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# Antiradical and Chelating Effects in Flavonoid Protection against Silica-Induced Cell Injury

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**Quercetin, dihydroquercetin, and rutin are capable of scavenging superoxide anion (rate constants of the reaction with superoxide at pH 10 were 1.7 x $10^5$, 1.5 x $10^5$, and 0.5 x $10^5$ $M^{-1}$ $s^{-1}$ respectively). At the same time rutin and quercetin but not dihydroquercetin are iron ion chelators. These substances were used to elucidate the role of radical scavenging and iron chelating in flavonoid protection against asbestos-induced oxidative cellular injury. Exposure of rat peritoneal macrophages to chrysotile asbestos fibers resulted in "frustrated" phagocytosis, cell injury, and a LDH release. Quercetin, dihydroquercetin, and rutin were effective in protecting the phagocytic cells against injury caused by asbestos. Moreover, these flavonoids exhibited cellular protection in the same order of effectiveness as that observed for the quenching of superoxide: quercetin > dihydroquercetin > rutin. Exposure of human red blood cells to asbestos fibers also caused progressive cell injury and lysis. Quercetin and rutin protected the red cells (quercetin > rutin), whereas dihydroquercetin was ineffective in preventing asbestos-induced hemolysis. The protective ability of quercetin and rutin may be related to their iron-chelating activity. Due to this these flavonoids can be located on asbestos surface in sites of initiation of free radical reactions and their antiradical moieties can scavenge reactive oxygen species immediately after the appearance. Thus, both antiradical and chelating effects appear to be involved in the flavonoid protection against silica-induced cell injury.** 

*Key Words:* **flavonoids; oxidative injury; asbestos; free radicals; rutin; quercetin; dihydroquercetin.**

Several lines of evidence strongly suggest that reactive oxygen species (ROS)[2] and intermediates which appear during free radical reactions mediate asbestos-induced pathological processes such as asbestosis, malignant mesothelioma, or bronchogenic carcinoma. At present two mechanisms by which asbestos and other mineral fibers can initiate free radical reactions are mainly discussed. The first is catalysis of electron transfer reactions and then generation of hydroxyl radicals through mechanism of Fenton chemistry in cell-free aqueous solutions (1, 2). This ability is due to the chemical properties of mineral fibers and especially their iron content and probably is the predominant cause of cellular oxidant stress induced by the fibers in epithelial (3) and red blood cells (4). The other mechanism operates only with phagocytic cells and involves release of ROS and other oxidants by NADPH-oxidase during so-called respiratory burst in response to "frustrated" phagocytosis of mineral particles (5-8). The overproduction of ROS leads to polymer degradation, dysfunction, death, or neoplastic transformation of immune and epithelial cells. In previous work we found that rutin and quercetin, widespread plant pigments possessing vitamin P activity, were effective in inhibiting superoxide production and respiratory burst in macrophages and completely protected the cells against injury caused by chrysotile asbestos (9). The protective effect of these compounds can be due to their ability to scavenge ROS (9-13). At the same time rutin and quercetin are capable of chelating iron ions (10) which play a vital role in the initiation of free radical reactions, including oxidative injury of cells by asbestos (2). The present work was undertaken in an attempt to clarify the role of antiradical and chelating

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[2] Abbreviations used: ROS, reactive oxygen species; BHT, butylated hydroxytoluene; TBA, 2-thiobarbituric acid; TBARS, TBA-reactive substances; LDH, lactate dehydrogenase; NBT, nitroblue tetrazolium; TMEDA, *N,N,N′,N′*-tetramethylethylenediamine.

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properties in the protection of rat peritoneal macrophages and human red blood cells by flavonoids against asbestos-induced injury.

## MATERIALS AND METHODS

*Chemicals.* Quercetin, dihydroquercetin, rutin, butylated hydroxytoluene (BHT), ethylenediaminetetraacetic acid (EDTA), deferoxamine, riboflavin, 2-thiobarbituric acid (TBA), superoxide dismutase (CuZnSOD) were from Sigma (U.S.A.). a-Tocopherol was from Henkel Corp. (U.S.A.). Nitroblue tetrazolium (NBT) and NADH were from Reanal (Hungary). Asbestos fibers (5-10 /Am) were natural chrysotile asbestos (Tuva, Russia).

*Macrophages oxidative injury assay.* Peritoneal macrophages were isolated from Wistar rats by the slightly modified method described by Korkina *et al.* (14) and washed with an isotonic phosphate buffer (pH 7.3). After being washed the cells were resuspended in the same buffer and used immediately. Two milliliters of macrophage suspension (8 X $10^6$ cells/ml) in the isotonic phosphate buffer (pH 7.3) was preincubated at 37°C for 2 min. At zero time 50 ml of water suspension of asbestos fibers was added (the final concentration was 2 mg/ml). The mixture was incubated at 37°C under continual stirring for 20 min and samples were taken for determination of TBA-reactive substances (TEARS) formation and lactate dehydrogenase (LDH) release. Flavonoids were added as a dimethyl sulfoxide solution; BHT and a-tocopherol were added as an ethanol solution (the final concentration of dimethyl sulfoxide and ethanol not exceeding 2% of the final volume).

*TEARS formation assay.* A portion (0.5 ml) of the incubation mixture containing macrophages was mixed with 0.5 ml 30% (w/v) trichloroacetic acid and 2.5 ml 0.5% (w/v) solution of 2-thiobarbituric acid, and the mixture was heated at 100°C for 15 min. After centrifugation of precipitated proteins, the TEARS content was determined by measuring the absorbance at 532 nm. An extinction coefficient of 1.56 x $10^5$ $M^{-1}$ $cm^{-1}$ was used for quantifying TEARS.

*LDH release assay.* A portion (0.5 ml) of incubation mixture containing macrophages was centrifuged at 1000 rpm for 5 min and 0.2 ml of supernatant was measured for LDH content. The assay was based on the measurement of the rate of NADH oxidation in the presence of pyruvate at 30°C. LDH values were expressed as changes in absorbance at 340 nm for 2 min.

*Red cells injury assay.* Standard healthy donor blood samples (sodium citrate was used as anticoagulant) were obtained from a hospital blood bank and used within 1 week. Erythrocytes were separated from plasma after centrifugation at 1000 rpm for 5 min and washed three times with a 0.9% saline solution. After being washed cells were resuspended in a fourfold volume of the same solution and used immediately. Red cell injury was carried out in 0.9% saline solution at 37°C. The final concentration of erythrocytes in the reaction mixture was 2%; asbestos fibers were 2 mg/ml. Flavonoids were added as a dimethyl sulfoxide solution (the final concentration of dimethyl sulfoxide not exceeding 2% of the final volume). Lysis of erythrocytes in the presence of asbestos was followed by changing turbidity at 740 nm.

*Superoxide-driven reduction of nitroblue tetrazolium by photochemically reduced riboflavin.* Reduction of NET was carried out at room temperature (22°C) under fluorescent lighting (20 W, 20 cm). The standard incubation mixture contained 6 $\mu$M riboflavin, 0.8 mM of *N,N,N¹,N¹*-tetramethylethylenediamine (TMEDA), 0.08 mM EDTA in 0.016 M phosphate buffer (pH 7.8), and 85 p,M NET (15). Flavonoids were added as a dimethyl sulfoxide solution (the final concentration of dimethyl sulfoxide not exceeding 2% of the final volume). After 5 min incubation, the reaction was stopped by switching off the light and the addition of 0.5 ml 10% (w/v) KI solution and absorbance was measured at 550 nm.

**TABLE I**

Concentrations Inhibiting the Superoxide-Driven Reduction of NET by 50% and Rate Constants of Selected Flavonoids with Superoxide (at pH 10.0)

| Flavonoids (fl) | $I_{50}$ values (/J.M) | Rate constants", $10^5$ $M^{-1}$ $s^{-1}$ |
|---|---|---|
| Quercetin | 4.6 | 1.7 |
| Rutin | 15.5 | 0.50 |
| Dihydroquercetin | 5.4 | 1.5 |
| SOD | 0.0004 | |

$K_{(fl)} \sim K_{(SOD)} \cdot I_{50\ (SOD)}/I_{50\ (fl)}$; $K_{(SOD)}$ — 2 X 10 M S

## RESULTS

*Antiradical properties of flavonoids.* Interaction of flavonoids with superoxide anion was quantitatively evaluated using the illumination of riboflavin in the presence of tetramethylethylenediamine (15). Upon reoxidation in air, photochemically reduced flavin generated $O'_2$ . Radical scavenging efficiencies of flavonoids toward superoxide were determined from competition kinetics with SOD as the reference scavenger using NET as the superoxide detector. The concentrations that inhibited superoxide-driven reduction of NET by 50% ($I_{50}$ values) and rate constants of flavonoids with superoxide are shown in Table I. It should be pointed out that the rate constant of quercetin in our study (1.7 X $10^5$ $M^{-1}$ $s^{-1}$) is very similar to the value that was received with pulse radiolysis experiments by Bors *et al.* (0.9 X $10^5$ $M^{-1}$ $s^{-1}$) (12).

*Chelating properties of flavonoids.* The ability of rutin to form complexes with iron ions was documented (10). In this work chelating properties of quercetin, dihydroquercetin, and rutin were compared using the method of competitive replacement. The addition of $Fe_2(SO_4)_3$ to rutin or quercetin in 0.9% saline solution resulted in the shift of maximum at 360 nm (rutin) or at 370 nm (quercetin) to the long-wave region (Figs. 1 and 2, trace 2). In the case of the iron-rutin complex the absorbance alterations were completely reversed by the addition of EDTA at a concentration two times more than rutin (Fig. 1, traces 3 and 4). The iron-quercetin interaction was stronger and the addition of EDTA at a concentration two times more than quercetin only slowly turned the absorbance alterations (Fig. 2, traces 3 and 4). The addition of $Fe_2(SO_4)_3$ to dihydroquercetin did not result in substantial absorbance alterations, and to reveal chelating properties of this flavonoid the competition for $Fe^{3+}$ between dihydroquercetin and rutin was spectrophotometrically evaluated. It was found that dihydroquercetin did not compete with rutin for ferric ions since no substantial alteration of the absorption spectrum of iron-rutin complex in the presence of dihydroquercetin was found

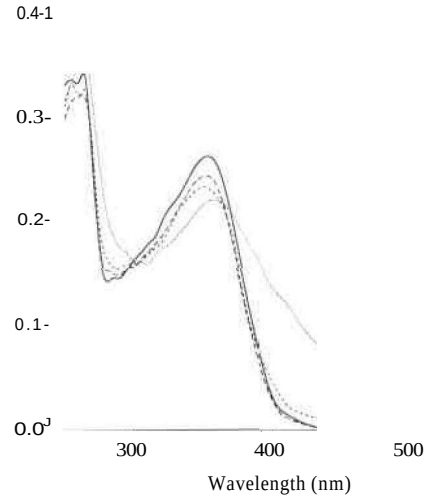


FIG. 1. Absorption spectrum of rutin (15 juM) in 0.9% saline solution (trace 1); differential spectrum of rutin-ferric ion complex against the solution with ferric ion (trace 2); differential spectrum of rutin-ferric ion complex immediately after addition EDTA at concentration two times more than rutin against the solution with ferric ion and EDTA (trace 3); the same as for trace 3 after 15 min incubation (trace 4).

(Fig. 3, traces 3 and 4). At the same time dihydroquercetin was found to be capable of forming the complexes with $SiO_3^{2-}$. Addition of this anion to dihydroquercetin resulted in the shift of a maximum at 290 nm to the long-wave region (Fig. 4, trace 2). The similar shift of the maximum at 290 nm was found after the addition of chrysotile asbestos to dihydroquercetin (data not shown).

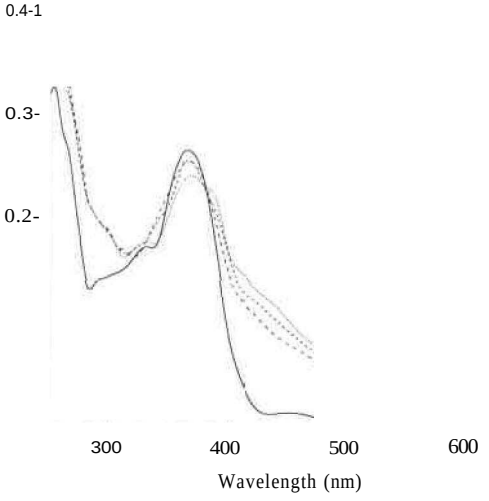


FIG. 2. Absorption spectrum of quercetin (15 $\mu$M) in 0.9% saline solution (trace 1); differential spectrum of quercetin-ferric ion complex against the solution with ferric ion (trace 2); differential spectrum of quercetin—ferric ion complex immediately after addition of EDTA at a concentration two times more than quercetin against the solution with ferric ion and EDTA (trace 3); the same as for trace 3 after 15 min incubation (trace 4).

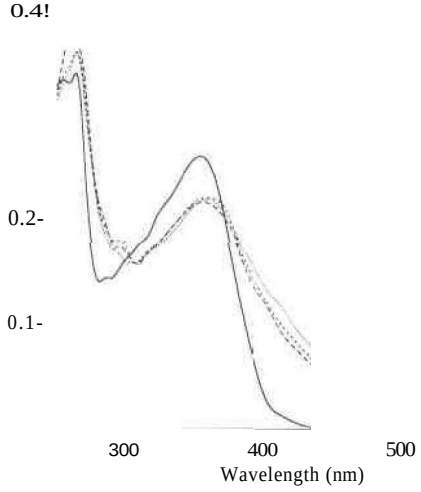


FIG. 3. Absorption spectrum of rutin (15 ju,M) in 0.9% saline solution (trace 1); differential spectrum of rutin-ferric ion complex against the solution with ferric ion (trace 2); differential spectrum of rutin-ferric ion complex after the addition of dihydroquercetin at concentration two times more than rutin against the solution with ferric ion and dihydroquercetin (trace 3); the same as for trace 3 except that dihydroquercetin was added to the ferric solution before rutin (trace 4).

*Influence of flavonoids on cytotoxic effects of chrysotile asbestos.* Exposure of rat peritoneal macrophages to asbestos fibers increased the TEARS content and resulted in the cell injury evaluated in terms of LDH release. Rutin, dihydroquercetin, and quercetin inhibited the formation of TEARS and were effective in protecting the phagocytic cells against asbestos-induced injury (Table II). The protective effect of these flavonoids against asbestos-induced injury evaluated

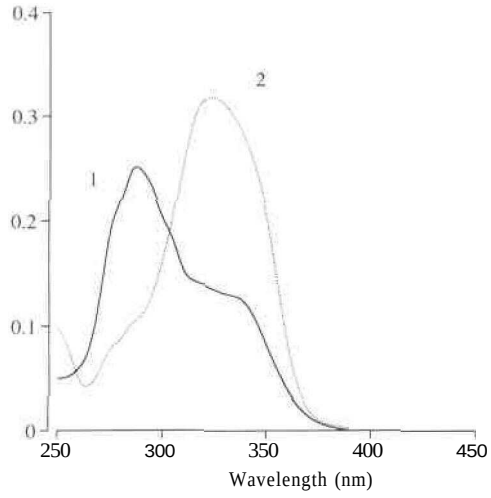


FIG. 4. Absorption spectrum of 15 /u,M dihydroquercetin (trace 1); differential spectrum of *dihydroquercetin-*$SiO_3^{2-}$ ion complex against

**TABLE II**

Effects of Selected Compounds on TBAES Formation and LDH Release in Rat Peritoneal Macrophages Following Exposure to Asbestos

| Additive | TEARS formation (nmol/ml) | LDH release (% to control)" |
|---|---|---|
| Without inhibitor" | 0.78 ± 0.05 | 95 ± 4 |
| Without inhibitor" (2% DMSO) | 0.79 ± 0.06 | 9 7 + 4 |
| Without inhibitor" (2% ethanol) | 0.76 ± 0.08 | 95 ± 6 |
| + 30 μM quercetin | 0.23 ± 0.12* | 56 ± 18* |
| + 60 /xM quercetin | 0.08 ± 0.10** | 36 + 12** |
| + 300 μM quercetin | 0 | 11 + 21** |
| + 120 juM rutin | 0.35 ± 0.14* | 67 + 11* |
| + 240 μM rutin | 0.28 ± 0.13* | 52 ± 15* |
| + 1200 μM rutin | 0.08 ± 0.12* | 11 ± 12** |
| + 120 μM dihydroquercetin | 0.42 ± 0.10* | 57 ± 7* |
| + 240 \|U,M dihydroquercetin | 0.33 ± 0.08* | 37 ± 5** |
| + 1200 /xM dihydroquercetin | 0.29 ± 0.06* | 0 |
| + 1 mM a-tocopherol | 0.05 ± 0.01** | 92 ± 6 |
| + 400 /jVl BHT | 0.11 ± 0.01** | 95 ± 8 |
| + 240 \|iiM deferoxamine | ND | 88 ± 8 |
| + 240 μM EDTA | ND | 96 ± 8 |

*Note.* ND, not determined. Results are the mean ± SD *(n* = 4-6).

" In the control experiments, macrophages were incubated at 37°C in isotonic phosphate buffer (pH 7.3) for 20 min without asbestos. In samples without inhibitor, only the corresponding solvent was added.

*$P < 0.01$.

** $P < 0.001$ compared to samples without inhibitor.

in terms of LDH release was classified as follows: quercetin > dihydroquercetin > rutin, respectively. BHT and a-tocopherol, which were used as reference scavengers of lipid radicals completely inhibited the formation of TEARS but did not influence the release of LDH (Table II). EDTA and deferoxamine, which were used as reference chelator agents, had no effect on the cytotoxic consequences of exposure of rat peritoneal macrophages to chrysotile asbestos (Table II).

Incubation of 2% suspension of human red blood cells with 2 mg/ml asbestos fibers in the 0.9% saline solution at 37°C caused progressive cell injury and lysis. Quercetin and rutin strongly protected the red cells, whereas dihydroquercetin, a-tocopherol, EDTA, and deferoxamine were ineffective in preventing asbestos-induced hemolysis (Table III). In these experiments the addition of additives was as follows: asbestos, drugs, and red blood cells. In some experiments asbestos fibers were precipitated by centrifugation after the addition of drugs and the precipitates were resuspended in new portions of the 0.9% saline solution and then mixed with red blood cells. It is important that in this case quercetin and rutin also were effective in preventing asbestos-induced hemolysis (Table III).

To evaluate the adsorption of flavonoids by asbestos, drug solutions (15 μM) were incubated for 1 min with asbestos and then the fibers were precipitated and removed. The amount of flavonoids was checked spectrophotometrically before and after the treatment. It was found that 1 mg of chrysotile asbestos adsorbed approximately 20, 12, and 9 nmol of quercetin, dihydroquercetin, and rutin, respectively.

The influence of iron chelator EDTA on efficacy of quercetin and rutin in preventing asbestos-induced hemolysis was also studied. It was found that the addition of EDTA to asbestos fibers 1 min before the flavonoids resulted in a decrease of the quercetin and rutin protective efficacy near by 60 and 70%, respectively, if flavonoids and EDTA were used in equal concentrations, and quercetin and rutin were completely ineffective if EDTA was added to asbestos in a concentration four times higher than flavonoids (Table IV).

## DISCUSSION

Asbestos-induced injury of rat peritoneal macrophages is related to NADPH-oxidase activation and superoxide production during respiratory burst in response to "frustrated" phagocytosis of mineral particles (5-8). Therefore, protection against asbestos-induced macrophages injury by flavonoids may be due to their influence on superoxide mediated free radical reactions. Flavonoids can directly inhibit the mechanism of

**TABLE III**

Effects of Selected Compounds on Asbestos-Induced Hemolysis

| Additive | Hemolysis (% to control)" |
|---|---|
| Without inhibitor | 69 ± 5 |
| Without inhibitor (2% DMSO) | 66 ±8 |
| + 40 μM quercetin | 31 \| 3* |
| + 80 /j,M quercetin | 23 \| 9* |
| + 160 juM quercetin | Q \| 3** |
| + 80 juM rutin | 42 \| 3* |
| + 160 \|u,M rutin | 37 \| 6* |
| + 320 μM rutin | 32 \| 6* |
| + 320 μM dihydroquercetin | 69 \| 5 |
| + 1600 /j,M dihydroquercetin | 65 \| 6 |
| + 200 μM EDTA | 63 ± 6 |
| + 200 μM deferoxamine | 68 ± 5 |
| + 160 jitM quercetin[6] | 8 ± 3** |
| + 320 M,M rutin[b] | 34 ± 9* |
| + 320 μM dihydroquercetin[6] | 66 ± 8 |
| + 200 juM EDTA[6] | 64 ± 5 |

*Note.* Results are the mean ± SD (re = 4—6).

" In the control experiments, human red blood cells were incubated at 37°C in 0.9% saline solution for 20 min without asbestos.

[6] Asbestos fibers were precipitated by centrifugation after 1-min incubation with drugs and the precipitates were resuspended in new portions of the 0.9% saline solution and then mixed with red blood cells.

*$P < 0.05$.

** $P < 0.01$ compared to samples without inhibitor.

**TABLE IV**

Effects of EDTA on Protective Efficacy of Quercetin and Rutin against Asbestos-Induced Hemolysis

| Additive | Hemolysis (% to control)[a] |
|---|---|
| Without inhibitor | 66 ± 5 |
| Without inhibitor (2% DMSO) | 63 ± 8 |
| + 200 μM quercetin | 12 ± 4 |
| +200ju,Mrutin | 35 ± 4 |
| + 200 /jM EDTA | 62 ±7 |
| + 200 juM EDTA + 200 *fiM* quercetin | 43 ±5* |
| + 200 juM EDTA + 200 /xM rutin | 55 ±5* |
| + 800 juM EDTA | 61 ±8 |
| + 800 μM EDTA + 200 /nM quercetin | 63 ± 6** |
| + 800 ju,M EDTA + 200 /u,M rutin | 67 ± 8* |

*Note.* Results are the mean ± SD ($n$ = 4-6).

[a] In the control experiments, human red blood cells were incubated at 37°C in 0.9% saline solution for 20 min without asbestos.

*$P < 0.05$.

** $P < 0.01$ compared to samples with flavonoid only.

Fenton chemistry as superoxide scavengers or iron chelators (Table I, Figs. 1 and 2). However, the results of this study indicate that the ability to chelate iron is not essential for protection of macrophages exposed to asbestos since dihydroquercetin which is not iron chelator protected macrophages against asbestos-induced injury, whereas EDTA and deferoxamine were ineffective. Flavonoids were also shown to be capable of inhibiting lipid peroxidation (10). In our experiments, they inhibited the asbestos-induced formation of TEARS. Nevertheless, the ability to inhibit lipid peroxidation is not essential for protection of macrophages exposed to asbestos (Table II). Therefore, the protective effect of flavonoids is more likely attributed to the properties of flavonoids to scavenge superoxide ion. In the present study, this ability was quantitatively evaluated and rate constants of flavonoids with superoxide were determined. It must be mentioned that the protective efficacy of flavonoids against injury caused by asbestos in peritoneal macrophages correlates quite closely with the rate constants.

Asbestos-induced red cell injury is associated with electron transfer reactions and the generation of hydroxyl radicals through the mechanism of Fenton chemistry (4), probably due to iron catalytic centers on surface of mineral fibers (16, 17). The difference in the ability of flavonoids to prevent asbestos-induced hemolysis is quite unexpected because quercetin and dihydroquercetin have similar scavenging properties in relation to superoxide anion (Table I) as well as hydroxyl radicals and lipid radicals (12, 13). We suggested that the difference in protective effect might be related to mechanisms of flavonoid adsorption by asbestos fibers. A comparison of the chelating properties of these flavonoids provides indirect evidence for this suggestion. Indeed, quercetin and rutin are capable of forming complexes with iron ions, whereas dihydroquercetin is not at all or is a very poor iron chelator. Nevertheless, the ability to chelate iron ions is not sufficient to explain fully the protection of red blood cells against asbestos-induced hemolysis by quercetin and rutin since EDTA and deferoxamine were ineffective as red cell protectors under the same conditions (Table III). Based on these data we concluded that there is a difference in localization between asbestos-associated dihydroquercetin, on the one hand, and asbestos-associated quercetin and rutin, on the other hand. Probably, only quercetin and rutin molecules are sorbed on the asbestos surface as a result of iron-chelating interaction. Because of this these flavonoids are located directly in sites of initiation of free radical reactions and their antiradical moieties can scavenge reactive oxygen species immediately after the appearance. Unlike this, dihydroquercetin is sorbed by asbestos as a result of other chelating interaction (e.g., with silicate) and therefore is located rather far from sites of initiation of free radical reactions.

To further support the "site-specific" effects of flavonoids, we attempted to replace quercetin or rutin associated with asbestos in sites of initiation of free radical reactions by another drug. In this way EDTA, which is strong iron chelator but not a radical scavenger, was used. It was found that the addition of EDTA to asbestos fibers before quercetin or rutin resulted in a complete avoidance of flavonoid protection against asbestos-induced hemolysis.

Thus, both antiradical and chelating effects appear to be involved in the flavonoid protection against silica-induced cell injury. The natural flavonoids, quercetin and rutin, were found to possess the most suitable combination of antiradical and chelating properties to prevent the cytotoxic action of asbestos fibers.

## ACKNOWLEDGMENT

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## REFERENCES

1. Weitzman, S. A., and Graceffa, P. (1984) *Arch. Biochem. Biophys.* 228, 373-376.
2. Kamp, D. W., Graceffa, P., Pryor, W. A., and Weitzman, S. A. (1992) *Free Radical Biol. Med.* 12, 293-315.
3. Mossman, B. T., and Landesman, J. M. (1983) *Chest* 83, 50-51.
4. Kennedy, T. P., Dodson, R., Rao, N. V., *et al.* (1989) *Arch. Biochem. Biophys.* 269, 359-364.
5. Hatch, G. E., Gardner, D. E., and Menzel, D. E. (1980) *Environ. Res.* 23, 121-133.
6. Velichkovsky, B. T., Vladimirov, Yu, A., Korkina, L. G., and Suslova, T. B. (1981) *Vestri. Akad. Med. Nauk SSSR* **10,** 45-51. [Russian]

7. Donaldson, K, and Cullen, R. T. (1984) *Br. J. Exp. Pathol.* **65,** 81-90.
8. Hansen, K, and Mossman, B. T. (1987) *Cancer Res.* 47,1681-1686.
9. Kostyuk, V. A., Potapovich, A. I., Speransky, S. D., and Maslova, G. T. (1996) *Free Radical Biol. Med.* 21, 487-493.
10. Afanas'ev, I. B., Dorozhko, A. I., Brodskii, A. V., Kostyuk, V. A., and Potapovitch, A. I. (1989) *Biochem. Pharmacol.* **38,** 1763-1769.
11. Kostyuk, V. A., and Potapovich, A. I. (1989) *Biochem. Int.* **19,** 1117-1124.
12. Bors, W., Heller, W., Michel, C., and Saran, M. (1990) *Methods Enzymol.* **186,** 343-354.
13. Bors, W., Heller, W., Michel, C., and Saran, M. (1992) *in* Free Radicals and Liver Damage (Csomos, G., and Feher, J., Eds.), pp. 77-95, Springer, Berlin.
14. Korkina, L. G., Suslova, T. B., Cheremisina, Z. P., and Velichkovsky, B. T. (1988) *Stud. Biophys.* **126,** 99-104.
15. Beauchamp, C., and Fridovich, I. (1971) *Anal. Biochem.* 44, 276-287.
16. Lund, L. G., and Aust, A. E. (1990) *Arch. Biochem. Biophys.* 278, 60-64.
17. Lund, L. G., and Aust, A. E. (1991) *Arch. Biochem. Biophys.* 287, 91-96.