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A kinetic and mechanistic approach to protection and repair of *tert*-butoxyl radicals induced adenine radicals by chlorogenic acid

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ABSTRACT

The rates of oxidation of adenine and chlorogenic acid by tert-butoxyl radicals have been studied by measuring the absorbance of adenine at 260 nm and chlorogenic acid at 328 nm spectrophotometrically. tert-butoxyl radicals are generated by the photolysis of tert-butyl hydroperoxide 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 have been determined in the absence and presence of varying concentrations of adenine. An increase in the concentration of adenine has been found to decrease the rate of oxidation of chlorogenic acid suggesting that adenine and chlorogenic acid compete for t-BuO• radicals. From competition kinetics, the rate constant of chlorogenic acid reaction with adenine has been calculated to be 4.43 x 10^8 dm^3 mol^{-1} s^{-1}. The quantum yields (ϕ_{expt}) have been 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-butoxyl radicals only, the quantum yields (ϕ_{cal}) have been theoretically calculated. ϕ_{expt} and ϕ_{cal} values suggest that chlorogenic acid not only protects adenine from t-BuO• radicals but also repairs adenine radicals formed by the reaction of adenine with t-BuO• radicals.

Keywords: chlorogenic acid, adenine, *t*-BuO• radicals, oxidation, protection, repair

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INTRODUCTION

Reactive Oxygen Species (ROS) are continuously released from various endogenous and exogenous sources resulting in continuous and accumulative oxidative damage to cellular components and alters many cellular functions [1]. Oxidative DNA damage has been thought to be an important source of mutation leading to aging [2] and a wide range of degenerative diseases such as cardiovascular disease, immune-system decline, brain dysfunction and cataracts [3].Among the biological targets most vulnerable to oxidative damage are proteinaceous enzymes, lipid membranes and DNA [4-7]. 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 peroxides produce alkoxyl and hydroxyl radicals. DNA is one of the main molecular targets of toxic effects of free radicals formed in mammalian cells during respiration, metabolism and phagocytosis. The lethal effects of the hydroxyl radicals on DNA and its constituents have been extensively studied [2] but 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 [8-10] though the mechanism of damage has not been elucidated. Organic oxygen radicals in particular alkoxyl radicals may participate in metabolic and pathological processes [11]. 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 [12]. The experimental evidence indicates that base

radicals also contribute to it by transfer of their radical sites from base moiety to sugar moiety. Strand breaks are considered to be a very serious kind of damage to DNA [13,14].

A large amount of evidence suggests that the dietary intake of phytochemicals plays an important role in maintaining health and protecting against degenerative process including cardiovascular diseases and certain cancers. Antioxidants are substances, when present in small quantities prevent the oxidation of cellular organelles by minimizing the damaging effects of oxidative stress. 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 [15]. They exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions [16-19]. Many of these biological functions have been attributed to their antioxidant activity, free radical scavenging, chelation of redox active metal ions, modulation of gene expression and interaction with the cell signaling pathways [20,21].Chlorogenic acid is a major polyphenol compound found in various plant products such as coffee, beans, potatoes and apples [22,23]. In vivo, when added to the diet, it inhibits chemically induced carcinogenesis of the large intestine, liver and tongue in rats and hamsters [24,25]. It is reported to prevent different cancers and cardiovascular diseases in several experimental studies in animal models. Chlorogenic acid is also found to have antioxidant [26] and anti-inflammatory properties [27]. Recently, in vivo studies suggested that chlorogenic acid provides beneficial effects duringischemia-reperfusion injury of rat liver and paraquat-induced oxidative stress in rats [28]. Chlorogenic acid is found to be a potent ROS and RNS radical scavenger. From our laboratory, caffeic acid has been reported [29, 30] to repair adenine radicals in addition to efficiently scavenging of $SO_4^{\bullet-}$ and *tert*-butoxyl radicals. Thus, studies involving chlorogenic acid assume importance due to its presence in many dietary phytochemicals in higher concentrations.It is in this background the kinetic study of oxidation of chlorogenic acid in the presence of adenine have been carried out to evaluate the extent of protection offered by chlorogenic acid against *t*-BuO$^{\bullet}$ radicals and also to characterize the nature of transient radicals formed on adenine.

The *t*-BuO$^{\bullet}$radicals have been generated by steady state photolysis of *tert*-butyl hydroperoxide in the presence of *t*-BuOH to scavenge the hydroxyl radicals in aqueous solution [31]. The reactions of *t*-BuO$^{\bullet}$radicals with adenine have been studied in the presence of chlorogenic acid with a view to assess the protection by chlorogenic acid towards oxidation of adenine by *t*-BuO$^{\bullet}$radicals and also repair, if any, offered by chlorogenic acid towards adenine radicals. Adenine is used as a model for DNA to understand the protection and repair by chlorogenic acid in the present study.

EXPERIMENTAL SECTION

Adenine and chlorogenic acid were purchased from sigma 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 [32]. 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 lamp. 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 (254nm) was measured using peroxydisulphate chemical actinometry [33]. On photolysis, *t*-BuOOH is activated at 254 nm to generate $^{\bullet}$OH and *t*-BuO$^{\bullet}$ radicals by homolytic cleavage of –O-O-bond [34]. The $^{\bullet}$OH radicals produced have been scavenged using sufficient concentration of *t*-BuOH [31]. In a typical kinetic run the aqueous reaction mixture of adenine, *t*-BuOOH and *t*-BuOH was taken in a specially designed one-centimeter path length quartz cuvette, suitable for both irradiations and absorbance measurements. The absorbance measurements were made at the λ_{max} of adenine (260 nm) on a Chemito UV-Visible spectrophotometer (model 2100).

The photochemical reaction of chlorogenic acid in the presence of *t*-BuOOH and other additives, viz., *t*-BuOH and adenine, has been followed by measuring the absorbance of chlorogenic acid at 328 nm at which adenine is totally transparent.

It is known that *t*-BuOOH is activated to radical reaction by the absorption of light at 254 nm [33]. However, the substrates used in the present work, viz., chlorogenic acid and adenine have strong absorption in this region. But in the absence of *t*-BuOOH, chlorogenic acid, adenine or chlorogenic acid-adenine mixture has not undergone any observable chemical change on shining the light. Even though a small fraction of the total light intensity is absorbed by *t*-BuOOH directly in the presence of adenine and/or chlorogenic acid, a considerable chemical change has been observed with adenine as well as chlorogenic acid. If adenine and chlorogenic acid act as only inner filters, the rates of the reaction of adenine or chlorogenic acid with *t*-BuO$^{\bullet}$radicals would have been decreased with increase in concentration of adenine or chlorogenic acid. But the results in Table 1 and 2 are contrary to this. One another fact

against the inner filter concept is that the rate of oxidation of chlorogenic acid in the presence of adenine would have been much less than the experimentally observed values (Table 4). Hence, we propose that the excited states of chlorogenic acid and adenine act as sensitizers to transfer energy to *t*-BuOOH to produce radical species. This type of sensitizing effect has been proposed in similar systems earlier [29]. Therefore, the light intensity at 254 nm has been used to calculate the quantum yields of oxidation of adenine as well as chlorogenic acid under different experimental conditions.

RESULTS AND DISCUSSION

The oxidation of adenine by *t*-BuO$^{\bullet}$radicals has been carried out by irradiating the reaction mixture containing known concentrations of adenine and *t*-BuOOH in the presence of sufficient amount of *t*-BuOH to scavenge the $^{\bullet}$OHradicals completely [31].The reaction was followed by measuring the absorbance of adenine at 260 nm (λ_{max} of adenine) with time. The initial rates and quantum yields of oxidation of adenine by *t*-BuO$^{\bullet}$ are presented in Table 1. The initial rates of photooxidation of chlorogenic acid by *t*-BuOOH in presence of *t*-BuOH have been 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.1). In order to find the protection offered to adenine by chlorogenic acid towards oxidation by *t*-BuO$^{\bullet}$, the reaction mixture containing known concentrations of adenine, *t*-BuOOH and *t*-BuOH was irradiated in presence of varying concentrations of chlorogenic acid. The reactions were followed by measuring the absorbance of chlorogenic acid at 328 nm (Fig.2) at which adenine is transparent and the rate data are presented in Table 3. The photooxidation of chlorogenic acid by *t*-BuO$^{\bullet}$radicals at different concentrations of adenine was also studied (Fig.3) and the data are presented in Table 4.

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

Table 1-Effect of [*t*-BuOOH] and [adenine] on the rate and quantum yield of photooxidation of adenine by *t*-BuOOH in the presence of light in aqueous neutral medium

$10^5\times$ [adenine] (mol dm^{-3})	$10^3\times$ [*t*-BuOOH] (mol dm^{-3})	$10^{10}\times$ Rate (mol dm^{-3} s^{-1})	Quantum Yield (ϕ)
2.0	5.00	5.54	0.000285
4.0	5.00	6.16	0.000317
6.0	5.00	6.80	0.000350
8.0	5.00	7.50	0.000386
4.0	10.0	7.87	0.000405
4.0	15.0	9.36	0.000482

Light Intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 260 nm, pH ~ 7.5, Temperature = 298 K, [t-BuOH] = 1.0 mol dm^{-3}

Table 2 - Effect of [*t*-BuOOH] and [chlorogenic acid] on the rate and quantum yield of photooxidation of chlorogenic acid by *t*-BuOOH in the presence of light in *t*-BuOH-water 1:4 (v/v) medium

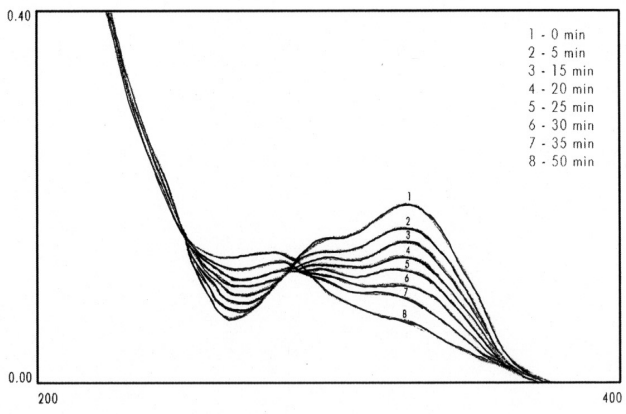
$10^6\times$ [chlorogenic acid] (mol dm^{-3})	$10^3\times$ [*t*-BuOOH] (mol dm^{-3})	$10^9\times$ Rate (mol dm^{-3} s^{-1})	Quantum Yield ϕ
20.0	5.00	9.6908	0.006445
10.0	5.00	7.0008	0.004656
8.00	5.00	5.2798	0.003511
5.00	5.00	2.7845	0.001852
2.00	5.00	2.2974	0.001528
20.0	10.0	11.2030	0.007451
20.0	15.0	13.1571	0.008750

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

The rate of oxidation of chlorogenic acid has been found to increase with increase in concentration of chlorogenic acid (Table 2). The quantum yields of oxidation of chlorogenic acid have been calculated from the initial rates and the light intensity at 254 nm. These values are also found to increase with increase in concentration of chlorogenic acid (Table 2). Having known the rates of *t*-BuO$^{\bullet}$ radical reactions with adenine as well as chlorogenic acid under varying experimental conditions, both adenine and chlorogenic acid are introduced for the competitive studies with *t*-BuO$^{\bullet}$ radical. Aqueous solutions of reaction mixture containing chlorogenic acid, *t*-BuOOH and *t*-BuOH were irradiated in presence of varying concentrations of adenine (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 adenine (Table 4). Comparison of the initial rates and quantum yields of oxidation of chlorogenic acid in presence and absence of adenine clearly indicate that the initial rates and quantum yields of oxidation of chlorogenic acid are substantially

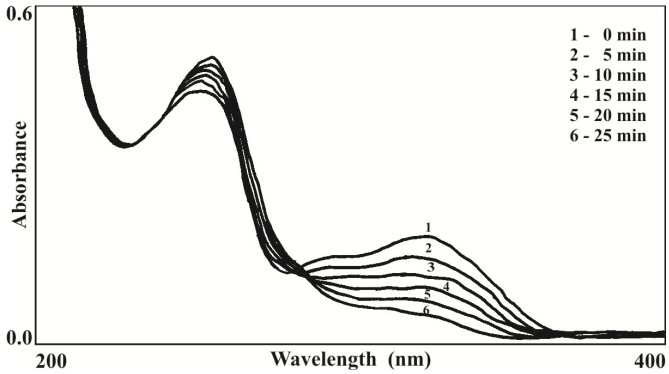
decreased in presence of adenine (Table 4). These observations clearly demonstrate that adenine and chlorogenic acid are in competition for *t*-BuO$^{\bullet}$ radicals.

Fig. 1



Absorption spectra of photooxidation of chlorogenic acid in the presence of tert-butyl hydroperoxide at different irradiation times; [chlorogenic acid] = 1 × 10^{-5}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 = 298K

Fig. 2



Absorption spectra of photooxidation of chlorogenic acid in the presence of tert-butyl hydroperoxide and adenine at different irradiation times; [chlorogenic acid] = 1 × 10^{-5}mol dm^{-3}, [t-BuOOH] = 5 × 10^{-3}mol dm^{-3}, [adenine] = 5 × 10^{-5} mol dm^{-3}, Light Intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, temperature = 298K

The rate constant of the reaction of *t*-BuO$^{\bullet}$with chlorogenic acid has been reportedto be 3.20×10^{9} dm^{3} mol^{-1} s^{-1} under similar experimental conditions of the present work [35]. The rate constant for the reaction of *t*-BuO$^{\bullet}$ with adenine has been calculated by the chlorogenic acid competition method, which is very similar to the one chosen by Akhalaq*et al*[36]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 adenine in presence of *t*-BuOOH and *t*-BuOH were irradiated for two minutes and the decrease in absorbance of chlorogenic acid was measured. The decrease in absorbance of chlorogenic acid reflects the amount of *t*-BuO$^{\bullet}$ radicals that has reacted with chlorogenic acid. From the known rate constant of the reaction of chlorogenic acid with *t*-BuO$^{\bullet}$ radical [35] under similar experimental conditions of the present work ($k_{\text{chlorogenic acid}}$ = 3.20× 10^{9} dm^{3} mol^{-1}s^{-1}), the rate constant of t-BuO$^{\bullet}$ radical reaction with adenine (k_{adenine}) can be calculated using the following equation:

$$\frac{[\text{Absorbance of chlorogenic acid}]_o}{[\text{Absorbance of chlorogenic acid}]_{\text{adenine}}} = 1 + \frac{k_{\text{adenine}}\,[\text{adenine}]}{k_{\text{chlorogenic acid}}\,[\text{chlorogenic acid}]} \qquad (1)$$

In Eq. (1), [Absorbance of chlorogenic acid]$_o$ and [Absorbance of chlorogenic acid]$_{\text{adenine}}$ are the absorbance values of chlorogenic acid in the absence and presence of adenine 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 adenine (k_{adenine}) has been calculated at different concentrations of chlorogenic acid and adenine and the

average of these is found to be $4.43\times 10^8 dm^3\ mol^{-1}\ s^{-1}$. As chlorogenic acid has strong absorption at 260nm, it is not possible for the direct determination of protection and repair offered to adenine by chlorogenic acid. However, one can calculate indirectly the extent of protection offered to adenine by chlorogenic acid from competition kinetic studies measured at 328 nm, λ_{max} of chlorogenic acid. The method is as follows:

When the system containing adenine, chlorogenic acid and *t*-BuOOH in the presence of *t*-BuOH is irradiated, the probability of *t*-BuO$^\bullet$ radicals reacting with adenine { $p_{(t\text{-}BuO^\bullet + adenine)}$ }is calculated using the following equation:

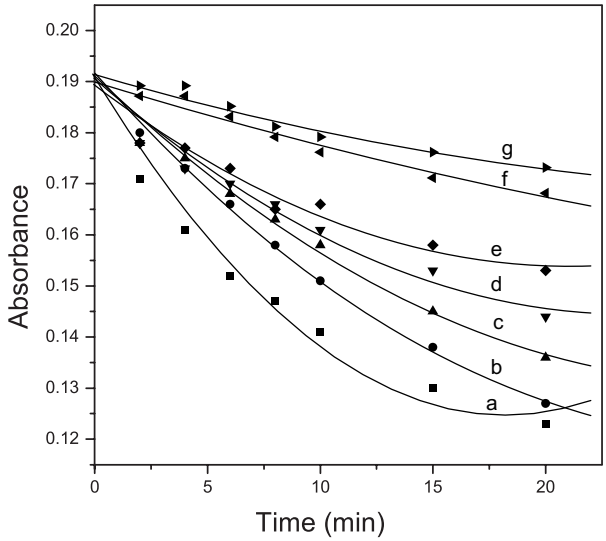
$$p_{(t\text{-}BuO^\bullet + adenine)} = \frac{k_{adenine}\ [adenine]}{k_{adenine}\ [adenine] + k_{chlorogenic\ acid}\ [chlorogenic\ acid]} \quad (2)$$

If chlorogenic acid scavenges only *t*-BuO$^\bullet$ radicals and does not give rise to any other reaction (e.g. reaction with adenine radicals), the quantum yield of oxidation of chlorogenic acid (ϕ_{cal}) at each concentration of adenine may be given by equation:

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

where$\phi^o_{expt}$is the quantum yield of oxidation of chlorogenic acid in the absence of adenine, and *p* is the probability given by Eq. (2).

Fig. 3



Effect of varying concentrations of adenine on the photooxidation of chlorogenic acid (1.0×10^{-5} mol dm^{-3}) in the presence of t-BuOOH (5×10^{-3}mol dm^{-3}) at 298 K. [adenine] = (a) 0.0, (b) 5×10^{-5}mol dm^{-3}, (c) 8×10^{-5}mol dm^{-3}, (d) 1×10^{-4}mol dm^{-3}, (e) 5×10^{-4}mol dm^{-3}, (f) 8×10^{-4}mol dm^{-3}, (g) 1×10^{-3}mol dm^{-3}, Light Intensity = 2.7168×10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5

The calculated quantum yield (ϕ_{cal}) values at different adenine concentrations are presented in Table 4. The data show that the ϕ_{cal}values are lower than the experimentally measured quantum yield (ϕ_{expt}) values. This indicates that more number of chlorogenic acid molecules is consumed in the system than expected and the most likely route for this is H atom donation by chlorogenic acid to adenine radicals. In Table 4, are presented the fraction of *t*-BuO$^\bullet$ radicals scavenged by chlorogenic acid at different concentrations of adenine. These values refer to the measure of protection offered toadenine due to scavenging of *t*-BuO$^\bullet$ radicals by chlorogenic acid. Using the ϕ_{exptl} values, a set of values, viz., ϕ'values have been calculated from Eq. (4) and are presented in Table 4.

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

whereϕ's represent the experimentally found quantum yieldvalues if no scavenging of adenine radicals by chlorogenic acid occurs. In the absence of any "repair" of adenine radicals by chlorogenic acid, the ϕ'values should all be equal to ϕ^o_{expt}. The observed increase in ϕ' with increasing adenine concentration (Table 4) clearly indicates that repair of adenine radicals does occur. The extent of repair may be quantified by the following equation:

$$\% \text{ Repair} = \frac{(\phi' - \phi^{o}_{expt})}{\phi^{o}_{expt}} \times 100 \qquad (5)$$

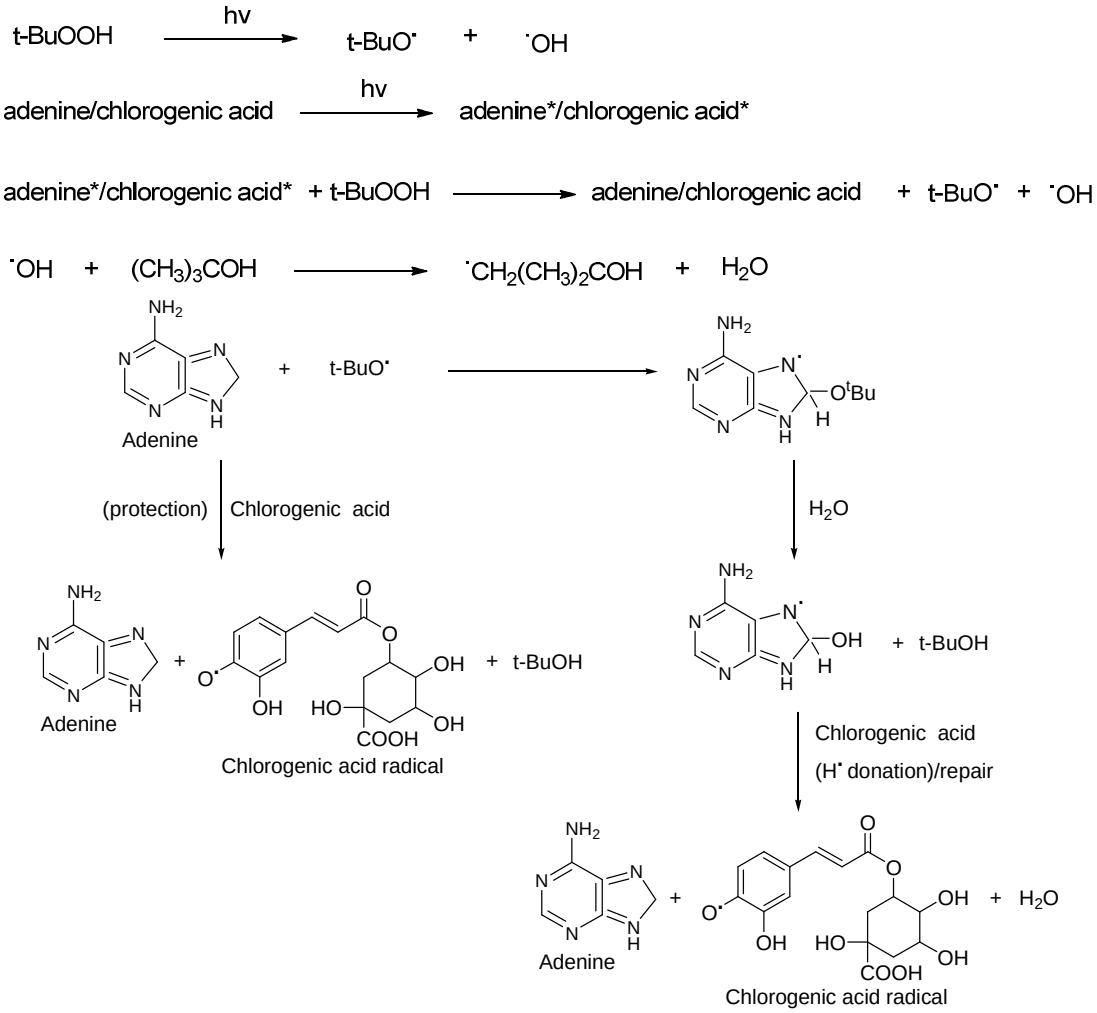
Table – 3: Effect of varying [chlorogenic acid] on the rate and quantum yield of photooxidation of chlorogenic acid by *t*-BuOOH in the absence and presence of adenine in *t*-BuOH-water (1:4 v/v) medium

$10^5\times$ [chlorogenic acid] (mol dm^{-3})	$10^5\times$ [adenine] (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
2.0	5.0	8.1370	0.00541
1.0	5.0	5.9565	0.00396
0.8	5.0	2.3726	0.00158
0.5	5.0	1.6541	0.00110

[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

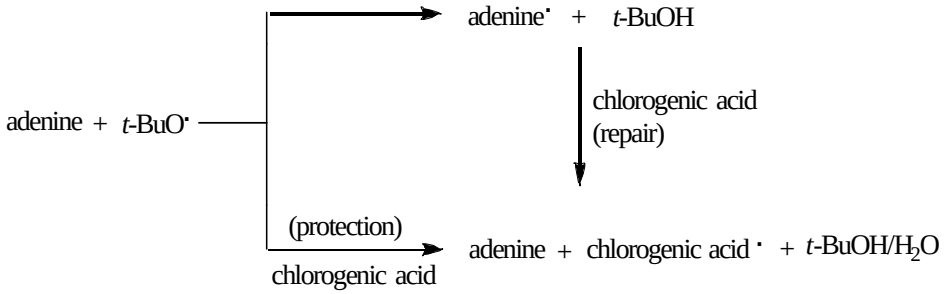
The data on percentage repair is presented in Table 4. The experimentally determined quantum yield (ϕ_{expt}) values are higher than the quantum yield (ϕ_{cal}) values calculated using Eq. (3) under the assumption that chlorogenic acid acts only as a *t*-BuO$^{\bullet}$radical scavenger. This shows that chlorogenic acid acts not only as an efficient scavenger of *t*-BuO$^{\bullet}$ radicals, but also as an agent for the repair of adenine radicals. The repair reaction of chlorogenic acid is explained in terms of the H donation as shown in scheme 1.

Scheme 1



The results obtained in the present study (Table 4) indicate that adenine radicals are efficiently repaired by chlorogenic acid to the extent of ~43 % at about 10μM of chlorogenic acid concentration. This type of repair

reactions by caffeic acid has been reported in the oxidation of adenine by *t*-BuO$^{\bullet}$ radicals [29]. The percentage repair found in the present study (Table 4) is similar to the one reported [29] with caffeic acid. Thus the results indicate that the oxidation of adenine by *t*-BuO$^{\bullet}$ radicals is *via* oxidizing transient radicals of adenine as suggested earlier [29]. The electron density calculations show that C_8 in adenine is more electron rich compared to C_4 or C_5 [37]. The bulkiness of the *t*-BuO$^{\bullet}$ radical is another reason that it prefers C_8 position where no steric hindrance is present due to other groups. The attack of *t*-BuO$^{\bullet}$ radical at C_8 leads to the formation of N_7-centered radical the nature of which has been reported to be oxidizing in nature. The percentage repair obtained in the present study further supports our contention that *t*-BuO$^{\bullet}$ radicals preferentially react at C_8 position leading to the formation of N_7-centered oxidizing radicals to the extent of ~43 %. The other aspect is thatchlorogenic acid is also found to repair the transient oxidizing radicals of adenine very efficiently similar to caffeic acid [29]. The protection of adenine and repair of adenine radicals are summarized in the following scheme.



The chlorogenic acid radicals were generated in the process of protection of adenine and repair of adenine radicals. These radicals were reported [38,39] to have short life time, extremely unstable and rapidly converted to unknown compounds of physiological pH [40]. If chlorogenic acid radicals reacted with adenine, then ϕ_{expt} would have been less than ϕ_{cal}values. Contrary to this, the ϕ_{cal}values were lower than the experimentally found quantum yield values (ϕ_{expt}) (Table 4). This supported our contention that the chlorogenic acid radicals might not be involved in oxidative stress in our experimental conditions.

Table 4 - Effect of varying [adenine] on the rate and quantum yield of photooxidation of chlorogenic acid in the presence of *t*-BuOOH in *t*-BuOH-water 1:4 (v/v) neutral medium

$10^5\times$ [adenine] (mol dm^{-3})	$10^9\times$ Rate (mol dm^{-3} s^{-1})	ϕ_{expt}	ϕ_{cal}	p	ϕ'	% scavenging	% repair
0.0	7.0008	0.004656	0.004656	1.0000	0.004656	100.0	0.00
5.0	5.9565	0.003962	0.003859	0.8290	0.004778	82.90	2.64
8.0	5.4804	0.003645	0.003500	0.7518	0.004848	75.18	4.13
10.0	5.3049	0.003528	0.003296	0.7079	0.004984	70.79	7.05
20.0	4.1938	0.002789	0.002551	0.5479	0.005091	54.79	9.34
50.0	2.8404	0.001889	0.001520	0.3265	0.005785	32.65	24.3
80.0	2.1303	0.001417	0.001082	0.2325	0.006094	23.25	30.9
100.0	1.9548	0.001300	0.000908	0.1951	0.006664	19.51	43.1

[chlorogenic acid] = 1.0 × 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

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