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Antioxidative Effects of Delphinidin under *in vitro* and Cellular System

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Abstract This study examined the antioxidative activity of delphinidin, a kind of anthocyanidin from eggplant. Cellular protective potential from oxidative damage by nitric oxide (NO), superoxide anion (O_2^-), and peroxynitrite ($ONOO^-$) using epithelial cell line LLC-PK1 cell as well as *in vitro* radical scavenging effects were investigated. Delphinidin showed strong *in vitro* radical scavenging effects against NO, O_2^-, and hydroxyl radical (·OH) in dose-dependent manners. In addition, delphinidin increased cell viability in LLC-PK1 cells in a concentration-dependent manner when viability was reduced by $ONOO^-$-induced oxidative damage. To elucidate the protective mechanisms of delphinidin from $ONOO^-$, sodium nitroprusside (SNP), and pyrogallol were also employed to generate NO and O_2^-, respectively. The treatment of delphinidin recovered reductions in cell viability caused by SNP and pyrogallol, indicating that delphinidin can attenuate oxidative stress induced by NO and O_2^-. The present study suggests that delphinidin is a promising antioxidative agent.

Keywords: delphinidin, antioxidative activity, peroxynitrite, oxidative stress, LLC-PK1 cell

Introduction

The overproduction of reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) mediates damage to cell structures, nucleic acids, lipids, and proteins (1). The harmful effects of free radicals, causing potential biological damage, are termed oxidative stress and nitrosative stress. Free radical-mediated oxidative and nitrosative stress leads to pathological conditions and has been implicated in a variety of degenerative diseases as well as in the aging process (2-5). Therefore, antioxidants that prevent free radical damage have attracted much attention, and great efforts have been made to find safe and effective therapeutic agents for oxidative stress-related various diseases. Compelling evidences indicate that increased consumption of dietary antioxidants or vegetables with antioxidant properties may contribute to improve quality of life by delaying the onset and reducing risk of degenerative diseases (6-8).

Among the vegetable species, eggplant is one of the most common, widespread vegetables in the world, and it is the anthocyanins in eggplant that produce its color. Our previous study demonstrated that eggplant scavenged free radical and exerted protective effect against oxidative stress under *in vitro* and cellular system (9). In addition, the beneficial potential of eggplant is attributed to its anthocyanin content, which includes delphinidin and nasunin. There is an increasing interest in anthocyanidins for their use as natural food colorants as well as potential health promoting properties. Numerous studies have shown the positive therapeutic effects of anthocyanins, such as antioxidative, anti-inflammatory, vision improvement, anticancer, and cardiovascular properties (10-13). Eggplant's major anthocyanin pigment component is nasunin, and several reports demonstrated that it possesses biological activities, including antioxidative and anticancer properties. However, studies examining the protective activity of delphinidin from free radical-induced oxidative stress have rarely been carried out. Therefore, the present study was focused on the radical scavenging effect of delphinidin under *in vitro* and protective potential from oxidative stress under cellular system.

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Materials and Methods

Materials Delphinidin was purchased from Sigma-Aldrich (St. Louis, MO, USA), and the structure was shown in Fig. 1. 3-Morpholinosydnonimine (SIN-1) was also obtained from Sigma-Aldrich. Sodium nitroprusside (SNP), pyrogallol and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H* tetrazolium bromide (MTT) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The LLC-PK1 cell, porcine renal epithelial cell, was provided from ATCC (Manassas, VA, USA). Dulbecco's modified Eagal medium/nutrient mixture F-12 (DMEM/F-12) and fetal bovine serum (FBS) were purchased from Invitrogen Co. (Grand Island, NY, USA).

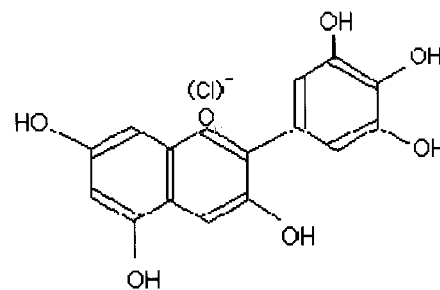

Fig. 1. Structure of delphinidin.

Nitric oxide (NO) scavenging activity NO was generated from SNP and measured by the Griess reaction, according to the method of Sreejayan and Rao (14). SNP (5 mM), in phosphate buffered saline (PBS), was mixed with different concentrations of delphinidin and then incubated at 25°C for 150 min. The amount of NO produced by SNP was assayed by measuring the accumulation of nitrite, based on the Griess reaction, using a microplate assay method.

Superoxide anion (O_2^-) scavenging activity The level of O_2^- was measured following the method as described by Ewing and Janero (15). For the assay, delphinidin was added to microplate wells containing 200 μL of freshly prepared 0.125 mM ethylenediamine tetraacetic acid (EDTA), 62 μM nitro blue tetrazolium (NBT), and 98 M NADH in 50 mM phosphate buffer, pH 7.4. The reaction was initiated with the addition of 25 μL of freshly prepared 33 μM 5-methylphenazium methyl sulfate (PMS) in 50 mM phosphate buffer (pH 7.4). The absorbance was continuously monitored at 540 nm over 5 min as an index of NBT reduction using a microplate reader (Model 3550-UV; Bio-Rad, Tokyo, Japan).

Hydroxyl radical (·OH) scavenging activity The reaction mixture contained 0.45 mL of 0.2 M sodium phosphate buffer (pH 7.0), 0.15 mL of 10 mM 2-deoxyribose, 0.15 mL of 10 mM $FeSO_4$-EDTA, 0.15 mL of 10 mM H_2O_2, 0.525 mL of H_2O, and 0.075 mL of sample solution. The reaction was initiated by the addition of H_2O_2. After incubation at 37°C for 4 hr, the reaction was stopped by adding 0.75 mL of 2.8% trichloroacetic acid and 0.75 mL of 1.0% of 2-tribarbituric acid in 50 mL NaOH. The solution was boiled for 10 min, and then cooled in water. The absorbance of the solution was measured at 520 nm. ·OH scavenging activity was evaluated as the inhibition rate of 2-deoxyribose oxidation by ·OH (16).

Cell culture Commercially available LLC-PK_1 cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 in culture plates with 5% FBS-supplemented DMEM/F-12 medium. The cells were subcultured weekly with 0.05% trypsin-EDTA in calcium- and magnesium-free phosphate buffer.

Treatment of free radical generators To investigate delphinidin's protective activity against oxidative damage, we employed SIN-1, SNP, and pyrogallol-induced cellular oxidative models (17,18). After confluence had been reached, the cells were seeded into 96-well culture plates at 10^4 cells/mL. Two hr later, 1.0 mM of SIN-1, 1.2 mM of SNP, or 1.2 mM of pyrogallol was added to all of the wells for 24 hr, followed by treating the test wells with delphinidin for 24 hr.

MTT assay Cell viability was determined using a MTT colorimetric assay (19). Fifty μL of MTT (1 mg/mL) solution was added to the each well. After incubation for 4 hr at 37°C, the MTT solution was removed from the medium. The resultant formazan crystals in the renal cells were solubilized with 100 μL of dimethyl sulfoxide (DMSO). The absorbance of each well was then read at 540 nm using a microplate reader (Model 3550-UV; Bio-Rad).

Statistical analysis The results for each group were expressed as mean±standard deviation (SD) values. The data were analyzed by the one-way analysis of variance (ANOVA) using SAS (SAS Institute Inc., Cary, NC, USA). To compare differences between control and sample treated groups. Significant differences among the groups were determined at $p<0.05$.

Results and Discussion

Radical scavenging activity Because the negative correlation between antioxidative status and various pathological conditions is clearly recognized, there has been much interest focused on the safe and effective therapeutic agents for oxidative stress-related diseases (6,7). Biologically active compounds found in vegetables, may play roles in reducing the risk of degenerative diseases caused by oxidative stress (20). Our previous study demonstrated that among different vegetables, eggplant, with high polyphenol content, exerted the strongest antioxidative effects within *in vitro* and cellular systems (9). In addition, much evidence suggests that the biological effects of eggplant are attributed to its antocyanins, especially delphinidin that is the main anthocyanin in eggplant. Anthocyanins, the reddish-blue pigments present in a variety of plant tissues, play a significant role in the antioxidant properties of vegetables and fruits. In particular, delphinidin is contained especially in the peel of eggplant and the extract from eggplant peel contains 0.43% delphinidin (21). However, the protective activity of delphinidin from free radical-induced oxidative stress under cellular system has not been studied.

Table 1 shows the NO scavenging activity of delphinidin, indicating that it effectively scavenged NO in a concentration-dependent manner. Although delphinidin's NO scavenging effect was lower than 10% at 5 μg/mL concentration, it exerted strong NO scavenging effect at the 25 μg/mL

Table 1. Protective effect of delphinidin from NO scavenging activity

Concentration (μg/mL)	NO scavenging effect[1)] (%)
2.5	1.3±2.2[d]
5	5.5±1.9[c]
10	32.8±3.3[b]
25	95.0±4.3[a]

[1)]Values are mean±SD; [a-d]Means with different letters are significantly different ($p<0.05$) by Duncan's multiple-range test.

Table 2. Protective effect of delphinidin from O_2^- scavenging activity

Concentration (μg/mL)	O_2^- scavenging effect[1)] (%)
2.5	35.6±1.6[e]
5	48.5±2.7[d]
10	60.7±1.8[c]
25	81.4±0.8[b]
50	89.1±0.2[a]
100	94.1±4.5[a]

[1)]Values are mean±SD; [a-e]Means with different letters are significantly different ($p<0.05$) by Duncan's multiple-range test.

Table 3. Protective effect of delphinidin from ·OH scavenging activity

Concentration (μg/mL)	·OH scavenging effect[1)] (%)
2.5	75.3±0.3[e]
5	81.9±0.6[d]
10	82.0±0.2[d]
25	90.0±0.5[c]
50	92.0±0.4[b]
100	99.1±0.6[a]

[1)]Values are mean±SD; [a-e]Means with different letters are significantly different (p<0.05) by Duncan's multiple-range test.

concentration (95.0±4.3%). As shown in Table 2, delphinidin showed strong and significant O_2^- scavenging effects in a concentration-dependent manner. At the low concentration of 5 mg/mL, it scavenged approximately 50% of O_2^-. In addition, when delphinidin was treated at 10 and 100 μg/mL, its O_2^- scavenging activities were 60.7 ±1.8 and 94.1±4.5%, respectively. Table 3 shows delphinidin's *in vitro* protective effects against ·OH. When compared with its NO and O_2^- scavenging activities, the effects for ·OH were markedly strong. At the low concentration of 5 μg/mL, delphinidin exerted greater than 80% scavenging activity against ·OH. Moreover, at 50 and 100 μg/mL, it scavenged ·OH more than 92.0±0.4 and 99.1±0.6%, respectively.

Oxygen free radicals are products of normal cellular metabolism and are well recognized for playing dual roles as both deleterious and beneficial species, since they can either be harmful or beneficial to living systems (1,22). Their beneficial effects occur at low/moderate concentrations and involve physiological roles in cellular responses. On the other hand, their harmful effects are due to their overproduction, which leads to oxidative stress and contributes to disease pathology. Many types of oxygen free radicals are produced through the cellular reactions, and O_2^-, ·OH, and NO account for the majority. O_2^- and NO are generated under normal metabolism, and have biological and beneficial roles in the body at low levels; when in excess, however, they can cause damage. Unlike O_2^- and NO, ·OH always plays a harmful role. ·OH is the most powerful and deadly oxygen free radical, providing no benefit to the body, even in small amounts. It is highly reactive, destroying everything in its path by altering DNA and contributing to plaque formation in blood vessels. Based on this evidence, it is well established that the excessive production of ROS, including O_2^-, ·OH, and NO, plays a critical role in the development of various diseases (1,23). ROS may induce tissue damage either directly through interacting with and destroying cellular proteins, lipids, and DNA, or indirectly by affecting normal cellular signaling pathways and gene regulation. Superoxide anion, arising either through metabolic processes or ischemia-reperfusion, is considered the primary ROS and can further interact with other molecules including NO. The *in vitro* results of this study demonstrate that delphinidin exerted strong radical scavenging effects against NO, O_2^-, and ·OH in concentration-dependent manners. In particular, the highest scavenging activity by delphinidin was against ·OH, the most reactive and toxic among the radicals.

Table 4. Protective effect of delphinidin under LLC-PK1 cells treated with SIN-1

Material		Cell viability[1)] (%)
SIN-1-treated control		44.8±1.8
Delphinidin (μg/mL)	2.5	60.4±1.0[e]
	5	66.6±1.9[e]
	10	70.0±0.8[d]
	25	72.0±1.4[c]
	50	75.6±1.2[b]
	100	81.8±0.6[a]
Normal		100.0±1.0

[1)]Values are mean±SD; [a-e]Means with different letters are significantly different (p<0.05) by Duncan's multiple-range test.

Protective effect against LLC-PK$_1$ cellular damage Protective effects of delphinidin against $ONOO^-$ under cellular system are shown in Table 4. While the treatment of well-known $ONOO^-$ generator, SIN-1, reduced LLC-PK1 cell viability by 44.8±1.8%, the addition of delphinidin significantly recovered LLC-PK1 cell viability in a dose-dependent manner, in which the treatments of 10 and 100 μg/mL of delphinidin increased cell viability to 70.0±0.8 and 81.8±0.6%, respectively.

The reaction of O_2^- and NO produces the more toxic radical, peroxynitrite ($ONOO^-$). $ONOO^-$ has been suggested as an important pathogenic causative agent of cellular damage; the pathological effects of $ONOO^-$ and ·OH, the decomposition product of $ONOO^-$, are attributed to antioxidant depletion, the alterations of protein structure and function by tyrosine nitration, and oxidative damage (24-26). Therefore, RNS are considered potential targets for therapeutic intervention in the prevention and treatment of oxidative stress-related diseases. The development of agents that possess protective activity against $ONOO^-$ offers great potential for treating oxidative stress-related pathological conditions that play crucial roles in the development of tissue injury.

In this study, we evaluated cytotoxicity by employing a cellular oxidative system using LLC-PK1 cells, which have proximal tubules known to be susceptible to ischemic renal failure (27). Because SIN-1 has been used as an experimental model for the simultaneous generation of NO and O_2^- in chemical and biological systems (28), we evaluated the cytoprotective effects of delphinidin against SIN-1 using cultured epithelial cell line, LLC-PK1. The excessive generation of $ONOO^-$, and its related damaging effects, ultimately induce the cellular losses (29,30). Our present investigation showed that LLC-PK$_1$ cell viability was reduced by SIN-1-induced oxidative damage. The cells were cultured with various concentrations of delphinidin after oxidative stress was induced by SIN-1. The cells that were exposed to SIN-1 showed a marked decrease in viability, suggesting that the radicals generated by SIN-1 caused cellular injury. Delphinidin scavenged SIN-1-generated radicals in a cell model, leading to the elevation of LLC-PK1 cell viability that had been damaged by oxidative stress. The detailed mechanisms causing biomolecule modifications by $ONOO^-$ are still not completely understood, but there is little doubt of the cytotoxicity

Table 5. Protective effect of delphinidin under LLC-PK1 cells treated with SNP

Material		Cell viability[1)] (%)
SNP-treated control		30.0±0.7
Delphinidin (μg/mL)	2.5	50.5±2.3[f]
	5	61.3±1.0[e]
	10	69.3±0.8[d]
	25	74.3±1.8[c]
	50	78.0±0.9[b]
	100	81.3±1.2[a]
Normal		100.0±1.0

[1)]Values are mean±SD; [a-f]Means with different letters are significantly different ($p<0.05$) by Duncan's multiple-range test.

Table 6. Protective effect of delphinidin under LLC-PK1 cells treated with pyrogallol

Material		Cell viability[1)] (%)
Pyrogallol-treated control		33.8±1.1
Delphinidin (μg/mL)	2.5	41.4±0.9[f]
	5	50.3±1.5[e]
	10	54.4±1.7[d]
	25	59.8±0.8[c]
	50	65.6±2.0[b]
	100	75.8±2.6[a]
Normal		100.0±1.0

[1)]Values are mean±SD; [a-f]Means with different letters are significantly different ($p<0.05$) by Duncan's multiple-range test.

associated with $ONOO^-$ formation at physiological pH (29). The contribution of $ONOO^-$ has been ascribed to induction of DNA damage, inhibition of DNA repair, and promotion of cell death due to either apoptotic or necrotic responses (30-32). Therefore, much attention has been given to preventing the formation of these radicals and to scavenging them. To clarify the protective effects of delphinidn against free radical-induced oxidative damage, we attempted to determine whether it could protect susceptible renal cells from oxidative injury generated by free radical donors.

Protective effects of delphinidin against cellular damage induced by SNP, a well-known NO generator, in LLC-PK1 cells are shown in Table 5. SNP treatment at 1.2 mM reduced LLC-PK1 cell viability to 30.0±0.7% as compared with cells not treated with SNP. However, the addition of delphinidin significantly recovered LLC-PK1 cell viability in a dose-dependent manner, in which 5 and 50 μg/mL of delphinidin, treated after SNP-induced oxidative stress, increased cell viability to 61.3±1.0 and 78.0±0.9%, respectively. As shown in Table 6, pyrogallol treatment reduced LLC-PK1 cell viability to 33.8±1.1% as compared to 100% for normal cell viability. Delphinidin recovered the cellular damage induced by pyrogallol dose-dependently, and at the concentrations of 5 and 50 μg/mL, cell viability increased to 50.3±1.5 and 65.6±2.0%, respectively. To elucidate the protective mechanisms of delphinidin against $ONOO^-$, we used SNP and pyrogallol under the cellular system to generate NO and O_2^-, respectively, the precursors of $ONOO^-$. The treatments of SNP and pyrogallol led to the significant decreases in cell viability, indicating oxidative stress by these NO and O_2^- generators. However, the addition of delphinidin significantly elevated the cell viability in a concentration-dependent manner. These results suggest that delphinidin can ameliorate oxidative stress induced by NO and O_2^-. Taken together, we could propose the several feasible mechanisms for delphinidin's protective activity against $ONOO^-$ under cellular system. Firstly, delphinidin scavenges NO and O_2^-, which are precursors of $ONOO^{-\cdot}$, secondly, $ONOO^-$ itself may be scavenged; thirdly, delphinidin scavenges ·OH and nitrogen dioxide, which are known to be decomposition products of ONOOH. From the present results, we could confirm the strong antioxidative activity of delphinidin. Our previous result showed that the antioxidative effect of delphinidin was as strong as that of vitamin C; IC_{50} value against 1,1-diphenyl-2-picrylhydrazyl of delphinidin was 6.59 μg/mL, while that of vitamin C was 1.10 μg/ mL (data not shown).

For better understanding the role of anthocyanins as antioxidants, structure-antioxidative activity relationship has been suggested (33-35). The number of OH substituent in the B ring primarily determines the reactivity of anthocyanins toward free radicals. In addition, *O*-methylation at 4'-OH position is critical in reducing the scavenging activity against free radical. This is probably because the methoxyl groups in the B ring increased the stability of the resulting phenoxyl radical. The more OH substituents and the lower *O*-methylation in the B ring, the higher antoxidative effect. Delphinidin has 3 free OH group in the B ring without *O*-methylation. Based on these evidences, the protective activity of delphinidin against oxidative stress was mainly responsible for higher hydroxylation and lower methoxylation in the B ring. To support the information on the consumption amount of eggplant for the protective effect of delphinidin against oxidative stress, the further study on the confirmation of its beneficial role under *in vivo* and the related protective mechanisms of delphinidin from oxidative stress has to be carried out.

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References

1. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mawur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39: 44-84 (2007)
2. Yu BP, Chung HY. Oxidative stress and vascular aging. Diabetes Res. Clin. Pr. 54: S73-S80 (2001)
3. Bokov A, Chaudhuri A, Richardson A. The role of oxidative damage and stress in aging. Mech. Ageing Dev. 125: 811-826 (2004)
4. Scott JA, King GL. Oxidative stress and antioxidant treatment in diabetes. Ann. NY Acad. Sci. 1031: 204-213 (2004)
5. Gibson GE, Huang HM. Oxidative stress in Alzheimer's disease. Neurobiol. Aging 26: 575-578 (2005)
6. Meydani M, Lipman RD, Han SN, Wu D, Beharka A, Martin KR, Bronson R, Cao G, Smith D, Meydani SN. The effect of long-term dietary supplementation with antioxidants. Ann. NY Acad. Sci. 854:

352-360 (1998)
7. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. P. Natl. Acad. Sci. USA 90: 7915-7922 (1993)
8. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention. J. Am. Diet. Assoc. 96: 1027-1039 (1996)
9. Lee YA, Kim HY, Cho EJ. Comparison of methanol extracts from vegetables on antioxidative effect under *in vitro* and cell system. J. Food Sci. Nutr. 34: 1151-1156 (2005)
10. Noda Y, Kneyuki T, Igarashi K, Mori A, Packer L. Antioxidant activity of nasunin, an anthocyanin in eggplant peels. Toxicology 148: 119-123 (2000)
11. Chang HJ, Choi EH, Chun HS. Quantitative structure-activity relationship (QSAR) of antioxidative anthocyanidins and their glycosides. Food Sci. Biotechnol. 17: 501-507 (2008)
12. Kahkonen MP, Heinonen M. Antioxidant activity of anthocyanins and their aglycons. J. Agr. Food Chem. 51: 628-633 (2003)
13. Hou DX. Potential mechanisms of cancer chemoprevention by anthocyanins. Curr. Mol. Med. 3: 149-159 (2003)
14. Sreejayan N, Rao MN. Nitric oxide scavenging by curcuminoids. J. Pharm. Pharmacol. 49: 105-107 (1997)
15. Ewing JF, Janero DR. Microplate superoxide dismutase assay employing a nonenzymatic superoxide generator. Anal. Biochem. 232: 243-248 (1995)
16. Gutteridge JM. Ferrous-salt-promoted damage to deoxyribose and benzoate. The increased effectiveness of hydroxyl-radical scavengers in the presence of EDTA. Biochem. J. 243: 709-714 (1987)
17. Yokozawa T, Ishida A, Kashiwada Y, Cho EJ, Kim HY, Ikeshiro Y. Coptidis Rhizoma: Protective effects against peroxynitrite-induced oxidative damage and elucidation of its active components. J. Pharm. Pharmacol. 56: 547-556 (2004)
18. Yokozawa T, Cho EJ, Nakagawa T, Terasawa K, Takeuchi S. Inhibitory effect of green tea tannin on free radical-induced injury to the renal epithelial cell line, LLC-PK1. Pharm. Pharmacol. Commun. 6: 521-526 (2006)
19. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65: 55-63 (1993)
20. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Res. 22: 375-383 (1995)
21. Azevedo L, Alves de Lima PL, Gomes JC, Stringheta PC, Ribeiro DA, Salvadori DMF. Differential response related to genotoxicity between eggplant (*Solanum melanogena*) skin aqueous extract and its main purified anthocyanin (delphinidin) *in vivo*. Food Chem. Toxicol. 45: 852-858 (2007)
22. Darley-Usmar V, Wiseman H, Halliwell B. Nitric oxide and oxygen radicals: A question of balance. FEBS Lett. 369: 131-135 (1995)
23. Halliwell B. Oxidants and human disease: Some new concepts. FASEB J. 1: 358-364 (1987)
24. Fukuyama N, Takebayashi Y, Hida M, Ishida H, Ichimori K, Nakazawa H. Clinical evidence of peroxynitrite formation in chronic renal failure patients with septic shock. Free Radical Bio. Med. 22: 771-774 (1997)
25. Ischiropoulos H. Biological tyrosine nitration: A pathophysiological function of nitric oxide and reactive oxygen species. Arch. Biochem. Biophys. 356: 1-11 (1998)
26. Nakazawa H, Fukuyama N, Takizawa S, Tsuji C, Yoshitake M, Ishida H. Nitrotyrosine formation and its role in various pathological conditions. Free Radical Res. 33: 771-784 (2000)
27. Schena FP, Grandaliano G, Gesualdo L. The role of tubular cells in the progression of renal damage: Guilty or innocent? Renal Failure 23: 589-596 (2001)
28. Lipton SA, Choi YB, Pan ZH, Lei SZ, Chen HSV, Sucher NJ, Loscalzo J, Singel DJ, Stamler JS. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and relateed nitroso-compounds. Nature 364: 626-632 (1993)
29. Zhu L, Gunn C, Beckman JS. Bactericidal activity of peroxynitrite. Arch. Biochem. Biophys. 298: 452-457 (1992)
30. Patel RP, McAndrew J, Sellak H, White CR, Jo H, Freeman BA, Darley-Usmar VM. Biological aspects of reactive nitrogen species. Biochim. Biophys. Acta 1411: 385-400 (1999)
31. Sandoval M, Zhang XJ, Liu X, Mannick EE, Clark DA, Miller MJ. Peroxynitrite-induced apoptosis in T84 and RAW 264.7 cells: Attenuation by L-ascorbic acid. Free Radical Bio. Med. 22: 489-495 (1997)
32. Doulias PT, Barbouti A, Galaris D, Ischiropoulos H. SIN-1-induced DNA damage in isolated human peripheral blood lymphocytes as assessed by single cell gel electrophoresis (comet assay). Free Radical Bio. Med. 30: 679-685 (2001)
33. Rahman MM, Ichiyanagi T, Komiyama T, Hatano Y, Konishi T. Superoxide radical- and peroxynitrite scavenging activity of anthocyanins; structure-activity relationship and their synergism. Free Radical Res. 40: 993-1002 (2006)
34. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Bio. Med. 20: 933-956 (1996)
35. Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. J. Agr. Food Chem. 45: 304-309 (1997)