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A Mixture of Curcumin, Vitamin C, and E Prolongs the Antioxidant Effect to Beyond That of Each Component Alone *in Vivo*

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Abstract This study aimed to investigate the alterations in plasma antioxidant activity after the consumption of a single oral dose of curcumin, vitamin C, and E administered individually or in combination to (i) assess possible synergies or antagonism between the antioxidants and (ii) determine the optimal composition of the antioxidant mixture such that the duration of action is prolonged to beyond that of individual antioxidants. Each antioxidant was administered to male Sprague-Dawley rats, and blood samples were drawn at different time points up to 180 min to measure the plasma total antioxidant capacity (TAC). Five antioxidant compositions (M1-M5) were evaluated to assess the possible synergies or antagonisms among them and to determine the optimal composition of the antioxidant mixture. Blood samples were collected up to 360 min post-consumption. A single oral dose of individual antioxidants significantly increased the TAC values; however, the time to reach the peak TAC value varied. Among the 5 antioxidant compositions, M2 exhibited the highest and most prolonged antioxidant effect in plasma; this was greater than the proportional sum of the effects of the individual antioxidants in the composition. This result indicates a synergistic interaction among antioxidants in the optimal composition M2.

Keywords: *in vivo*, total antioxidant capacity, duration of action, oxidative stress

Introduction

For decades, fruits and vegetables rich in antioxidants and dietary antioxidants have been known to prevent aging or diminish the risk of various diseases; these preventive effects have been attributed to their antioxidant action. Externally supplied antioxidants countervail the deleterious consequences of oxidative stress by counteracting free radicals that react with biological targets such as proteins, lipids, enzymes, and DNA (1,2). Although some chemically pure antioxidants are available as supplements, most of antioxidants are probably ingested as crude mixtures together with other dietary antioxidants (3-5). Antioxidants may compete with or assist other antioxidants commonly found in foods; thus, the degree of increase in antioxidant capacity, time to peak increase, and dosage effect vary with antioxidant composition (5-7). Nevertheless, few *in vivo* studies have investigated the combined or synergistic effects of antioxidants (8).

Moreover, individual antioxidants possess different pharmacokinetic properties; thus, they vary with regard to time of the onset and duration of action (9-17). Serafini *et al.* (18) reported that of 300 mL of green tea ingestion increased plasma total antioxidant capacity (TAC) with the highest increment of 40% at 30 min and decreased thereafter; reaching pre-ingestion values at 80 min. Ko *et al.* (19) evaluated the effect of consumption of 9 types of fruit juice on the antioxidant activity in human plasma. They reported that single servings of fruit juices increased the plasma TAC within 30 min after consumption; however, the activities could not be sustained for longer than 2 hr, except by grape juice that exhibited a persistent effect for up to 2 hr. Further, variations were observed in the degree of increment in the antioxidant capacity and time to peak. These differences enable us to make antioxidant combinations with prolonged effect. Since oxidative stress is continuously produced in the body during metabolic processes, it is required to maintain the antioxidant defense system in a steady state in order to prevent oxidative damage (20-22). With respect to the fact that antioxidant supplements are usually consumed 2 or 3 times a day, maintaining the effect until the next ingestion is advantageous. In drug development, combinations of short-acting drugs are used to prolong the duration of action (23,24). Further, it has been proved that when administered in combination, chemically dissimilar drugs interact synergistically to extend the duration of action to beyond that of the individual drugs (25).

In this study, we investigated the changes in plasma antioxidant activity after the administration of a single dose of curcumin, vitamin C, and vitamin E individually or in combination to (i) assess possible synergies or antagonism between the antioxidants and (ii) determine the optimal composition of the antioxidant mixture (curcumin, vitamin C, and E) that prolongs the duration of action to beyond that of the individual antioxidants. Curcumin is a powerful antioxidant derived from the rhizome *Curcuma longa* and has been used in Asian medicine since the second millennium BC (26,27). Interest in curcumin has increased due to its promising biological activities (28,29). The time to attain the maximum concentration after curcumin administration is estimated as 45 min, and its elimination half-life ($t_{1/2}$) ranges between 28 and 45 min (15). Vitamin C and E are the most common dietary antioxidants found in foods and vegetables and are often referred to as antioxidant vitamins (4). Vitamin C is a predominant water-soluble antioxidant associated with a broad range of

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biological activities. Its $t_{1/2}$ has been estimated as 10 hr (3). Vitamin E is a prototype of a radical chain breaker in the lipid phase, comprising 4 tocopherol and 4 tocotrienol homologues (α, β, γ, and δ). In nature, α-tocopherol is the most abundantly found tocopherol (11). Plasma concentrations of vitamin E after α-tocopherol administration peak at 12-14 hr, while its $t_{1/2}$ is approximately 81 hr (12-14).

Few studies have investigated the changes in the postdose levels of plasma TAC *in vivo*, and to our knowledge, this is the first study attempting to determine the optimal antioxidant composition for prolonging the duration of antioxidant action (19).

Materials and Methods

Chemicals Vitamin C (>98%) and vitamin E (DL-α-tocopheryl acetate, >50%) were obtained from DSM Nutrional Products Ltd. (Basel, Switzerland). Curcumin (>95%) was obtained from Phytotech Extract Pvt., Ltd. (Bangalore, India). All other chemicals used were of analytical grade or high performance liquid chromatography (HPLC) grade.

Animals Male Sprague-Dawley (SD) rats (10 weeks old) weighing 210-240 g were obtained from Orient Bio Inc. (Gyeonggi, Korea). The animals were acclimatized for 1 week in an animal facility prior to the experiments and housed under controlled conditions of temperature (23±2°C), humidity (55±10%), and light (12-hr light/dark, without any ultraviolet exposure). The animals had free access to food and water until 18 hr prior to being used in the experiments, at which time only food was restricted. Food and water intake was restrained throughout the experiments.

Experimental design In the first experiment (postdose experiment), 16 male SD rats were randomly divided into 4 groups: control, curcumin-, vitamin C-, and vitamin E-treated groups (each group, n=4). Each group was housed in a separate cage. The test groups received curcumin, vitamin C, or vitamin E at doses of 300 mg/kg of body weight (BW), dissolved in saline, by gastric gavage. The control group received an equivalent volume of the vehicle. To measure the plasma TAC, blood samples were collected from the orbital sinus in heparinized tubes at 10, 40, 90, and 180 min after the administration of antioxidants.

In the second experiment (optimization experiment), a 4-run, 3-factor, and 1-level Doehlert experimental design was employed for determining the experimental conditions. The following equation was used.

$$Y=A_0+A_1X_1+A_2X_2+A_3X_3+A_{12}X_1X_2+A_{23}X_2X_3+A_{13}X_1X_3+A_{11}X_1^2+A_{22}X_2^2+A_{33}X_3^2$$

Where, A_j are the coefficients of the respective variables and their interaction terms and X_1, X_2, and X_3 are the experimental factors corresponding to curcumin, vitamin C, and vitamin E, respectively (30-32). Table 1 summarizes the variables. Based on preliminary studies, the range for each factor was selected. The experimental design was generated using MINITAB 14 (University Park, PA, USA). Antioxidant mixtures were prepared according to the predefined composition provided by the statistical design; an antioxidant mixture with equal concentrations of each component was also prepared (Table 2).

Table 1. Variables of the Doehlert experimental design

Variables	Low level	High level
X_1=Curcumin (mg/kg BW)	50	250
X_2=Vitamin C (mg/kg BW)	50	250
X_3=Vitamin E (mg/kg BW)	10	150

Table 2. Composition of individual antioxidants in the mixtures

Mixture	Experimental combinations (mg/kg BW)		
	Curcumin	Vitamin C	Vitamin E
M1	100	50	150
M2	50	240	10
M3	240	50	10
M4	50	100	150
M5	100	100	100

Twenty-four male SD rats were randomly divided into 6 groups, i.e., 1 control group and 5 groups treated with antioxidant mixtures (M1-M5) (each group, n=4). Antioxidant samples were administered in a manner similar to that described above for the first experiment. To measure the plasma TAC, blood samples were obtained at 10, 40, 90, 180, and 360 min after administration.

The blood samples were centrifuged at 3,000×g for 10 min and the plasma separated to measure TAC. The samples were stored at –70°C until used.

Biochemical assays The plasma TAC was measured as Trolox equivalent antioxidant capacities (TEAC) according to Miller *et al.* (33,34). The assay is based on the principle that antioxidants inhibit the absorbance of the radical cation of 2,2'-azino-di-2-ethylbenzthiazoline sulfonate (ABTS). ABTS is incubated with a peroxidase (metmyoglobin) and H_2O_2 to yield the radical cation $ABTS^+$. This cation is relatively stable, blue-green, and can be measured at 405 nm. The antioxidants in a sample suppress the production of this color to a degree proportional to their concentration. The assay was calibrated against the α-tocopherol analogue Trolox, and the results were expressed as μmol TEAC/L of plasma.

Statistics The results are expressed as mean±standard deviation (SD, n=4). Statistical analysis was performed using the SPSS program (SPSS 12.0). One-way analysis of variance (ANOVA) with Duncan's multiple-range tests was used to examine the difference in TAC between the control group and antioxidant-treated groups. A p value of <0.01 was considered statistically significant.

Results and Discussion

Changes in the plasma TAC of the rats after the administration of a single dose of antioxidants Curcumin, vitamin C, and E were orally administered to investigate the changes in the plasma TAC after the administration of a single oral dose of antioxidants. No

significant change was observed in the plasma TAC of the control group at all time points (data not shown). The data shown in Table 3 represent differences in the plasma TEAC between the control and antioxidant-treated groups. The plasma TAC level significantly increased 10 min after the administration of vitamin C and peaked at 40 min; it then returned to the control level at 90 min. Administration of vitamin E resulted in a pattern similar to that observed with vitamin C until 90 min post-comsumption, but increased the plasma TAC again at 180 min. On the other hand, curcumin first caused a significant increase in the plasma TAC at 90 min post-consumption and the activity was sustained until 180 min. Curcumin exhibited delayed onset of antioxidant activity as compared to vitamins C or E.

With regard to the time to reach the maximum plasma concentration (T_{max}) of curcumin, vitamin C, and E, which is 3-4 (35), 12-14 (13), and 1-2 hr (36), respectively, in humans, our result is not consistent with the pharmaco-kinetic properties of each compound. The time of onset and duration of action are determined not only by pharmaco-kinetic properties but also by pharmacodynamic processes (37). The plasma TAC is affected by the parent compound or its metabolites. In the case of curcumin, the fact that the efficacy of tetrahydrocurcumin, a major metabolite of curcumin, is comparable to that of curcumin is believed to be one of the major reasons for curcumin's chemopreventive potential in certain tissues despite its low bioavailability (27,38-40). Therefore, differences between compound pharmacokinetics and antioxidant response is a result of variability in either pharmacokinetic or pharmacodynamic processes or a combination of both.

Although the antioxidant responses did not correspond to the compound pharmaco-kinetics, individual antioxidants showed different patterns of changes in the plasma TAC. These results indicate that the antioxidant responses by antioxidant mixtures may differ from those caused by a single antioxidant in plasma. Therefore, we subsequently proceeded to perform an optimization experiment to develop an antioxidant mixture of a composition that prolongs the duration of action to beyond that of individual antioxidants while maintaining a similar strength of antioxidant activity.

Optimization of antioxidant composition A Doehlert experimental design was applied to determine the optimal composition of the antioxidant mixture. This tool enables evaluation of the effect of each component (vitamin C, vitamin E) and that of the interactions between them on the plasma TAC by performing a minimal number of experiments. No significant change in the plasma TAC of the control group was observed at all time points (data not shown).

Five compositions (M1-M5, 300 mg/kg) were evaluated to determine the optimal composition of the antioxidant mixture. The data obtained from all groups are shown in Table 4. Interestingly, the plasma TAC changes of each composition appear similar to the patterns of the major component in the mixture. Both M1 and M4, whose major component is vitamin E, showed a maximal effect at 40 min, and M1 maintained a significant increase until 360 min. However, after M4 consumption, there was a momentary lull at 90 min post-consumption, after which it then slightly increased again. The fact that the second major component of M1 and M4 is curcumin and vitamin C, respectively, may account for the differences. Until 180 min post-consumption, M3, 80% of which is curcumin, exhibited no significant effect, but demonstrated antioxidant effect at 360 min. Since curcumin shows its first effect at 90 min post-consumption, the action of M3 was delayed. This may be due to competition for absorption or metabolism with other components. Indeed, dietary antioxidants have been reported to compete with each other for absorption,

Table 3. Changes in the plasma total antioxidant capacity (TAC) in male Sprague-Dawley rats after the administration of curcumin, vitamin C, and E

Time (min)	Plasma TAC (μmol TEAC/L)			
	10	40	90	180
Control	0.0±44.3$^{a1)}$	0.0±26.6^{a}	0.0±30.3^{a}	0.0±11.6^{a}
Curcumin	12.5±69.3^{a}	−14.5±23.3^{a}	173.7±55.9^{b}	150.9±20.0^{b}
Vitamin C	109.3±22.3^{b}	213.0±31.2^{b}	15.9±12.1^{a}	−38.0±29.6^{a}
Vitamin E	119.4±47.5^{b}	153.4±10.8^{c}	26.5±11.1^{a}	86.4±23.3^{c}

$^{1)}$At a given point in time, within the same column, values not sharing the same letter are significantly different, $p<0.01$.

Table 4. Changes in the total antioxidant capacity (TAC) in male Sprague-Dawley rats after the administration of an antioxidant mixture (M1-M5)

Time (min)	Plasma TAC (μmol TEAC/L)				
	10	40	90	180	360
Control	0.0±44.3$^{a1)}$	0.0±26.6^{a}	0.0±30.3^{a}	0.0±11.6^{a}	0.0±36.3^{a}
M1	22.8±22.0^{a}	104.7±45.1^{b}	91.8±61.7^{b,c}	77.7±43.6^{b,c}	61.0±19.0^{b}
M2	103.5±35.0^{b}	197.8±57.9^{c}	161.0±33.8^{c}	126.6±22.4^{c}	147.3±30.8^{c}
M3	36.5±21.7^{a}	50.6±21.9^{a,b}	33.0±56.3^{a,b}	24.2±27.5^{a,d}	108.9±29.0^{b,c}
M4	48.8±45.2^{a,b}	114.0±19.7^{b}	60.2±57.1^{a,b}	88.5±11.1^{b,c}	76.1±21.4^{b}
M5	51.6±27.6^{a,b}	84.1±53.8^{b}	86.1±38.2^{b,c}	61.8±42.7^{b,d}	83.9±39.7^{b}

$^{1)}$At a given point in time, within the same column, values not sharing the same letter are significantly different, $p<0.01$.

thus affecting the bioavailability of the compound (7,41,42). M5, in which each component is present in equal concentrations, exhibited stable antioxidant activity with medium efficacy until 360 min. M2 showed the highest plasma TAC increase at all time points and reached the maximum at 40 min. M2 is composed of 80% vitamin C, 3% vitamin E, and 17% curcumin. The change in the plasma TAC in the first 40 min was similar to the effect of vitamin C; however, M2 exhibited a considerably higher and prolonged effect as compared to vitamin C. The effect was greater than the proportional sum of the individual antioxidants in the composition. A synergistic effect appears to have occurred among the 3 components of M2.

Some part of observed synergism can be explained by the synergy between vitamin C and E. Ascorbate (vitamin C) regenerates vitamin E from the tocopherol radical formed during the antioxidant activity of vitamin E (43,44). However, limited information is available regarding the synergy between curcumin and vitamin C or E. It might be related to different physicochemical properties and/or mechanism of antioxidant action which simultaneously enhance the antioxidant mechanism and promotes antioxidant effectiveness. It also can be postulated that there is reciprocal protective interaction between curcumin and vitamin C or E, since cooperative interactions are very common mechanisms in the action of biological antioxidants. Further investigation of the roles of each component is required. Although the mechanism of the manner in which each component mediates its synergistic effect is unclear, our results show that the optimized antioxidant mixture provides prolonged high antioxidant effects when administered as an oral supplement. Further, the synergistic effect appears to depend on not only the combination of antioxidants but also the concentrations of each antioxidant.

In conclusion, the current study suggests that consuming different antioxidants causes diverse patterns of changes in the plasma TAC, and an appropriate combination of antioxidants may prolong the duration of action to beyond that of an individual antioxidant. The optimized composition M2 developed by using a Doehlert experimental design exhibited a prolonged duration of action with superior efficacy as compared to other antioxidant mixtures. These results indicate that the positive effect of M2 persists for longer than that of any individual antioxidant and that the components in the mixture synergistically contribute to the inhibition of oxidation *in vivo*. Based on the present results, we suggest that the optimized antioxidant composition M2 could be used as an effective antioxidant supplement, the effect of which persists for up to 6 hr, thus maintaining a steady state of antioxidant defense until the next ingestion. However, the antioxidant properties of each antioxidant in the mixture should be confirmed in cellular systems, and long-term studies using a larger number of subjects are required to provide additional supportive evidence. Nevertheless, this study serves as an important starting point for further *in vivo* analyses of the changes in the post-dose plasma TAC.

References

1. Ratnam DV, Ankola DD, Bhardwaj V, Sahana DK, Kumar MN. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. J. Control Release 113: 189-207 (2006)
2. Choi JM, Han J, Yoon BS, Chung JH, Shin DB, Lee SK, Hwang JK, Ryang R. Antioxidant properties of tannic acid and its inhibitory effects on paraquat-induced oxidative stress in mice. Food Sci. Biotechnol. 15: 728-734 (2006)
3. McArdle F, Rhodes LE, Parslew RAG, Close GL, Jack CIA, Friedmann PS, Jackson MJ. Effects of oral vitamin E and β-carotene supplementation on ultraviolet radiation-induced oxidative stress in human skin. Am. J. Clin. Nutr. 80: 1270-1275 (2004)
4. Schwedhelm E, Maas R, Troost R, Boger RH. Clinical pharmacokinetics of antioxidants and their impact on systemic oxidative stress. Clin. Pharmacokinet. 42: 437-459 (2003)
5. Chen CY, Milbury PE, Lapsley K, Blumberg JB. Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. J. Nutr. 135: 1366-1373 (2005)
6. Richelle M, Sabatier M, Steiling H, Williamson G. Skin bioavailability of dietary vitamin E, carotenoids, polyphenols, vitamin C, zinc, and selenium. Brit. J. Nutr. 96: 227-238 (2006)
7. Reboul E, Thap S, Tourniaire F, André M, Juhel C, Morange S, Amiot MJ, Lairon D, Borel P. Differential effect of dietary antioxidant classes (carotenoids, polyphenols, vitamins C and E) on lutein absorption. Brit. J. Nutr. 97: 440-446 (2007)
8. Yogeeta SK, Gnanapragasam A, Kumar SS, Subhashini R, Sathivel A, Devaki T. Synergistic interactions of ferulic acid with ascorbic acid: Its cardioprotective role during isoproterenol induced myocardial infarction in rats. Mol. Cell. Biochem. 283: 139-146 (2006)
9. Blanchard J. Depletion and repletion kinetics of vitamin C in humans. J. Nutr. 121: 170-176 (1991)
10. Hornig D. Metabolism and requirements of ascorbic acid in man. S. Afr. Med. J. 60: 818-823 (1981)
11. Cohn W. Bioavailability of vitamin E. Eur. J. Clin. Nutr. 51: S80-S85 (1997)
12. Ferslew KE, Acuff RV, Daigneault EA. Pharmacokinetics and bioavailability of the RRR and all racemic stereoisomers of alpha-tocopherol in humans after single oral administration. J. Clin. Pharmacol. 33: 84-88 (1993)
13. Cheeseman KH, Holley AE, Kelly FJ. Biokinetics in humans of RRR-alpha-tocopherol: The free phenol acetate ester and succinate ester forms of vitamin E. Free Radical Bio. Med. 19: 591-598 (1995)
14. Julianto T, Yuen KH, Noor AM. Improved bioavailability of vitamin E with self emulsifying formulation. Int. J. Pharm. 200: 53-57 (2000)
15. Yang KY, Lin LC, Tseng TY, Wang SC, Tsai TH. Oral bioavailability of curcumin in rat and the herbal analysis from *Curcuma longa* by LC-MS/MS. J. Chromatogr. B 853: 183-189 (2007)
16. Levine M, Conry-Cantilena C, Wang Y. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. P. Natl. Acad. Sci. USA 93: 3704-3709 (1996)
17. MacMahon MT, Neale G. The absorption of alpha-tocopherol in control subjects and in patients with intestinal malabsorption. Clin. Sci. 38: 197-210 (1970)
18. Serafini M, Ghiselli A, Ferro-Luzzi A. *In vivo* antioxidant effect of green and black tea in man. Eur. J. Clin. Nutr. 50: 28-32 (1996)
19. Ko SH, Choi SW, Ye SK, Cho BL, Kim HS. Comparison of the antioxidant activities of nine different fruits in human plasma. J. Med. Food. 8: 41-46 (2005)
20. Banfi G, Malavazos A, Iorio E, Dolci A, Doneda L, Verna R, Corsi MM. Plasma oxidative stress biomarkers nitric oxide and heat shock protein 70 in trained elite soccer. Eur. J. Appl. Physiol. 96: 483-486 (2006)
21. Sies H. Strategies of antioxidant defense. Eur. J. Biochem. 215: 213-219 (1993)
22. Bray TM. Dietary antioxidants and assessment of oxidative stress. Nutrition 16: 578-581 (2000)
23. Flood P. The importance of myorelaxants in anesthesia. Curr. Opin. Pharmacol. 5: 322-327 (2005)
24. Ermer JC, Shojaei A, Pennick M, Anderson CS, Silverberg A,

Youcha SH. Bioavailability of triple-bead mixed amphetamine salts compared with a dose-augmentation strategy of mixed amphetamine salts extended release plus mixed amphetamine salts immediate release. Curr. Med. Res. Opin. 23: 1067-1075 (2007)
25. Breslin DS, Jiao K, Habib AS, Schultz J, Gan TJ. Pharmacodynamic interactions between cisatracurium and rocuronium. Anesth. Analg. 98: 107-110 (2004)
26. Eybl V, Kotyzová D Lešetický L, Bludovská M, Koutenský J. The influence of curcumin and manganese complex of curcumin on cadmium-induced oxidative damage and trace elements status in tissues of mice. J. Appl. Toxicol. 26: 207-212 (2005)
27. Sharma RA, Gescher AJ, Steward WP. Curcumin: The story so far. Eur. J. Cancer. 41: 1955-1968 (2005)
28. Calabrese V, Butterfield DA, Stella AM. Nutritional antioxidants and the heme oxygenase pathway of stress tolerance: Novel targets for neuroprotection in Alzheimer's disease. Ital. J. Biochem. 52: 177-181 (2003)
29. Tayyem RF, Heath DD, Al-Delaimy WK, Rock CL. Curcumin content of turmeric and curry powders. Nutr. Cancer. 55: 126-131 (2006)
30. Maneä C, Souquet JM, Olleä D, Verrieäs C, Veäran F, Mazerolles G, Cheynier V, Fulcrand H. Optimization of simultaneous flavanol phenolic acid and anthocyanin extraction from grapes using an experimental design: Application to the characterization of champagne grape varieties. J. Agr. Food Chem. 55: 7224-7233 (2007)
31. Leichtnam ML, Rolland H, Wüthrich P, Guy RH. Formulation and evaluation of a testosterone transdermal spray. J. Pharm. Sci. 95: 1693-1702 (2006)
32. El-Malah Y, Nazzal S, Khanfar NM. D-Optimal mixture design: Optimization of ternary matrix blends for controlled zero-order drug release from oral dosage forms. Drug Dev. Ind. Pharm. 32: 1207-1218 (2006)
33. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. 84: 407-412 (1993)
34. Miller NJ, Johnston JD, Collis CS, Rice-Evans C. Serum total antioxidant activity after myocardial infarction. Ann. Clin. Biochem. 34: 85-90 (1997)
35. Blanchard J, Conrad KA, Mead RA, Garry PJ. Vitamin C disposition in young and elderly men. Am. J. Clin. Nutr. 51: 837-845 (1990)
36. Cheng AL, Hsu CH, Lin JK. Phase I clinical trial of curcumin a chemopreventive agent in patients with high-risk or pre-malignant lesions. Anticancer Res. 21: 2895-2900 (2001)
37. Lin JH. Pharmacokinetic and pharmacodynamic variability: A daunting challenge in drug therapy. Curr. Drug Metab. 8: 109-136 (2007)
38. Ma Z, Shayeganpour A, Brocks DR, Lavasanifar A, Samuel J. High performance liquid chromatography analysis of curcumin in rat plasma: Application to pharmacokinetics of polymeric micellar formulation of curcumin. Biomed. Chromatogr. 21: 546-552 (2007)
39. Somparn P, Phisalaphong C, Nakornchai S, Unchern S, Morales NP. Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. Biol. Pharm. Bull. 30: 74-78 (2007)
40. Murugan P, Pari L. Effect of tetrahydrocurcumin on plasma antioxidants in streptozotocin-nicotinamide experimental diabetes. J. Basic Clin. Physiol. Pharmacol. 17: 231-244 (2006)
41. Tyssandier V, Cardinault N, Caris-Veyrat C, Amiot MJ, Grolier P, Bouteloup C, Azais-Braesco V, Borel P. Vegetable-borne lutein lycopene and β-carotene compete for incorporation into chylomicrons with no adverse effect on the medium-term (3-wk) plasma status of carotenoids in humans. Am. J. Clin. Nutr. 75: 526-534 (2002)
42. van den Berg H. Carotenoid interactions. Nutr. Rev. 57: 1-10 (1999)
43. Kadoma Y, Ishihara M, Okada N, Fujisawa S. Free radical interaction between vitamin E (alpha-, beta-, gamma-, and delta-tocopherol) ascorbate and flavonoids. In Vivo 20: 823-827 (2006)
44. Niki E. Interaction of ascorbate and alpha-tocopherol. Ann. NY Acad. Sci. 498: 186-199 (1987)