ABSTRACT
The rates of oxidation of α-tocopherol and chlorogenic acid by tert-butoxy radicals (t-BuO•) were studied by measuring the absorbance of α-tocopherol at 294 nm and chlorogenic acid at 328 nm spectrophotometrically. Radicals (t-BuO•) were generated by the photolysis of tert-butyl hydroperoxide (t-BuOOH) in presence of tert-butyl alcohol to scavenge OH• radicals. The rates and the quantum yields (φ) of oxidation of chlorogenic acid by t-BuO• radicals were determined in the absence and presence of varying concentrations of α-tocopherol. An increase in the concentration of α-tocopherol was found to decrease the rate of oxidation of chlorogenic acid, suggesting that α-tocopherol and chlorogenic acid competed for t-BuO• radicals. From competition kinetics, the rate constant of α-tocopherol reaction with t-BuO• was calculated to be 7.28 x 10^8 dm^3 mol^-1 s^-1. The quantum yields (φ_{exp}) 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• radicals only, the quantum yields (φ_{cal}) were theoretically calculated. φ_{exp} and φ_{cal} values suggested that chlorogenic acid not only protected α-tocopherol from t-BuO• radicals, but also regenerated α-tocopherol from α-tocopheroxyl radicals, formed by the reaction of α-tocopherol with t-BuO• radicals. Results indicated a possible synergistic interaction between chlorogenic acid and α-tocopherol in which 52.1% of α-tocopherol was regenerated by chlorogenic acid.

Keywords: chlorogenic acid, α-tocopherol, regeneration, synergism, tert-butoxy radicals, oxidation

[I] INTRODUCTION
Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) have been shown to possess many characteristics of carcinogens [1, 2]. In living aerobic cells, ROS are continuously generated by inflammatory, ischemia-reperfusion injury, xenobiotic metabolism as well as physiological mitochondria respiration. Organic peroxides form an important part of various chemical, pharmaceutical and cosmetic products.
Upon reduction or oxidation by the cytochrome P450 enzyme family, by other heme proteins and by low molecular weight metal ion complexes, these hydroperoxides produce alkoxyl and hydroxyl radicals. Although DNA is a stable and well-protected molecule, these radicals can interact with it and cause several types of damage: modification of DNA bases, single and double strand breaks, loss of purines (apurinic sites), damage to the deoxyribose sugar, DNA-purine cross-linkage and damage to the DNA repair system [3,4]. Oxidative DNA damage has been thought to be an important source of mutation leading to aging [5] and a wide range of degenerative diseases such as cardiovascular disease, immune-system decline, brain dysfunction and cataracts [6]. 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. Recent studies have demonstrated that the exposure of cultured cells to alkoxyl radicals resulted in the generation of DNA strand breaks [7-10], though the mechanism of damage has not been elucidated. tert-Butyl hydroperoxide (t-BuOOH) has been chosen as a model peroxide which on homolysis gives •OH and t-BuO• 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 [11]. The experimental evidence indicates that base radicals also contribute to strand breaks by transfer of their radical sites from base moiety to sugar moiety.

The implication of oxidative and nitrosative stress in the etiology and progression of several acute and chronic clinical disorders has led to the suggestion that antioxidants can have health benefits as prophylactic agents. In a biological system, an antioxidant can be defined as “any substance that when present at low concentrations compared to that of an oxidizable substrate would significantly delay or prevent oxidation of that substrate.” The oxidizable substrate may be any molecule that is found in foods or biological materials, including carbohydrates, DNA, lipids and proteins. The function of an antioxidant is to retard the oxidation of an organic substance, thus increasing the useful life or shelf life of that material.

Antioxidants, such as phenolics, are widely distributed in the plant kingdom and are therefore, an integral part of the diet, with significant amounts being reported in fruits, vegetables and beverages [12]. Several studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk of cardiovascular diseases and certain forms of cancer [13, 14]. Accumulating chemical, biochemical, clinical and epidemiological evidence supports the chemoprotective effects of phenolic antioxidants against oxidative stress-mediated disorders.

Chlorogenic acid (CGA), an ester of caffeic acid with quinic acid, is found in a wide range of fruits and vegetables and coffee. Phenolcarboxylic acids such as CGA exert beneficial effects on human health through prevention of degenerative pathologies such as cardiovascular diseases and cancer, scavenges radicals generated in the aqueous phase [15], increases the resistance of LDL to lipid peroxidation [16] and inhibits DNA damage [17]. In vivo, when added to the diet, it inhibits chemically induced carcinogenesis of large intestine, liver and tongue in rats and hamsters [18, 19].

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 components when used separately [20]. Two types of synergism are observed: one involving primary antioxidants only and other involving a combination of primary antioxidants with metal chelators or peroxy scavengers. 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. Several mechanisms are involved in synergism among antioxidants: one among them involves combination of two or more different free radical scavengers in which one antioxidant is regenerated by others. Regeneration of a more effective free radical scavenger (primary antioxidant) by a less effective free radical scavenger (coantioxidant, synergist) occurs mostly when one free radical scavenger has a higher reduction potential than the other. The antioxidant system of ascorbic acid and tocopherols is an example in which tocopherols (E = 500 mV) are primary antioxidant and ascorbic acid (E = 330 mV) acts as a synergist [21, 22].

During the past two decades, intensive research has been carried out on naturally occurring antioxidative compounds from different sources. The main drive behind this search was to reduce the use of synthetic compounds as food additives because of their potential negative health effects and as a result of consumer demand. In this context, studies involving chlorogenic acid assume importance due to its presence in many dietary phytochemicals in higher concentrations. The \( t \)-BuO• radicals have been generated by steady-state photolysis of \( t \)-butyl hydroperoxide in the presence of \( t \)-BuOH to scavenge the hydroxyl radicals in aqueous solution [23]. In the present paper, the reactions of \( t \)-BuO• radicals with \( \alpha \)-tocopherol have been studied in the presence of chlorogenic acid to assess the protection by chlorogenic acid towards oxidation of \( \alpha \)-tocopherol by \( t \)-BuO• radicals and also regeneration, if any offered by chlorogenic acid towards \( \alpha \)-tocopherol radicals.

**[II] MATERIALS AND METHODS**

CGA and \( \alpha \)-tocopherol were purchased from Sigma Chemical Co., St. Louis, USA and used as received. All solutions were prepared afresh using double-distilled water. \( t \)-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 [24]. 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 [25]. On photolysis, \( t \)-BuOOH was activated at 254 nm to generate \(^\cdot\)OH and \( t \)-BuO• radicals by homolytic cleavage of –O-O-bond [26]. The \(^\cdot\)OH radicals produced were scavenged using sufficient concentration of \( t \)-BuOH [23]. 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 \( \lambda_{\text{max}} \) 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 CGA at 328 nm at which \( \alpha \)-tocopherol was totally transparent. It is known that \( t \)-BuOOH is activated to radical reaction by the absorption of light at 254 nm [27]. However, the substrates used in the present work, viz., CGA and \( \alpha \)-tocopherol have strong absorption in this region. But, in the absence of \( t \)-BuOOH in the reaction mixture, CGA, \( \alpha \)-tocopherol or CGA-\( \alpha \)-tocopherol 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 \( \alpha \)-tocopherol
and/or CGA, a considerable chemical change was observed with α-tocopherol as well as CGA. If α-tocopherol and CGA acted as only inner filters, the rates of the reaction of α-tocopherol or CGA with t-BuO• radicals would have been decreased with increase in concentration of α-tocopherol or CGA. 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 CGA in the presence of α-tocopherol would have been much less than the experimentally observed values (Table 4). Hence, we proposed that the excited states of CGA and α-tocopherol 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 α-tocopherol as well as CGA under different experimental conditions.

[III] RESULTS AND DISCUSSION

The oxidation of α-tocopherol by t-BuO• radicals was carried out by irradiating the reaction mixture containing known concentrations of α-tocopherol and t-BuOOH in the presence of sufficient amount of t-BuOH to scavenge the \( \alpha \)OH radicals completely [23]. The reaction was followed by measuring the absorbance of α-tocopherol at 294 nm (\( \lambda_{\text{max}} \) of α-tocopherol) with time. The initial rates and quantum yields of oxidation of α-tocopherol by t-BuO• are presented in Table 1. The initial rates of photooxidation of CGA by t-BuOOH in presence of t-BuOH were calculated from the plots of absorbance of CGA at 328 nm vs time using microcal origin computer program on a personal computer (Table 2). UV-visible absorption spectra of CGA in presence of t-BuOOH and t-BuOH at different irradiation times were recorded (Fig. 1). In order to find the protection offered to α-tocopherol by CGA towards oxidation by t-BuO•, the reaction mixture containing known concentrations of α-tocopherol and t-BuOOH was irradiated in presence of varying concentrations of CGA. The reactions were followed by measuring the absorbance of CGA at 328 nm (Fig. 2) at which α-tocopherol was transparent and the rate data are presented in Table 3. The photooxidation of CGA by t-BuO• at different concentrations of α-tocopherol was also studied (Fig. 3) and the data are presented in Table 4.

The oxidation rate of α-tocopherol in the presence of t-BuOH refers exclusively to the reaction of t-BuO• with α-tocopherol. These rates were found to increase with increase in concentration of α-tocopherol as well as t-BuOOH. The quantum yield values were also increased with increase in α-tocopherol as well as t-BuOOH (Table 1). The rate of oxidation of CGA increased with increase in concentration of CGA (Table 2). The quantum yields of oxidation of CGA were calculated from the initial rates and the light intensity at 254 nm. These values were also increased with increase in concentration of CGA (Table 2). Having known the rates of t-BuO• radical reactions with α-tocopherol as well as CGA under varying experimental conditions, both α-tocopherol and CGA were introduced for the competitive studies with t-BuO• radical. Aqueous solutions of reaction mixture containing CGA, and t-BuOOH were irradiated in presence of varying concentrations of α-tocopherol (Fig. 3). The initial rates and quantum yields of oxidation of CGA by t-BuO• radicals were found to decrease with increase in concentration of α-tocopherol (Table 4). Comparison of the initial rates and quantum yields of oxidation of CGA in presence and absence of α-tocopherol clearly indicated that the initial rates and quantum yields of oxidation of CGA were substantially decreased in presence of α-tocopherol (Table 4). These observations clearly demonstrated that α-tocopherol and CGA was in competition for t-BuO• radicals.

The rate constant of the reaction of t-BuO• with CGA has been reported [28] to be \( 3.20 \times 10^9 \) dm\(^3\)
mol\(^{-1}\) s\(^{-1}\) under similar experimental conditions of the present work. The rate constant for the reaction of \(t\text{-BuO}\) with \(\alpha\)-tocopherol was calculated by the adenosine competition method, which was very similar to the method [29] used to determine the rate constant for the reaction of \(\text{OH}\) radicals with polyhydric alcohols in competition with KSCN. In the present study, solutions containing CGA and varying amounts of \(\alpha\)-tocopherol in presence of \(t\text{-BuOOH}\) was irradiated for 2 min and the decrease in absorbance of CGA was measured. The decrease in absorbance of CGA reflected the amount of \(t\text{-BuO}\) radicals that had reacted with CGA. From the known rate constant of the reaction of CGA with \(t\text{-BuO}\) radical under similar experimental conditions of the present work \((k_{\text{CGA}} = 3.20 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1})\), the rate constant of \(t\text{-BuO}\) radical reaction with \(\alpha\)-tocopherol \((k_{\alpha\text{-tocopherol}})\) can be calculated using the equation (1):

\[
\frac{[\text{Absorbance of chlorogenic acid}]_0}{[\text{Absorbance of chlorogenic acid}]_{\alpha\text{-tocopherol}}} = 1 + \frac{k_{\alpha\text{-tocopherol}} [\alpha\text{-tocopherol}]}{k_{\text{chlorogenic acid}} [\text{chlorogenic acid}]} \tag{1}
\]

Fig. 1 - Absorption spectra of photooxidation of \(\alpha\)-tocopherol in the presence of \(t\text{-butyl hydroperoxide}\) at different irradiation times; [\(\alpha\)-tocopherol] = 5 \times 10^{-5} \text{ mol d}m^{-3}, [\(t\text{-BuOOH}\)] = 5 \times 10^{-3} \text{ mol d}m^{-3}, \text{Light intensity} = 2.7168 \times 10^{15} \text{ quanta s}^{-1}, \lambda_{\text{max}} = 294 \text{ nm}, \text{pH} \sim 7.5, \text{temperature} = 298 \text{ K, [t-BuOH]} = 1.0 \text{ M

Fig. 2 - Absorption spectra of photooxidation of CGA in the presence of \(t\text{-butyl hydroperoxide}\) at different irradiation times in \(t\text{-BuOH-water} (4:1 \text{ v/v}) \text{ medium, [CGA]} = 10.0 \times 10^{-6} \text{ mol d}m^{-3}, [\(t\text{-BuOOH}\)] = 5 \times 10^{-3} \text{ mol d}m^{-3}, \text{Light intensity} = 2.7168 \times 10^{15} \text{ quanta s}^{-1}, \lambda_{\text{max}} = 328 \text{ nm, pH} \sim 7.5, \text{temperature} = 298 \text{ K}

Fig. 3 – Absorption spectra of photooxidation of CGA in the presence of \(t\text{-butyl hydroperoxide and \(\alpha\)-tocopherol at different irradiation times; [CGA]} = 2 \times 10^{-5} \text{ mol d}m^{-3}, [\(t\text{-BuOOH}\)] = 5 \times 10^{-3} \text{ mol d}m^{-3}, [\(\alpha\text{-tocopherol]} = 5 \times 10^{-5} \text{ mol d}m^{-3}, \text{Light Intensity} = 2.7168 \times 10^{15} \text{ quanta s}^{-1}, \lambda_{\text{max}} = 328 \text{ nm, pH} \sim 7.5, \text{temperature} = 298 \text{ K

Fig. 4 - Effect of [\(\alpha\)-tocopherol] on the oxidation of CGA by \(t\text{-BuO}^\bullet\) in \(t\text{-BuOH-water 4:1 (v/v)} \text{ neutral medium.

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In Eq. (1), [Absorbance of CGA]₀ and [Absorbance of CGA]₆-tocopherol are the absorbance values of CGA in the absence and presence of α-tocopherol, 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⁺ radical with α-tocopherol (k₆-tocopherol) was calculated at different concentrations of CGA and α-tocopherol and the average of these was found to be 7.28 × 10⁸ dm³ mol⁻¹ s⁻¹. As CGA had strong absorption at 294 nm, it is not possible for the direct determination of protection and repair offered to α-tocopherol by CGA. However, one could calculate indirectly the extent of protection offered to α-tocopherol from competition kinetic studies measured at 328 nm, λₘₐₓ of CGA. The method was as follows:

When the system containing α-tocopherol, CGA and t-BuOOH was irradiated, the probability of t-BuO⁺ radicals reacting with CGA {p(t-BuO⁺ + CGA)} was calculated using the following equation:

\[
p(t-BuO^+ + \text{chlorogenic acid}) = \frac{k_{\text{chlorogenic acid}}[\text{chlorogenic acid}]}{k_{\alpha\text{-tocopherol}}[\alpha\text{-tocopherol}]} + k_{\text{chlorogenic acid}}[\text{chlorogenic acid}]
\]  

(2)

If CGA scavenged only t-BuO⁺ radicals and did not give rise to any other reaction (e.g. reaction with α-tocopherol radicals), the quantum yield of oxidation of CGA (φ_cal) at each concentration of α-tocopherol may be given by equation:

\[
φ_{\text{cal}} = φ_{\text{expt}} p
\]  

(3)

where φ_{expt} is the quantum yield of oxidation of CGA in the absence of α-tocopherol, and p is the probability given by Eq. (2).

The calculated quantum yield (φ_cal) values at different α-tocopherol 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 CGA molecules was consumed in the system than expected and the most likely route for this was H atom donation by CGA to α-tocopherol radicals. In Table 4, are presented the fraction of t-BuO⁺ radicals scavenged by CGA at different concentrations of α-tocopherol. These values referred to the measure of protection offered to α-tocopherol due to scavenging of t-BuO⁺ radicals by CGA. Using the φ_{expt} values, a set of values, viz., φ’ values were calculated from Eq. (4) and are presented in Table 4

\[
φ' = \frac{φ_{\text{expt}}}{p}
\]  

(4)

where φ’s represent the experimentally found quantum yield values if no scavenging of α-tocopherol radicals by CGA occurs. In the absence of any “repair” of α-tocopherol radicals by CGA, the φ’ values should all be equal to φ_{expt}'s. The observed increase in φ’ with increasing α-tocopherol concentration (Table 4) clearly indicated the repair of α-tocopherol radicals. The extent of repair may be quantified by the following equation:

\[
\% \text{Repair} = \left( \frac{φ' - φ_{\text{expt}}}{φ_{\text{expt}}} \right) \times 100
\]  

(5)

Table 1 – Effect of [α-tocopherol] and [t-BuOOH] on the rates and quantum yields of photo oxidation of α-tocopherol by t-BuOOH in t-BuOH-water (4:1 v/v) medium. Light intensity = 2.7168 × 10¹⁵ quanta s⁻¹. λₘₐₓ = 294 nm, pH ~ 7.5, Temperature = 298 K
REGENERATION OF α-TOCOPHEROL FROM PHOTOCHEMICALLY GENERATED t-BUTOXYL RADICAL INDUCED α-TOCOPHEROXYL

Table 2 - Effect of [CGA] and [t-BuOOH] on the rates and quantum yields of photooxidation of CGA by t-BuOOH in t-BuOH-water (4:1 v/v) medium. Light intensity = 2.7168 × 10¹⁵ quanta s⁻¹, λmax = 328 nm, pH ∼ 7.5, Temperature = 298 K

<table>
<thead>
<tr>
<th>[CGA] (mol dm⁻³)</th>
<th>[t-BuOOH] (mol dm⁻³)</th>
<th>Initial rate (mol dm⁻³ s⁻¹)</th>
<th>Quantum yield (φ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
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<td>9.6908</td>
<td>0.006445</td>
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<tr>
<td>10.0</td>
<td>5.0</td>
<td>7.0008</td>
<td>0.004656</td>
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<td>0.003511</td>
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<td>5.0</td>
<td>2.7845</td>
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<td>5.0</td>
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<td>20.0</td>
<td>15.0</td>
<td>13.157</td>
<td>0.008750</td>
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</table>

Table 3 – Effect of [CGA] on the rates and quantum yields of oxidation of CGA by t-BuO⁺ in t-BuOH-water 4:1 (v/v) neutral medium in the presence and absence of α-tocopherol. Light intensity = 2.7168 × 10¹⁵ quanta s⁻¹, λmax = 328 nm, pH ∼ 7.5, Temperature = 298 K

<table>
<thead>
<tr>
<th>[α-tocopherol] (mol dm⁻³)</th>
<th>[CGA] (mol dm⁻³)</th>
<th>10⁷× Rate (mol dm⁻³ s⁻¹)</th>
<th>Quantum yields φ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
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<tr>
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<td>0.5</td>
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<td>1.3701</td>
<td>0.00097</td>
</tr>
</tbody>
</table>

Table 4 – Effect of varying [α-tocopherol] on the rate and quantum yield of photooxidation of CGA in the presence of t-BuOOH in t-BuOH-water 4:1 (v/v) neutral medium

<table>
<thead>
<tr>
<th>[α-tocopherol] (mol dm⁻³)</th>
<th>10⁷× Rate(mol dm⁻³ s⁻¹)</th>
<th>Quantum yield (φ)</th>
</tr>
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<tr>
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<td>10.0</td>
<td>6.8922</td>
<td>0.004589</td>
</tr>
</tbody>
</table>

The data on percentage repair is presented in Table 4. The experimentally determined quantum yield (φexpt) values were higher than the quantum yield (φcal) values calculated using Eq. (3) under the assumption that CGA acts only as a t-BuO⁺ radical scavenger. This showed that CGA acted not only as an efficient scavenger of t-BuO⁺ radicals, but also as an agent for the repair of α-tocopherol radicals.

\[
\text{CGA} + \alpha\text{-TO}^* \rightarrow \text{CGA}^* + \alpha\text{-TOH}
\]

The reduction potential of CGA and Trolox (vitamin E equivalent) at pH 7 is 0.54 V and 0.48 V respectively. α-Tocopherol regeneration by CGA is more preferable partly because of the higher standard reduction potential of the phenoxy radical of CGA (o-semiquinone) than that of the tocopheroxyl radical [30]. CGA regenerates α-tocopherol by reducing the α-tocopheroxyl radical as shown in the following equation. The proposed mechanism for the reaction between CGA and α-tocopherol may involve the reduction of α-tocopheroxyl radical by CGA at the LDL surface with concomitant production of CGA o-semiquinone (phenoxy radical). The molecular mechanisms involving the regeneration of α-tocopherol by CGA is given in the scheme 1.

**Scheme**

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On the basis of the experimental results and the above discussion, the protection of α-tocopherol and repair of α-tocopheroxyl radical by CGA is proposed as scheme 2.

[V]CONCLUSIONS
Comparison of the results for the photooxidation of CGA in the absence and presence of α-tocopherol suggested that a synergistic interaction existed between CGA and α-tocopherol. Peyrat-Maillard et al [31] have studied the synergistic and antagonistic effects occurring between pairs of phenolic antioxidants in a mixture. Their results indicated that significant mixture effect (ME) was found between α-tocopherol and cinnamic acids. α-tocopherol with low antioxidant power (AOP) would be regenerated by the more powerful phenolic acids. Regeneration of a more effective free radical scavenger (primary antioxidant) by a less effective free radical scavenger (coantioxidant, synergist) occurs mostly when one free radical scavenger has a higher reduction potential than the other [30]. The α-tocopherol with higher reduction potential acts as primary antioxidant and CGA acts as a synergist. Regeneration of primary antioxidants contributes to a higher net interactive antioxidant effect than the simple sum of individual effects.

Possible interactions occurring in α-tocopherol / CGA complex
Stable intermolecular complexes could be formed between α-tocopherol and CGA as already been suggested with anthocyanin and CA or rutin in the co-pigmentation mechanism [32]. These interactions could be due to π-π stacking between the aromatic ring of phenolic acid and the chroman moiety of α-tocopherol and hydrogen-bonding effects would also help in stabilize the complex [33]. This higher stability of the complex formed between CGA with α-tocopherol due to better structural analogy and additional bonding between the two molecules could partially explain that CGA regenerates 52.1% of α-tocopherol.
The CGA radicals were generated in the process of protection of α-tocopherol and repair of α-tocopherol radicals. These radicals were reported [34, 35] to have short lifetime, extremely unstable and rapidly convert to unknown compounds at physiological pH [36]. The nature of interactions of CGA with other antioxidants is essential for understanding the effects of this compound in oxidative stress conditions in vivo. This supported our contention that the CGA radicals might not involve in oxidative stress in our experimental conditions.

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