid for 10 minutes, was diluted with isotonic saline solution (1:2) to give a platelet concentration of 400,000/㎕. Adenosine diphosphate (ADP, 2μM) was added to initiate aggregation, and three readings of impedance changes were averaged for each sample. Increased platelet aggregation caused an increase in impedance across two platinum electrodes. The impedance method using the fresh whole blood has the advantage of measuring platelet aggregation under almost in vivo conditions in the presence of other blood components.

4. Erythrocyte Na-leak

Sodium leak is defined as sodium efflux through passive sodium channels occurring under inhibition of ouabain-sensitive Na-pumps and furosemide-sensitive Na^+-K^+ co-transport. Sodium leak can be increased through damage of erythrocyte membranes after exposure to a free radical generating system such as phenazine methosulfate (PMS).[17] Four ㎖ of blood, preincubated with 100μM of flavonoid for 10 minutes, was divided into two 2㎖ portions; one portion was treated with 0.5mM PMS while the other remained untreated, before both portions were incubated in a shaking water bath for 5 minutes. Blood was then centrifuged at 1,000×g for 10 minutes, and the plasma and buffy coat were removed. The red blood cells were subsequently washed 5 times with a cold isotonic washing solution [150mM choline chloride, 10mM Tris-4 morpholinopropane sulfonic acid (MOPS), pH 7.4 at 4℃], and were centrifuged at 1,000×g for 5 minutes after each wash. The final mass of erythrocytes was resuspended in the choline chloride washing solution to give a 40-50% hematocrit. A 50㎕ aliquot of the suspended erythrocytes was added to 5㎖ of 0.025% acationox (a metal free detergent from Scientific Products, McGaw Park Illinois, USA) for use in determining intracellular K and Na concentrations. Forty ㎖ of medium (150mM choline chloride, 10mM glucose, 1mM ouabain, 1mM furosemide, 10mM Tris-MOPS pH7.4 at 37℃) were added to each of two ㎖ of PMS-treated erythrocyte suspension and two ㎖ of untreated erythrocyte suspension; each of the two mixtures were then mixed gently and aliquoted to 12 tubes. The tubes were transferred in duplicates to an ice bath after incubation at 37℃ in a shaking water bath for 0, 10, 20, 30, 40 and 50min. Tubes were subsequently centrifuged at 1,000×g for 5 minutes, the supernatants were removed, and Na concentration was then measured.

Calculations:

Na μg/(ml×min)×60min×mmole/23mg×[44ml - (4ml ×hct)/(0.0041×hct)]×(mg/1000μg)=mmole/ℓ RBC /hour (Na leak)

Na μg/(m l×min)×mmole/23mg×101/hct×(mg/1000μg) =mmole/ℓ RBC (Intracelluar Na)

5. TBARS production

Platelet rich plasma (PRP) was obtained after centrifuging PMS treated and untreated whole blood at 300 ×g for 10 minutes. After incubation of each PRP sample

with 100μM of flavonoids for 24 hours at 37℃, the amount of thiobarbituric acid reactive substance (TBARS) was determined according to a modified Yagi's method.[18] Plasma lipids were precipitated with phosphotungstic acid, and then TBARS were measured by a fluometer (Kontron model SFM 25) using 1,1,3,3- tetraethoxypropane (Sigma-Aldrich) as a standard.

6. Statistical analysis

Values were analyzed using the SAS package (SAS, 1994). Analyses of variance were conducted in a completely randomized block design. Duncan's multiple test was applied to compare individual means when the F-value was significant ($P<0.05$).

RESULTS AND DISCUSSION

1. DPPH assay of flavonoids

Radical scavenging activity results are shown in Table 1. SC_{50} is the flavonoid concentration that can scavenge 0.1mM DPPH (2,2 Diphenyl 1-picryl hydrazyl) of radicals by 50% at 10 minutes after addition of flavonoids. Among the five flavonoids, Quercetin had the lowest SC_{50}, 7.4μM, meaning that quercetin had the strongest antioxidant activity. Catechin and morin also had comparatively low SC_{50} values, while hesperidin and naringin had high SC_{50} concentrations, of 0.4mM and 3.95mM respectively. Morin had the same antioxidant activity as vitamin C. SC_{50} can be useful in deciding the optimum concentration of flavonoids for in vitro experiments; our results supported the generally-used concentrations of 5-10μM for quercetin or catechin and 800-1200μM for hesperidin or naringin.[5),19)]

The patterns of decrease in absorbance after the addition of the flavonoids are shown in Fig 1. The time period during which the antioxidant activity lasted, when interpreted with the pattern of decrease in absorbance, was different between flavonoids. Like vitamin C, which completed its antioxidant activity in 10 seconds, morin showed 90% of its antioxidant activity in 10-15 seconds and completed all activity in 2 minutes. Catechin, hesperidin and naringin had slow but lasting activities, decreasing absorbance for 10 minutes and longer. This suggests that the activities scavenging 50% of the DPPH radicals by morin and vitamin C are their total antioxidant activity, while catechin, hesperidin and naringin have some remaining activity above scavenging 50% of the DPPH radicals. The length of time of lasting antioxidant activities of flavonoids is important for their concentration and incubation time in in vitro experiments. The potency and period of activity of different flavonoids may be associated with their chemical structures, and further study is needed regarding the relationship between the chemical characteristics of DPPH and its concentration.

Table 1. SC_{50} concentrations of flavonoid for 0.1mM DPPH

	Quercetin	Catechin	Morin	Hesperidin	Naringin	Vit. C[2)]
SC_{50} (μM)[1)]	7.4	10.6	22	400	3950	22

1) Flavonoid concentrations at which the decrease in absorbance at 517nm by 50% for
0.1mM DPPH in methanol at 10minutes after addition of flavonoid in methanol
2) Vitamin C was tested to compare with flavonoids in its radical scavenging activity

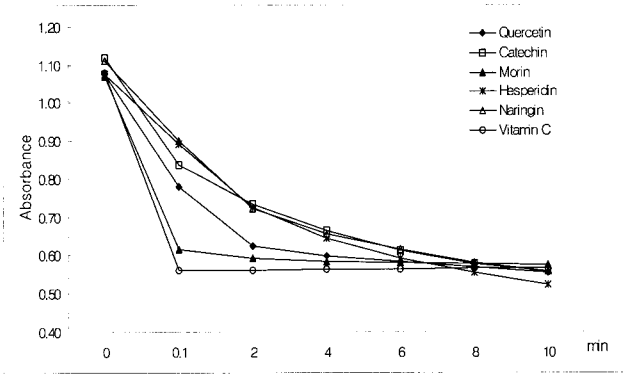

Fig 1. Patterns of decrease in absorbance after addition of SC50 concentration of flavonoid to 0.1mM DPPH

2. Platelet aggregation

Whole blood platelet aggregation results are shown in Table 2. All of the test flavonoids decreased the maximum platelet aggregation, and quercetin and catechin were significantly different compared with the control ($P<0.05$). Quercetin only significantly decreased the initial slope of platelet aggregation ($P<0.05$), while hesperidin tended to increase the initial slope.

The inhibitory effects of flavonoids on platelet aggregation may be implicated in oxidative events such as arachidonic acid metabolism and thromboxane production in platelets.[20)] Flavonids may interact with oxidant species formed on platelet activation or flavonoids may decrease the release of superoxide (a platelet stimulator) in platelets. Havsteen[21)] reported that flavonoids inhibited cyclooxygenase. Grape juice incubated with platelets enhanced platelet release of nitric oxide which is a platelet inhibitor.[22)] At levels of 10-20μM quercetin or 50-100μM catechin platelet aggregation is inhibited in in vitro systems using human PRP.[5)] Considering their scavenging activities in DPPH assays, the flavonoid concentrations of 100μM used in this experiment may be sufficient for quercetin and catechin, but not sufficient for naringin and hesperidin, to act as antioxidants.

Table 2. Whole blood platelet aggregation

	Control	Quercetin	Catechin	Morin	Hesperidin	Naringin
Maximum(Ω)[1]	12.10±2.05[a]	9.59±1.07[b]	10.28±0.87[b]	10.67±0.77[ab]	11.64±2.16[ab]	10.60±1.02[ab]
Initial slope[2] (Ω/min)	7.69±0.90[a]	6.58±0.70[b]	7.34±0.83[ab]	7.73±0.95[a]	7.84±0.83[a]	7.73±0.95[a]

1) Maximum aggregation in ohm at the point where aggregate dissociated.
2) Initial slope is ohm change for the first one minute.
Values in the same row not sharing the same superscript differ. (P<0.05)
Values are means±SD of ten samples.

Table 3. Intracellular Na and K concentrations and passive Na leak in intact or PMS treated red blood cell

	Intracellular (mmole/ℓ rbc)		Passive Na leak (mmole/ℓ rbc/hr)					
	Na	K	Control	Quercetin	Catechin	Morin	Hesperidin	Naringin
Intact	2.53±0.26	87.9±3.27	2.71±0.6[ab]	2.21±0.4[b]	2.89±0.1[ab]	3.06±0.9[a]	3.20±0.8[a]	3.21±0.9[a]
PMS treated	2.68±0.39	90.30±9.20	2.95±0.9[a]	2.69±0.3[ab]	3.09±0.5[a]	3.08±0.1[a]	3.06±0.2[a]	2.81±0.4[ab]

Values in the same row not sharing the same superscript differ (P<0.05)
Values are means±SD of ten samples

3. Erythrocyte Na leak

Intracellular Na and K levels, and Na leak, are shown in Table 3. Intracellular Na and K levels were not different between intact and PMS treated red blood cells. Na leak in intact red blood cells was significantly decreased when treated with quercetin, compared to the control or samples treated with other flavonoids (P<0.05). Although there is no statistical difference between flavonoids when treated with PMS, Na leak after treatment with PMS was increased with quercetin, catechin and morin, while Na leak was decreased with hesperidin and naringin. There may be some interactions between agents which generate and scavenge radicals.

PMS, which is used for radical production, can readily cross the cell membrane and generate free radicals in the presence of NADH, causing damage inside the cells.[18] Unlike vitamin E that is localized in the cell membrane, flavonoids present in their aqueous phase can scavenge intracellular and extracellular free radicals. Some flavonoids such as flavanol appear to pass through biological membranes.[23] In the present study, PMS did not cause loss of intracellular Na and K compared with those of intact cells. Cells may close channels through defense mechanisms, preventing toxic substances such as PMS entering. All of the test flavonoids except quercetin caused an increase in Na leak in intact cells, which can not be interpreted as the consequence of oxidative damage. Quercetin and catechin, with strong antioxidant activity, caused the increase in Na leak with PMS, while naringin and hesperidin inhibited the increase in Na leak with PMS. Maridonneau et al[24] reported that flavonoids have heterogenous effects such as protective, toxic, biphasic, effective or inactive, on K loss and lipid peroxidation induced by oxygen free radicals. Hydroxyl groups in the flavonoids and the polarity of flavonoids led to significant impacts on the flavonoid-biomembrane interaction, causing membrane leak in immobilized artificial membranes.[25] Radical scavenging may not be the sole mechanism by which flavonoids control Na efflux.[26] Further studies are needed to clarify whether flavonoid can act as an antioxidant in chain breaking in membrane lipid peroxidation, and prevent oxidative damage.

4. TBARS production

Results for TBARS production in platelet rich plasma (PRP) from PMS treated and untreated blood are shown in Table 4, and comparisons between TBARS production and the influence of PMS treatment on the effect of flavonoids are shown in Fig 2. All of the test flavonoids decreased TBARS production in untreated PRP samples, compared with the control, showing a significant difference in the cases of quercetin and hesperidin (P<0.05). TBARS production in PMS-treated PRP samples was generally increased compared with the untreated PRP samples, and there were two-fold increases in TBARS production in the quercetin and hesperidin treated samples, so that there were no differences between any of the groups.

Diet containing catechin and quercetin decreased vitamin E consumption and reduced TBARS production in vitamin E deficient rats.[27] Flavonoids can replace vitamin E as an antioxidant to some extent. Flavonoid-rich extr-

Table 4. TBARS production in platelet rich plasma

	Control	Quercetin	Catechin	Morin	Hesperidin	Naringin
Untreated[1)]	0.97±0.31[a]	0.55±0.05[b]	0.68±0.21[ab]	0.70±0.06[ab]	0.56±0.11[b]	0.64±0.05[ab]
PMS treated[1)]	1.15±0.38[a]	1.04±0.60[ab]	1.09±0.6[a]	1.13±0.71[a]	1.24±0.47[a]	0.98±0.26[ab]

1) TBARS concentration expressed in nmole/ml platelet rich plasma from intact or PMS treted and untreated blood
Values in the same row not sharing the same superscript differ (P<0.05)
Values are means±SD of ten samples

acts, in in vitro tests, reduced TBARS production and LDL oxidation, and retarded the lag time of conjugate diene production.[28)] In the present study, quercetin and hesperidin by themselves reduced TBARS production, but with PMS they increased TBARS production. Compared with the effects of PMS on TBARS production, the effects of PMS on Na leak were comparatively minor, which may imply that functioning cells such as erythrocytes and platelets have significant defence mechanisms against toxic substance or free radicals.

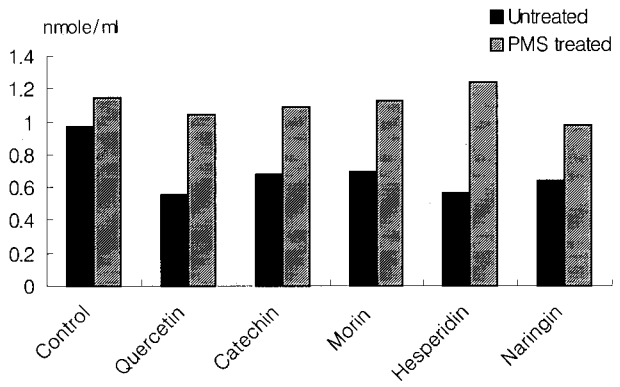

Fig 2. Comparison of TBARS production in PRPs from untreated and PMS treated blood

CONCLUSIONS

The purpose of the present study was to correlate the antioxidant capacity of flavonoids and their effects on biological systems. Quercetin and catechin have antioxidant activity of 40-400 times that of hesperidin and naringin. Of the five flavonoids tested, quercetin was the only one found to have a consistent antioxidant effect on platelet aggregation, Na leak and TBARS production. All of the tested flavonoids except quercetin caused increases in Na leak, which can not currently be explained. Recent interests focus on the structural features of flavonoids such as the hydroxyl groups, and on the polarity of flavonoids which is associated with their physiological functions in biomembranes.

It is as yet unclear whether flavonoids really can act as antioxidants in in vivo systems, or whether the doses of flavonoids effective in vitro are achievable in plasma in vivo. More in vitro and in vivo studies are needed to determine conclusively which flavonoids are bioavailable and what doses of flavonoids are beneficial for human health. Overall, further studies are necessary to elucidate the favorable mechanisms of flavonoids involved in degenerative diseases before recommending their therapeutic use.

Acknowledgements:
This work was supported by grant No R02-2001-00718 from the Basic Research Program of the Korea Science & Engineering Foundation

Litrature Cited

1) Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 342:1007-11, 1993
2) Imai K, Nakachi K. Croos sectional study of effects of drinking green tea on cardiovascular and liver diseases. *Br Med J* 310:693-696, 1995
3) Arai Y, Watanabe S, Kimira M, Shimoi K, Kinae N. Dietary intakes of flavonols, Flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J Nutr* 130:2243-2250, 2000
4) Lavy A, Fuhrman B, Markel A, Dankner G, Ben-Amotz A, Presser D, Aviram M. Effect of dietary supplementation of red or white wine on human blood chemistry, hematology and coagulation favorable effect of red wine on plasma high-density lipoprotein. *Ann Nutr Metab* 38(5):287-294, 1994
5) Pignatelli P, Pulcinelli P, Violi F. The flavonoids quercetin and catechein synergicall inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. *Am J Clin Nutr.* 72:1150-1155, 2000
6) Keevil JG, Osman HE, Reed JD and Folts JD. Grape fruit, but not orange juice or grapefruit juice inhibit human platelet aggregation. *J Nutr* 130:53-56, 2000
7) Atensvold I, Tverdal A, Solvoll K, Foss OP. Tea consump-

tion. Relationship to cholesterol, blood pressure, and coronary and total mortality. *Prev Med* 21:546-553, 1992

8) Ishigawa T, Suzugawa M, Ito T, Yoshida H, Hara Y, Nakamura H. Effect of tea flavonoid supplementation on the susceptibility of low density lipoprotein to oxidative modification. *Am J Clin Nutr.* 66(2)261-266, 1997
9) Van Het Hof KH, De Boer H, Wiseman SA, Weststrate JA, Tijburg LB. Consumption green or black tea does not increase resistance of low density lipoprotein to oxidation in humans. *Am J Clin Nutr.* 66:1125-1132, 1997
10) Young JF, Nielsen SE, Haraldsdottir J, Daneshvar B, Dragsted LO. Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status. *Am J Clin Nutr.* 69:87-94, 1999
11) Donovan JL, Bell JR, Kasim-Karakas S, German JB, Hansen RJ, Waterhouse AL. Catechin is present as metabolites in human plasma after consumption of red wine. *J Nutr* 129: 1662-1668, 1999
12) Olthof MR, Hollman PCH, Vree TB, Katan MB. Bioavailabilities of quecetin 3-glucoside and quecetin 4-glucoside do not differ in human. *J Nutr.* 130:1200-1203, 2000
13) Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. *J Nutr.* 130:2073S-2085S, 2000
14) Terao J, Pikula M, Yao Q. Protective effect of epicatechin, epicatechin gallate and quecertin on lipid peroxidation in phospholipid bilayers. *Arch Biochem Biophys.* 308(1):278-284, 1994.
15) Salah N, Miller NJ, Bolwell GP, Rice-Evan C. Polyphenolic flavanols as scavengers of aqueous phase radical and as chain breaking antioxidants. *Arch Biochem Biophys.* 322:339-346, 1995
16) Brand-Williams W, Cuvalier ME, Berset C. Use of free radical method to evaluate antioxidant activity. *Lebensm-Wiss Techol.* 28:25-30, 1995
17) Yagi K. A simple fluometric assay for lipoperoxide in plasma. *Biochem Med.* 15:212-216, 1976
18) Maridonneau I, Braquet P, Garay RP. Na+ and K+ transport damage induced by oxygen free radicals in human red blood cell membranes. *Biol Chem.* 285: 3107-3113, 1983
19) Bok SH, Lee SH, Choi MS. Plasma and hepatic activities of HMG coA reductase and acyl coA-cholesterol transferase are lower in rats fed citrus peel or a mixture of citrus bioflavonoids. J Nutr. 129;1182-1185, 1999
20) Landolfi R, Mower RL, Steiner M. Modification of platelet fuction and arachidonic acid metabolism by bioflavonoids. Structure-activity relations. *Biochem Pharmacol.* 33:1525-1530, 1984
21) Laughton MJ, Evans PJ, Moroney MA, Halliwell B. Inhibition of mammalian 5 lipooxigenase and cyclooxygenase by flavonoids and phenolic dietary additives; relationship to antioxidant activity and to iron ion reducing ability. *Biochem Pharmacol.* 42:1673-1681, 1991
22) Sauter R, Folts JD, Freedman JE. Purple grape juice inhibits platelet function and increases platelet derived nitric oxide release. *Circulation* 98:581-585, 1998
23) Scarbert A, Williams G. Dietary intake and bioavailability of polyphenols. *J Nutr* 130:2073S-2085S, 2000
24) Maridonneau-Parini I, Braquet P, Garay RP. Heterogeneous effect of flavonoids on K+ loss and lipid peroxidation induced by oxygen-free radicals in human red cells. *Pharmacol Res Comm.* 18(1): 61-72, 1986
25) Ollila F, Halling K, Vuorela P, Vuorela H, Slotte JP. Characterization of flavonoid- biomembrane interactions. *Arch Biochem Biophys.* 1:399(1):103-108, 2002
26) Umarova FT, Khushbactova ZA, Batirov EH, Mekler VM. Inhibition of Na-K ATPase by flavonoids and their inotropic effect. Investigation of the structure-activity relationship. *Membrane Cell Biol.* 12(1)27-40, 1998
27) Fremont L, Gozzelino MT, Franchi MP, Linard A. Dietary flavonoids reduce lipid peroxidation in rats fed polyunsaturated or monounsaturated fat diets. *J Nutr.* 128(9):1495-1502, 1998
28) Viana M, Barbas C, Bonet B, Bonet MV, Castro M Herrera E. In vitro effects of a flavonoid-rich extract on LDL oxidation. *Athrosclerosis.* 123(1-2):83-91, 1996