IJPSR (2014), Vol. 5, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 October, 2013; received in revised form, 19 November, 2013; accepted, 13 February, 2014; published 01 March, 2014

A SYNERGISTIC APPROACH TO KINETIC AND MECHANISTIC STUDIES OF REGENERATION OF β-CAROTENE FROM *TERT*-BUTOXYL RADICAL INDUCED β-CAROTENE RADICAL CATION BY CHLOROGENIC ACID

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Keywords:

Chlorogenic acid, β-carotene, regeneration, synergism, *tert*-butoxyl radicals, oxidation

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ABSTRACT: The rates of oxidation of β-carotene and chlorogenic acid by *tert*-butoxyl radicals (*t*-BuO$^{\bullet}$) were studied by measuring the absorbance of β-carotene at 491 nm and chlorogenic acid at 328 nm spectrophotometrically. Radicals (*t*-BuO$^{\bullet}$) were generated by the photolysis of *tert*-butyl hydroperoxide (*t*-BuOOH) in presence of *tert*-butyl alcohol to scavenge OH$^{\bullet}$ radicals. The rates and the quantum yields (ϕ) of oxidation of chlorogenic acid by *t*-BuO$^{\bullet}$ radicals were determined in the absence and presence of varying concentrations of β-carotene. An increase in the concentration of β-carotene was found to decrease the rate of oxidation of chlorogenic acid, suggesting that β-carotene and chlorogenic acid competed for *t*-BuO$^{\bullet}$ radicals. The quantum yields (ϕ_{expt}) were calculated from the experimentally determined rates of oxidation of chlorogenic acid under different experimental conditions. Assuming that chlorogenic acid acts as a scavenger of *t*-BuO$^{\bullet}$ radicals only, the quantum yields (ϕ_{cal}) were theoretically calculated. ϕ_{expt} and ϕ_{cal} values suggested that chlorogenic acid not only protected β-carotene from *t*-BuO$^{\bullet}$ radicals, but also regenerated β-carotene from β-carotene radical cation, formed by the reaction of β-carotene with *t*-BuO$^{\bullet}$ radicals. Results indicated a possible synergistic interaction between chlorogenic acid and β-carotene in which 35.4% of β-carotene was regenerated by chlorogenic acid.

INTRODUCTION: In living aerobic cells, oxidative stress caused by an imbalance between antioxidant systems and the production of oxidants, including Reactive Oxygen Species (ROS) is associated with many multifactorial diseases, especially cancers, cardiovascular diseases and inflammatory disorders [1-3]. The mechanisms by which these pathologies develop generally involve oxidative alteration of physiologically critical molecules, including proteins, lipids, carbohydrates and nucleic acids, along with modulation of gene expression and the inflammatory response [4-6].Oxidative DNA damage has been thought to be an important source of mutation leading to aging [7] and a wide range of degenerative diseases such as immune-system decline, brain dysfunction and cataracts [8].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(3).942-50

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(3).942-50

Although lethal effects of the hydroxyl radicals on DNA and its constituents have been studied [4] extensively, relatively little is known about the biological effects of alkoxyl radicals and the key cellular targets for these species. Organic oxygen radicals, particularly alkoxyl radicals may participate in metabolic and pathological processes [9]. *tert*-Butyl hydroperoxide (*t*-BuOOH) has been chosen as a model peroxide which on homolysis gives $^{\bullet}$OH and *t*-BuO$^{\bullet}$ radicals. Previous studies on the reactivity of *tert*-butoxyl radicals suggest that these species might be expected to attack both the sugar and the base moieties of DNA [10]. The experimental evidence indicates that base radicals also contribute to strand breaks by transfer of their radical sites from base moiety to sugar moiety.

Antioxidants are substances, when present in small quantities prevent or delay the oxidation of cellular organelles by minimizing the damaging effects of ROS/RNS or oxidative stress. Many studies have now confirmed that exogenic antioxidants, especially supplied by foods, are essential for counteracting oxidative stress [11-13]. These antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids. They are required in the management of pathophysiological conditions, most of which involve free radical damage. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last 20 years.

Synergism is the cooperative effect of antioxidants or an antioxidant with other compounds to produce enhanced activity than the sum of the activities of the individual component when used separately [14]. In a combination of two or more free radical scavengers, rapid reaction with free radicals occurs because of the differences in bond dissociation energies (BDE) or steric hindrance of free radical scavenger [15]. These differences result in one scavenger being used faster than the other and regenerate the primary scavenger by transferring its radical to another scavenger.

Antioxidants may act synergistically due to differences in reactivity towards different oxidants thereby yielding a better overall protection in combination than either could individually or may be due to direct interaction between them. Interaction among antioxidants can be synergistic, antagonistic or merely additive. Synergistic antioxidant effects can be achieved by the protective action of one antioxidant by means of its sacrificial oxidation [16]. The less effective antioxidant traps alkyl or alkyl peroxy radicals in foods resulting in protecting more effective antioxidant from the oxidation.

Peyrat-Maillard *et al* [17] have studied the synergistic and antagonistic effects occurring between pairs of phenolic antioxidants in a mixture. A synergistic or antagonistic effects occurring between pairs of antioxidants during the oxidation of linoleic acid in an aqueous dispersed system can be partly explained by regeneration mechanisms, depending on the chemical structure of molecules and on the possible formation of stable intermolecular complexes [18, 19].

Chlorogenic acid (CGA), an ester of caffeic acid with quinic acid, is found in a wide range of fruits and vegetables. Coffee, one of the most widely consumed beverages in the world, contains high amounts of CGA. Phenolcarboxylic acids such as CGA exert beneficial effects on human health through prevention of degenerative pathologies such as cardiovascular diseases and cancer [20, 21]. It was found that CGA inhibited NO production in lipopolysaccharide (LPS) stimulated mouse macrophage like cells (RAW 264.7 cells) and scavenged various radicals such as superoxide anions and hydroxyl radicals. It scavenges radicals generated in the aqueous phase [22], increases the resistance of LDL to lipid peroxidation [23] and inhibits DNA damage [24]. *In vivo*, when added to the diet, it inhibits chemically induced carcinogenesis of the large intestine, liver and tongue in rats and hamsters [25, 26].

Antioxidants such as chlorogenic acid do not act in isolation, but rather constitute an intricate network in the presence of coantioxidants such as glutathione (GSH), ascorbate or other phenolic compounds such as vitamin E [21]. There remains a great interest in the possible health promoting effects of antioxidants, but the synergistic mechanism by which these compounds coexist in foods is in need of further study.

The regeneration effect of one antioxidant by another antioxidant is a potentially beneficial reaction that needs to be further studied in human and animal models. It is in this context that a systematic kinetic study of interaction of CGA with β-carotene in the presence of *t*-BuO$^{\bullet}$ radicals was carried out to get an insight into the possible synergistic/antagonistic molecular mechanisms which help in selection of co-antioxidants while adding to the food preservatives.

MATERIALS AND METHODS: CGA and β-carotene were purchased from Sigma Chemical Co., St. Louis, USA and used as received. All solutions were prepared afresh using double-distilled water. *tert*-Butyl hydroperoxide (*t*-BuOOH) was used as received from Merck-Schuchardt of Germany. There is no contamination of other peroxides in the assay of the sample. *t*-BuOOH was estimated by iodometric method [27].

The irradiations were carried out at room temperature in a quantum yield reactor model QYR-20 supplied by Photophysics, England, attached with 400 W medium pressure mercury lamps. The quartz cuvette containing the sample was irradiated and the irradiations were interrupted at definite intervals of time and the absorbance was noted. The light intensity corresponding to the irradiating wavelength (254 nm) was measured using peroxydisulphate chemical actinometry [28]. On photolysis, *t*-BuOOH was activated at 254 nm to generate $^{\bullet}$OH and t-BuO$^{\bullet}$ radicals by homolytic cleavage of –O-O-bond [29]. The $^{\bullet}$OH radicals produced were scavenged using sufficient concentration of *t*-BuOH [30]. In a typical kinetic run, the aqueous reaction mixture of CGA and *t*-BuOOH was taken in a specially designed 1 cm path length quartz cuvette, suitable for both irradiations and absorbance measurements. The absorbance measurements were made at the λ_{max} of CGA (328 nm) on a Chemito UV-Visible spectrophotometer (model 2100).

The photochemical reaction of CGA in the presence of *t*-BuOOH was followed by measuring the absorbance of chlorogenic acid at 328 nm at which β-carotene was totally transparent.

It is known that *t*-BuOOH is activated to radical reaction by the absorption of light at 254 nm [28]. However, the substrates used in the present work, viz., chlorogenic acid and β-carotene have strong absorption in this region. But, in the absence of *t*-BuOOH in the reaction mixture, chlorogenic acid, β-carotene or chlorogenic acid-β-carotene mixture did not undergo any observable chemical change on shining the light. Even though a small fraction of the total light intensity was absorbed by *t*-BuOOH directly in the presence of β-carotene and/or chlorogenic acid, a considerable chemical change was observed with β-carotene as well as chlorogenic acid.

If β-carotene and chlorogenic acid acted as only inner filters, the rates of the reaction of β-carotene or chlorogenic acid with t-BuO$^{\bullet}$ radicals would have been decreased with increase in concentration of β-carotene or chlorogenic acid. But, the results in **Tables 1 and 2** were contrary to this. One another fact against the inner filter concept was that the rate of oxidation of chlorogenic acid in the presence of β-carotene would have been much less than the experimentally observed values (**Table 4**). Hence, we proposed that the excited states of chlorogenic acid and β-carotene acted as sensitizers to transfer energy to *t*-BuOOH to produce radical species. This type of sensitizing effect was proposed in similar systems earlier [28]. Therefore, the light intensity at 254 nm was used to calculate the quantum yields of oxidation of β-carotene as well as chlorogenic acid under different experimental conditions.

RESULTS AND DISCUSSIONS: The oxidation of β-carotene by *t*-BuO$^{\bullet}$ radicals was carried out by irradiating the reaction mixture containing known concentrations of β-carotene and *t*-BuOOH in the presence of sufficient amount of *t*-BuOH to scavenge the $^{\bullet}$OH radicals completely[28].The reaction was followed by measuring the absorbance of β-carotene at 451 nm (λ_{max} of β-carotene) with time. The initial rates and quantum yields of oxidation of β-carotene by *t*-BuO$^{\bullet}$ are presented in Table 1. UV-visible absorption spectra of β-carotene in presence of *t*-BuOOH and *t*-BuOH at different irradiation times were recorded (Fig. 1). The initial rates of photooxidation of chlorogenic acid by *t*-BuOOH in presence of *t*-BuOH were calculated from the plots of absorbance of chlorogenic acid at 328 nm *vs* time using microcal origin computer program on a personal computer

(Table 2). UV-visible absorption spectra of chlorogenic acid in presence of *t*-BuOOH and *t*-BuOH at different irradiation times were recorded (**Fig. 2**). In order to find the protection offered to β-carotene by chlorogenic acid towards oxidation by *t*-BuO$^{\bullet}$, the reaction mixture containing known concentrations of β-carotene and *t*-BuOOH was irradiated in presence of varying concentrations of chlorogenic acid. The photooxidation of chlorogenic acid by *t*-BuO$^{\bullet}$ at different concentrations of β-carotene was also studied (**Fig. 3**) and the data are presented in **Table 3**. The reactions were followed by measuring the absorbance of chlorogenic acid at 328 nm (**Fig. 4**) at which β-carotene was transparent and the rate data are presented in Table 4.

The oxidation rate of β-carotene in the presence of *t*-BuOH refers exclusively to the reaction of *t*-BuO$^{\bullet}$ with β-carotene. These rates were found to increase with increase in concentration of β-carotene as well as *t*-BuOOH. The quantum yield values were also increased with increase in [β-carotene] as well as [*t*-BuOOH] (Table 1).

The rate of oxidation of chlorogenic acid increased with increase in concentration of chlorogenic acid (Table 2). The quantum yields of oxidation of chlorogenic acid were calculated from the initial rates and the light intensity at 254 nm. These values were also increased with increase in concentration of chlorogenic acid (Table 2). Having known the rates of *t*-BuO$^{\bullet}$

TABLE 1: EFFECT OF [β-CAROTENE] ON THE RATES AND QUANTUM YIELDS OF PHOTO OXIDATION OF β-CAROTENE BY *t*-BuOOH IN *t*-BuOH-WATER (4:1 v/v) MEDIUM

10^5 × [β-carotene] (mol dm^{-3})	10^3 × [*t*-BuOOH] (mol dm^{-3})	10^9 × Initial rate (mol dm^{-3} s^{-1})	Quantum yield (φ)
0.5	5.0	0.316	0.00210
0.8	5.0	0.478	0.00318
1.0	5.0	0.532	0.00354
2.0	5.0	1.057	0.00703
5.0	5.0	2.500	0.01662
8.0	5.0	4.837	0.03216
10.0	5.0	6.790	0.04516
5.0	10.0	5.825	0.03874
5.0	15.0	8.024	0.05340

Light intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 294 nm, pH ~ 7.5, Temperature = 298 K

TABLE 2: EFFECT OF [CHLOROGENIC ACID] AND [t-BuOOH] ON THE RATES AND QUANTUM YIELDS OF PHOTO OXIDATION OF CHLOROGENIC ACID BY t-BuOOH IN t-BuOH-WATER (4:1 v/v) MEDIUM

10^6 × [chlorogenic acid] (mol dm^{-3})	10^3 × [*t*-BuOOH] (mol dm^{-3})	10^9 × Initial rate (mol dm^{-3} s^{-1})	Quantum yield (φ)
20.0	5.0	9.6908	0.00644
10.0	5.0	7.0008	0.00465
8.0	5.0	5.2798	0.00351
5.0	5.0	2.7845	0.00185
2.0	5.0	2.2974	0.00152
20.0	10.0	11.203	0.00745
20.0	15.0	13.157	0.00875

Light intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, Temperature = 298 K

Radical reactions with β-carotene as well as chlorogenic acid under varying experimental conditions, both β-carotene and chlorogenic acid were introduced for the competitive studies with *t*-BuO$^{\bullet}$ radical. Aqueous solutions of reaction mixture containing chlorogenic acid, and *t*-BuOOH were irradiated in presence of varying concentrations of β-carotene (Fig. 3). The initial rates and quantum yields of oxidation of chlorogenic acid by *t*-BuO$^{\bullet}$ radicals were found to decrease with increase in concentration of β-carotene (Table 4). Comparison of the initial rates and quantum yields of oxidation of chlorogenic acid in presence and absence of β-carotene clearly indicated that the initial rates and quantum yields of oxidation of chlorogenic acid were substantially decreased in presence of β-carotene (Table 4).

These observations clearly demonstrated that β-carotene and chlorogenic acid was in competition for *t*-BuO$^{\bullet}$ radicals.

The rate constant of the reaction of *t*-BuO$^{\bullet}$ with CGA has been reported[31] to be 3.20×10^9 dm^3 mol^{-1} s^{-1} under similar experimental conditions of the present work. The rate constant for the reaction of *t*-BuO$^{\bullet}$ with β-carotene was calculated by the adenosine competition method, which was very similar to the method[32] used to determine the rate constant for the reaction of $^{\bullet}$OH radicals with polyhydric alcohols in competition with KSCN. In the present study, solutions containing chlorogenic acid and varying amounts of β-carotene in presence of *t*-BuOOH was irradiated for 2 min and the decrease in absorbance of chlorogenic acid was measured.

The decrease in absorbance of chlorogenic acid reflected the amount of *t*-BuO$^{\bullet}$ radicals that had reacted with chlorogenic acid. From the known rate constant of the reaction of CGA with *t*-BuO$^{\bullet}$ radical under similar experimental conditions of the present work ($k_{CGA} = 3.20 \times 10^9$ dm^3 mol^{-1} s^{-1}), the rate constant of *t*-BuO$^{\bullet}$ radical reaction with β-carotene ($k_{\beta\text{-carotene}}$) can be calculated using the following equation:

$$\frac{[\text{Absorbance of chlorogenic acid}]_o}{[\text{Absorbance of chlorogenic acid}]_{\beta\text{-carotene}}} = 1 + \frac{k_{\beta\text{-carotene}}\,[\beta\text{–carotene}]}{k_{\text{chlorogenic acid}}\,[\text{chlorogenic acid}]} \quad (1)$$

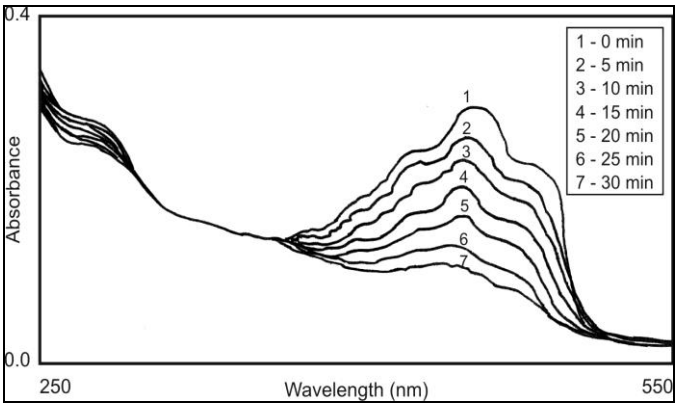


FIG. 1: ABSORPTION SPECTRA OF PHOTO-OXIDATION OF β-CAROTENE IN THE PRESENCE OF *TERT*-BUTYL HYDROPEROXIDE AT DIFFERENT IRRADIATION TIMES. [β-carotene] = 5×10^{-5} mol dm^{-3}, [*t*-BuOOH] = 5×10^{-3} mol dm^{-3}, Light intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 451 nm, pH ~ 7.5, temperature = 298K, [*t*-BuOH] = 1.0 M

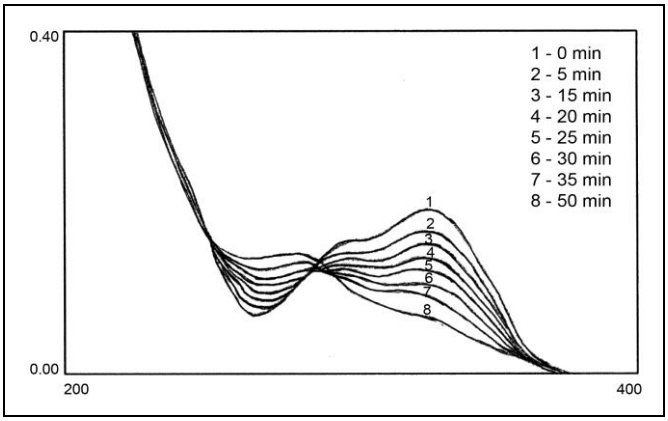


FIG. 2: ABSORPTION SPECTRA OF PHOTO-OXIDATION OF CGA IN THE PRESENCE OF *TERT*-BUTYL HYDROPEROXIDE AT DIFFERENT IRRADIATION TIMES IN; *t*-BuOH-water (4:1 v/v) medium, [chlorogenic acid] = 10.0×10^{-6} mol dm^{-3}, [*t*-BuOOH] = 5×10^{-3} mol dm^{-3}, Light intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, temperature = 298 K

In Eq. (1), [Absorbance of chlorogenic acid]$_o$ and [Absorbance of chlorogenic acid]$_{\beta\text{-carotene}}$ are the absorbance values of chlorogenic acid in the absence and presence of β-carotene, respectively at the same interval of time. Experiments of this kind can be carried out with great accuracy. Using Eq. (1), the rate constant for the reaction of *t*-BuO$^{\bullet}$ radical with β-carotene ($k_{\beta\text{-carotene}}$) was calculated at different concentrations of chlorogenic acid and β-carotene and the average of these was found to be 5.54×10^8 dm^3 mol^{-1} s^{-1} which is found to be same as earlier reported[33]. As chlorogenic acid had strong absorption at 294 nm, it is not possible for the direct determination of protection and repair offered to β-carotene by chlorogenic acid. However, one could calculate indirectly the extent of protection offered to β-carotene by chlorogenic acid from competition kinetic studies measured at 328 nm, λ_{max} of chlorogenic acid. The method was as follows:

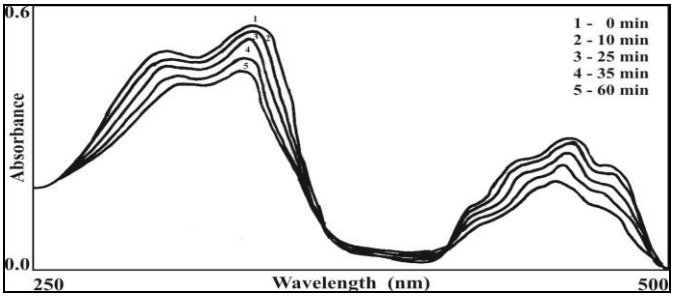


FIG. 3: ABSORPTION SPECTRA OF PHOTO-OXIDATION OF CHLOROGENIC ACID IN THE PRESENCE OF TERT-BUTYL HYDROPEROXIDE AND β-CAROTENE AT DIFFERENT IRRADIATION TIMES; [chlorogenic acid] = 2×10^{-5} mol dm^{-3}, [*t*-BuOOH] = 5×10^{-3} mol dm^{-3}, [β-carotene] = 5×10^{-5} mol dm^{-3}, Light Intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, temperature = 298 K

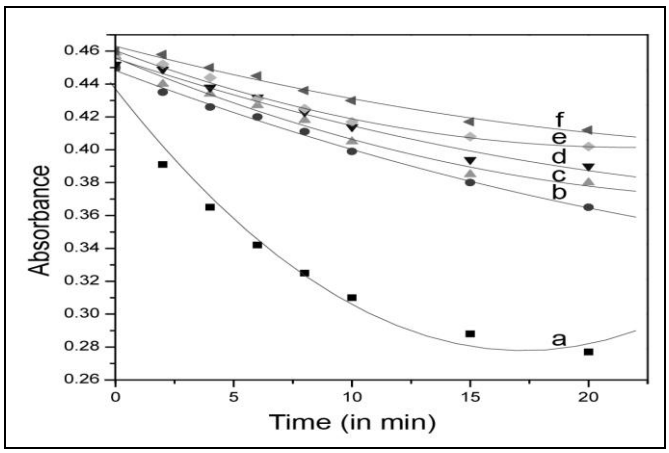


FIG. 4: EFFECT OF [β-carotene] ON THE OXIDATION OF CHLOROGENIC ACID BY *t*-BuO• IN *t*-BuOH-WATER 4:1 (v/v) NEUTRAL MEDIUM

When the system containing β-carotene, chlorogenic acid and *t*-BuOOH was irradiated, the probability of *t*-BuO• radicals reacting with chlorogenic acid $\{p_{(t\text{-BuO}^{\bullet} + \text{ chlorogenic acid})}\}$ was calculated using the following equation:

$$p_{(t\text{-BuO}^{\bullet} + \text{chlorogenic acid})} = \frac{k_{\text{chlorogenic acid}}\ [\text{chlorogenic acid}]}{k_{\beta\text{-carotene}}\ [\beta\text{-carotene}] + k_{\text{chlorogenic acid}}\ [\text{chlorogenic acid}]} \quad (2)$$

If chlorogenic acid scavenged only *t*-BuO• radicals and did not give rise to any other reaction (e.g. reaction with β-carotene radicals), the quantum yield of oxidation of chlorogenic acid (ϕ_{cal}) at each concentration of β-carotene may be given by equation:

$$\phi_{cal} = \phi^{o}_{expt} \times p \quad (3)$$

Where ϕ^{o}_{expt} is the quantum yield of oxidation of chlorogenic acid in the absence of β-carotene, and *p* is the probability given by Eq. (2).

TABLE 3: EFFECT OF [CHLOROGENIC ACID] ON THE RATES AND QUANTUM YIELDS OF OXIDATION OF CHLOROGENIC ACID IN THE ABSENCE AND PRESENCE OF β-CAROTENE BY *t*-BuO• IN *t*-BuOH-WATER (1:4 v/v) MEDIUM

$10^5 \times$ [chlorogenic acid] (mol dm^{-3})	$10^5 \times$ [β-carotene] (mol dm^{-3})	$10^9 \times$ Rate (mol dm^{-3} s^{-1})	Quantum yields ϕ
2.0	0.0	9.6908	0.00644
1.0	0.0	7.0008	0.00465
0.8	0.0	5.2798	0.00351
0.5	0.0	2.7845	0.00185
0.2	0.0	2.2974	0.00153
2.0	5.0	7.1010	0.00472
1.0	5.0	4.7953	0.00319
0.8	5.0	3.5255	0.00234
0.5	5.0	2.1888	0.00145
0.0	5.0	1.5455	0.00103

[*t*-BuOOH] = 5.0×10^{-3} mol dm^{-3}, Light intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, Temperature = 298 K

The calculated quantum yield (ϕ_{cal}) values at different β-carotene concentrations are presented in Table 4. The data showed that the ϕ_{cal} values were lower than the experimentally measured quantum yield (ϕ_{expt}) values. This indicated that more number of chlorogenic acid molecules was consumed in the system than expected and the most likely route for this was H atom donation by chlorogenic acid to β-carotene radicals. In Table 4, are presented the fraction of *t*-BuO• radicals scavenged by chlorogenic acid at different concentrations of β-carotene. These values referred to the measure of protection offered to β-carotene due to scavenging of *t*-BuO• radicals by chlorogenic acid. Using the ϕ_{exptl} values, a set of values, viz., ϕ' values were calculated from Eq. (4) and are presented in Table 4.

$$\phi' = \frac{\phi_{expt}}{p} \quad (4)$$

Where ϕ's represent the experimentally found quantum yield values if no scavenging of β-carotene radicals by chlorogenic acid occurs. In the absence of any "repair" of β-carotene radicals by chlorogenic acid, the ϕ' values should all be equal to ϕ^{o}_{expt}.

The observed increase in ϕ′ with increasing β-carotene concentration (Table 4) clearly indicated the repair of β-carotene radicals. The extent of repair may be quantified by the following equation:

$$\% \text{ Regeneration} = \frac{(\phi' - \phi^{o}_{expt})}{\phi^{o}_{expt}} \times 100$$

The data on percentage repair is presented in Table 4. The experimentally determined quantum yield (ϕ_{expt}) values were higher than the quantum yield (ϕ_{cal}) values calculated using Eq. (3) under the assumption that chlorogenic acid acts only as a *t*-$BuO^{\bullet}$ radical scavenger. This showed that chlorogenic acid acted not only as an efficient scavenger of *t*-$BuO^{\bullet}$ radicals, but also as an agent for the repair of β-carotene radicals.

The nature of interactions of CGA with other antioxidants is essential for understanding the effects of this compound in oxidative stress conditions *in vivo*. Whether a synergistic or antagonistic effect is observed for the mixtures derived from radical oxidation may depend on the chemical structure of the molecules, Bond Dissociation Energy (BDE), redox potentials, microenvironment of the reaction and the possible formation of stable intermolecular complexes.

TABLE 4: EFFECT OF VARYING [β-CAROTENE] ON THE RATE AND QUANTUM YIELD OF PHOTOOXIDATION OF CHLOROGENIC ACID IN THE PRESENCE OF *t*-BuOOH IN *t*-BuOH-WATER (4:1 v/v) MEDIUM

$10^5 \times$ [β-carotene] (mol dm^{-3})	$10^9 \times$ Rate (mol dm^{-3} s^{-1})	ϕ_{expt}	ϕ_{cal}	p	ϕ’	% scavenging	% regeneration
0.0	9.6909	0.00644	0.00644	1.0000	0.00644	100.0	0.00
1.0	9.4820	0.00630	0.00593	0.9203	0.00685	92.03	6.32
2.0	8.8722	0.00590	0.00549	0.8524	0.00692	85.24	7.41
5.0	8.3291	0.00553	0.00449	0.6979	0.00793	69.79	23.1
8.0	7.4264	0.00493	0.00380	0.5908	0.00835	59.08	29.7
10.0	7.0342	0.00467	0.00345	0.5360	0.00872	53.60	35.4

[chlorogenic acid] = 2.0×10^{-5} mol dm^{-3}, [*t*-BuOOH] = 5.0×10^{-3} mol dm^{-3}, Light intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, Temperature = 298 K

In general, regeneration between antioxidants occurs when BDE of an antioxidant is lower, or at least similar to that of other antioxidants [34, 35]. The BDE of CGA (~80.0 kcal/mol) is higher than that of β-carotene (74.0 kcal/mol) for the removal of weaker hydrogen in the molecule. CGA has more BDE hence its regeneration is unlikely by β-carotene. However, β-carotene which has less BDE might be regenerated by CGA.

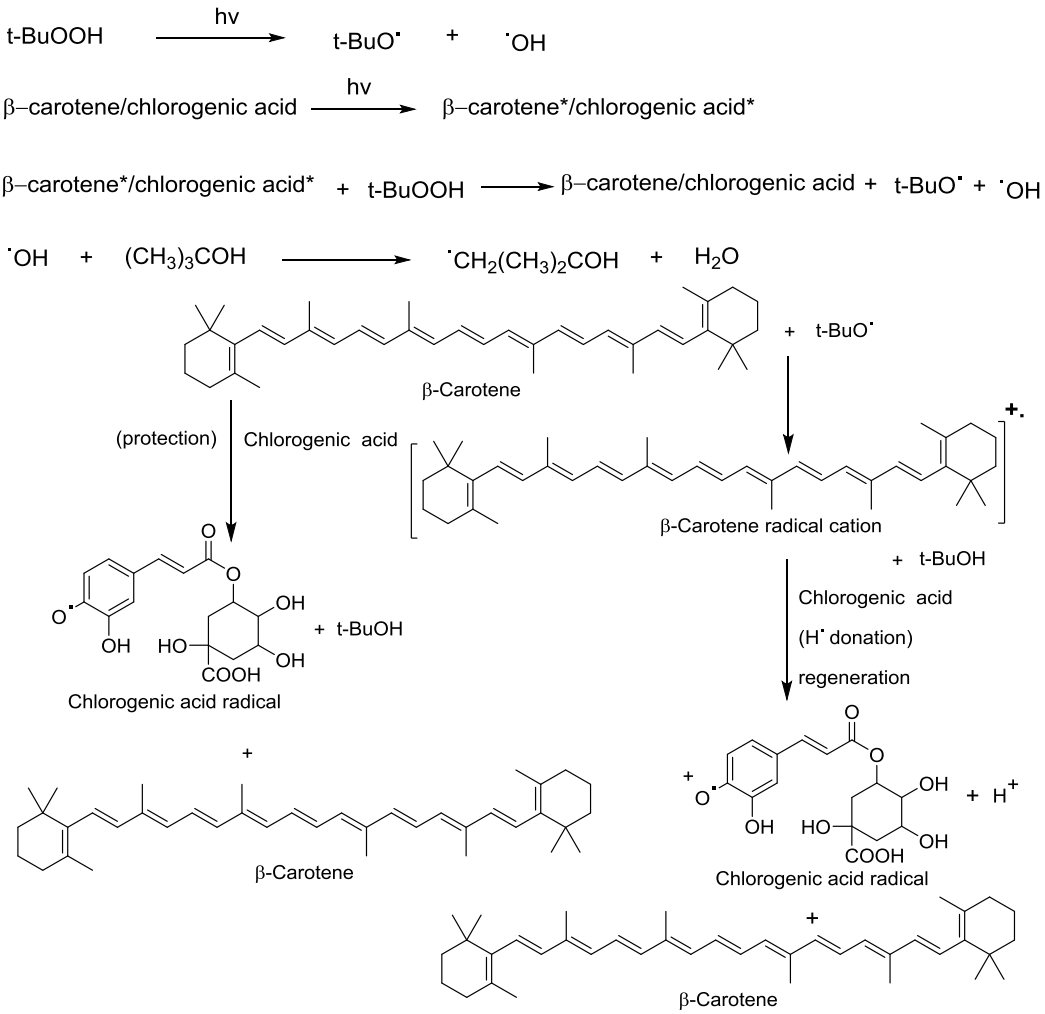
CGA has redox potential of 0.54 V vs SCE is lower compared to that of β-carotene (1.06V). The BDE of the OH bond of phenolics along with redox potentials control the regeneration of antioxidants. The CGA molecule with higher OH Bond Dissociation Energy and a redox potential of 0.54 V is capable of regenerating β-carotene as evident from the data in Table 4.

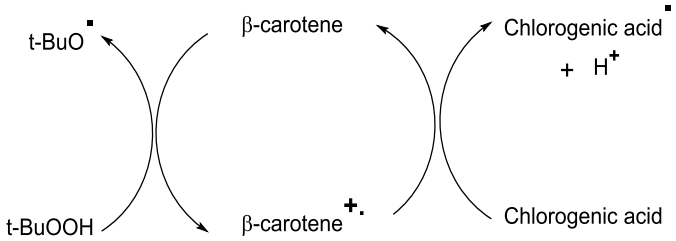
CGA regenerates β-carotene (β-CAR) by reducing the β-carotene radical cation (β-$CAR^{+\bullet}$) as shown in the equation below;

$$CGA + \beta\text{–}CAR^{+\cdot} \longrightarrow CGA^{\bullet} + \beta\text{–}CAR + H^{+}$$

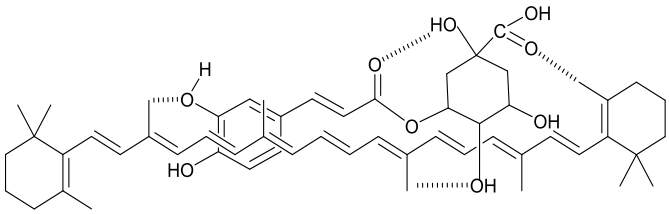
The proposed mechanism for the reaction between CGA and β-carotene may involve the reduction of β-carotene radical by CGA at the LDL surface with concomitant production of CGA o-semiquinone (phenoxyl radical).

On the basis of the experimental results and the above discussion, the scheme for the synergistic interaction of β-carotene with CGA and regeneration of β-carotene from β-carotene radical as a representative by CGA is given as follows:





Comparison of the results for the photooxidation of CGA in the absence and presence of β-carotene suggested that CGA acts as a synergist and β-carotene acts as a primary antioxidant. Stable intermolecular complexes could be formed between β-carotene and CGA as shown above which is similar to anthocyanin and CA or rutin in the co-pigmentation mechanism [36, 18]. These interactions could be due to π-π stacking between the aromatic ring of phenolic acid and β-carotene by hydrogen-bonding effects that would help in stabilizes the complex [19]. A higher stability of the complex formed between CGA with β-carotene due to better structural analogy and additional bonding between the two molecules, could explain 35.4% of β-carotene regeneration by CGA.



POSSIBLE INTERACTIONS OCCURRING IN β-CAROTENE / CGA COMPLEX

ACKNOWLEDGEMENT: Authors thank Head, Department of Chemistry, Osmania University, Hyderabad, for providing facilities to carry out the research work.

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How to cite this article:
Vijayalakshmi G, Adinarayana M and Rao PJ: A synergistic approach to kinetic and mechanistic studies of regeneration of β-carotene from *tert*-butoxyl radical induced β-carotene radical cation by chlorogenic acid. *Int J Pharm Sci Res* 2014; 5(3): 942-50.doi: 10.13040/IJPSR.0975-8232.5(3).942-50