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KINETICS AND MECHANISM OF PROTECTION AND REPAIR OF PYRIMIDINE NUCLEOSIDES BY CHLOROGENIC ACID FROM *TERT*-BUTOXYL RADICALS

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ABSTRACT

The rates of oxidation of chlorogenic acid (CGA) by *t*-BuO$^{\bullet}$ radicals in the presence of pyrimidine nucleosides *viz.*, thymidine, uridine and cytidine have been studied by measuring the absorbance at 328 nm (λ_{max} of CGA) spectrophotometrically. *tert*-butoxyl (*t*-BuO$^{\bullet}$) radicals are 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 CGA by *t*-BuO$^{\bullet}$ radicals have been determined in the absence and presence of varying concentrations of pyrimidine nucleosides. An increase in the concentration of nucleosides has been found to decrease the rate of oxidation of CGA suggesting that nucleosides and CGA have competed for *t*-BuO $^{\bullet}$radicals. From competition kinetics, the rate constant of *t*-BuO$^{\bullet}$radical reaction with thymidine, uridine and cytidine has been calculated to be 4.23 × 10^7, 6.52× 10^7 and 3.75×10^7 dm^3mol^{-1} s^{-1} respectively. The quantum yields (ϕ_{expt}) have been calculated from the experimentally determined rates of oxidation of CGA under different experimental conditions. Assuming that CGA acts as a scavenger *t*-BuO$^{\bullet}$ radicals only, the quantum yields (ϕ_{cal}) have been theoretically calculated. ϕ_{expt} and ϕ_{cal} values suggest that CGA not only protects nucleosides from *t*-BuO$^{\bullet}$ radicals but also repairs nucleoside radicals.

KEYWORDS: chlorogenic acid, pyrimidine nucleosides, *t*-BuO$^{\bullet}$ radicals, oxidation, protection, repair.

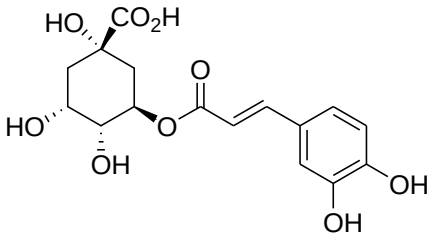
INTRODUCTION

The DNA of the cell nucleus is known to be the most sensitive target for the radicals generated in living systems. The DNA damage is attributed to reactive oxygen species (ROS) formed in the cellular oxygen metabolism.[1-4] A broad range of products are reported from the oxidative attack of DNA by these radicals which include base and sugar modifications, covalent cross links, single and double stranded breaks.[5-8]

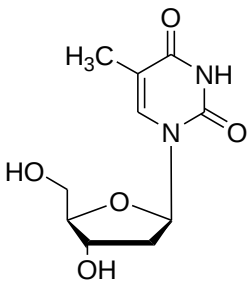
The purine,[9] and the pyrimidine,[10] bases are the most sensitive to the radiation induced modification or destruction of the components of DNA and DNA itself.When the reactions were initiated directly with UV light of λ>260 nm or photosensitized with acetone and light of λ>290 nm, pyrimidine dimers were formed in the irradiated DNA together with the 8-alkyl derivatives of adenine and guanine. Under these conditions, it was shown that pyrimidines exhibited higher reactivity than the purines.[11] Methylation of cytosine.[12] in DNA is important for the regulation of gene expression and normal methylation patterns can be altered during carcinogenesis. Conversion of guanine to 8-OHG,[13] frequent result of ROS attack, has been found to alter the enzyme-catalysed methylation of adjacent cytosines thus providing a link between oxidative DNA damage and altered methylation patterns.

During the oxidative stress and exposure to radiation, excessive free radicals are produced which are known to cause damage to biomolecules.[2,3] Recent studies showed that the exposure of cultured cells to hydroperoxides resulted in the generation of DNA strand breaks.[14,15] Although lethal effects of the hydroxyl radicals ($^{\bullet}$OH) on DNA and its constituents have been studied 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. Alkoxyl radicals are produced on UV-A irradiation or metal catalyzed decomposition of lipid hydroperoxides.[3,16,17] They have been reported to induce strand breaks in Supercoiled pBR322 DNA.[18] The addition of the radical scavengers, viz., *t*-butanol and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) are found to inhibit significantly the formation of strand breaks. This establishes,[19] the fact that strand breaks are caused not only by hydroxyl radicals but also by alkoxyl radicals in DNA and may play a significant role in the tumor promoting activity of peroxides.[20] *tert*-Butylhydroperoxide (*t*-BuOOH) has been chosen as a model peroxide which on homolysis gives $^{\bullet}$OH and *t*-BuO$^{\bullet}$ radicals.

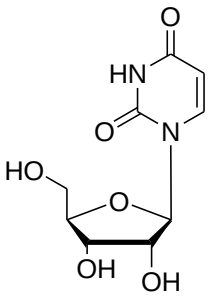
Plant phenolics are said to be multifunctional antioxidants and they might act at several levels in the oxidative sequence. The multiple potential mechanisms by which the antioxidants act make the diverse group of phenolic compounds an interesting target in the search of health-beneficial phytochemicals and also offer a possibility to use phenolic compounds to extend the shelf life of lipid-rich foods.[21] A variety of natural and synthetic antioxidants, especially phenolic antioxidants, have been found to protect biological molecules from oxygen radical injury.[22,23]



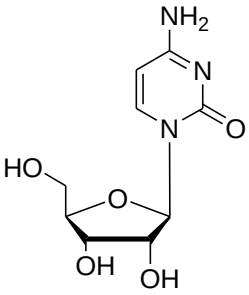
Chlorogenic acid



Thymidine



Uridine



Cytidine

Chlorogenic acid (CGA), an ester of caffeic acid with quinic acid, is found in a wide range of fruits and vegetables.[24] Coffee, one of the most widely consumed beverages in the world, contains high amounts of CGA. It scavenges radicals generated in the aqueous phase.[25] increases the resistance of LDL to lipid peroxidation.[26] and inhibits DNA damage.[27] *In vivo*, when added to the diet, it inhibits chemically induced carcinogenesis of the large intestine, liver and tongue in rats and hamsters.[28] It is reported.[29] to prevent different cancers and cardiovascular diseases in several experimental studies in animal models. 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 hydroxy radicals.

CGA is also found to have antioxidant,[30] and anti-inflammatory properties.[31] CGA possesses powerful radical scavenging properties as well as strong electron donating

properties to guaiacol-type peroxidases such as horseradish peroxidase (HRP).[32] It was shown that peroxynitrite causes extensive strand breaks in plasmic DNA.[33] CGA is found to be a potent ROS and RNS radical scavenger. In the presence of CGA, the extent of peroxynitrite mediated DNA damage decreased in a dose-dependent manner suggesting that CGA can inhibit the formation of single strand breaks in supercoiled pBR322 DNA by efficiently scavenging peroxynitrite radicals in peroxidase-containing systems in vivo.[34]

Hydroxycinnamic acid derivatives such as CGA have been identified as good antioxidants for reduction and repair of OH-adducts of pyrimidines via electron transfer. It has been demonstrated.[35] that CGA can either scavenge $^{\bullet}$OH radical or repair oxidizing $^{\bullet}$OH radical adduct of 2'-deoxyguanosine-5'-monophosphate (dGMP). Unlike most antioxidants, products of CGA formed by reaction with free radicals were rapidly broken down further to the products which were not able to generate any free radical which is the beneficial nature of the antioxidant. In this context, a systematic kinetic study of oxidation of pyrimidine nucleosides (thymidine, uridine and cytidine) by *t*-BuO$^{\bullet}$ radical has been carried out in presence of chlorogenic acid (CGA) to understand the nature of transient radicals formed and to evaluate the extent of protection and repair offered by CGA to pyrimidine nucleosides.

MATERIALS AND METHODS

CGA, thymidine, uridine and cytidine were purchased from Sigma Chemical Co., St. Louis, USA and used as received. All solutions were prepared afresh using double-distilled water. *tert*-Butylhydroperoxide (*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,[36] 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 (254 nm) was measured using peroxydisulphate chemical actinometry.[37] 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.[38] The $^{\bullet}$OH radicals produced were scavenged using sufficient concentration of *t*-BuOH.[39]

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 CGA at 328 nm at which nucleoside was totally transparent. It is known that *t*-BuOOH is activated to radical reaction by the absorption of light at 254 nm. However, the substrates used in the present work, *viz.*, CGA and nucleosides have strong absorption in this region. But, in the absence of *t*-BuOOH in the reaction mixture, CGA, nucleosides or CGA - nucleoside mixture did not undergo any observable chemical change on shining the light. From the results obtained, we proposed that the excited states of CGA and nucleosides acted as sensitizers to transfer energy to *t*-BuOOH to produce radical species. This type of sensitizing effect was proposed in similar systems earlier. Therefore, the light intensity at 254 nm was used to calculate the quantum yields of oxidation of nucleosides as well as CGA under different experimental conditions.

RESULTS AND DISCUSSION

The initial rates of photooxidation of CGA by *t*-BuOOH in presence of *t*-BuOH have been calculated from the plots of absorbance of CGA at 328 nm *vs* time using microcal origin computer program on a personal computer.[40] UV-visible absorption spectra of CGA in presence of *t*-BuOOH and *t*-BuOH at different irradiation times were recorded. In order to find the protection offered to pyrimidine nucleosides*viz*., thymidine, uridine and Cytidine by CGA towards oxidation by *t*-BuO$^{\bullet}$, the reaction mixture containing known concentrations of nucleoside, *t*-BuOOH and *t*-BuOH was irradiated in presence of varying concentrations of CGA. The reactions were followed by measuring the absorbance of CGA at 328 nm (Fig.1) at which nucleosides are transparent and the rate data are presented in Table 1-3.

The solutions of reaction mixture containing CGA, *t*-BuOOH and *t*-BuOH were irradiated in presence of varying concentrations of nucleosides (Fig.2). The initial rates and quantum yields of oxidation of CGA by *t*-BuO$^{\bullet}$ radicals were found to decrease with increase in concentration of nucleosides (Tables 1-3). Comparison of the initial rates and quantum yields of oxidation of CGA in presence and absence of nucleosides clearly indicated that the initial rates and quantum yields of oxidation of CGA have substantially decreased in presence of

nucleosides. These observations clearly demonstrated that nucleosides and CGA were in competition for *t*-BuO$^{\bullet}$ radicals.

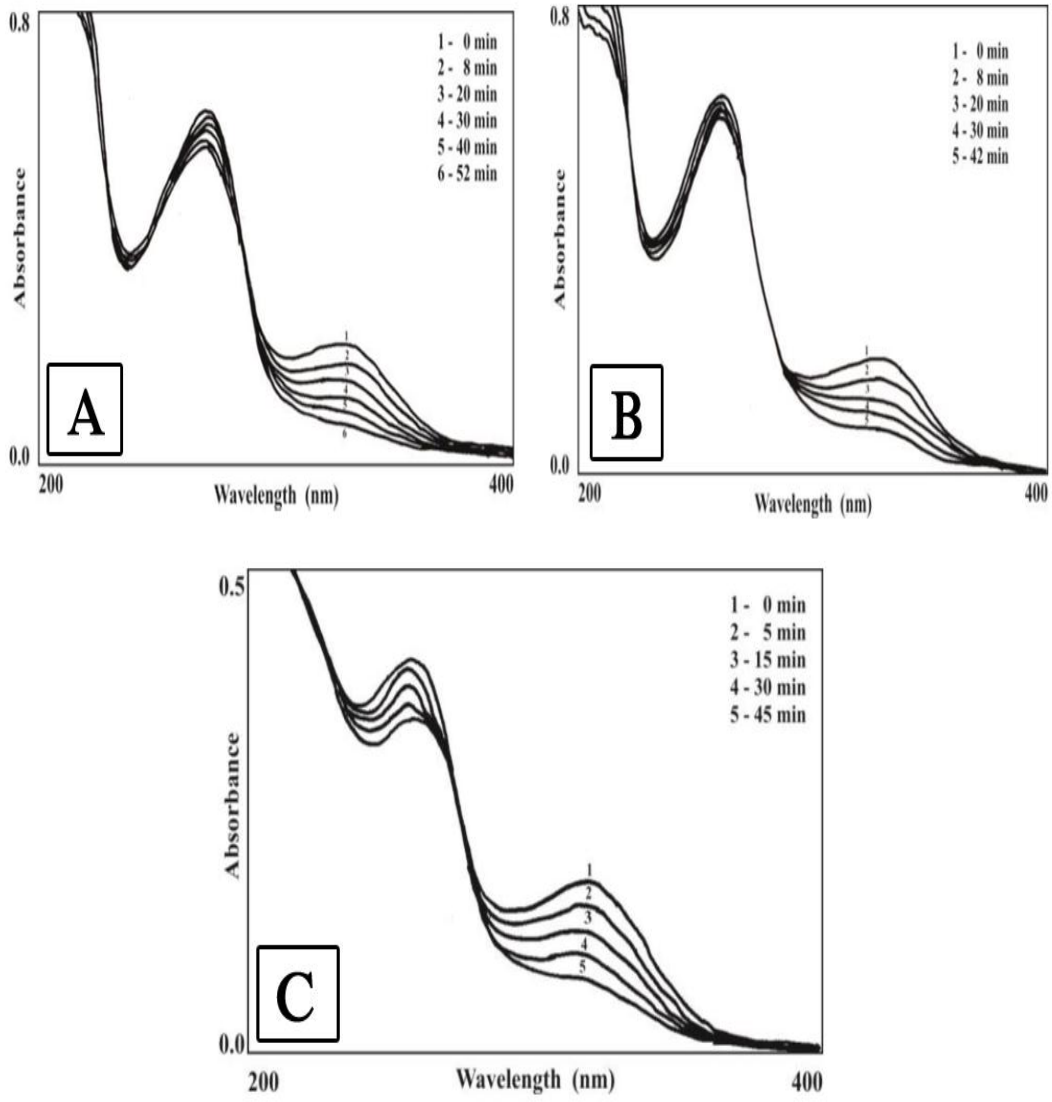
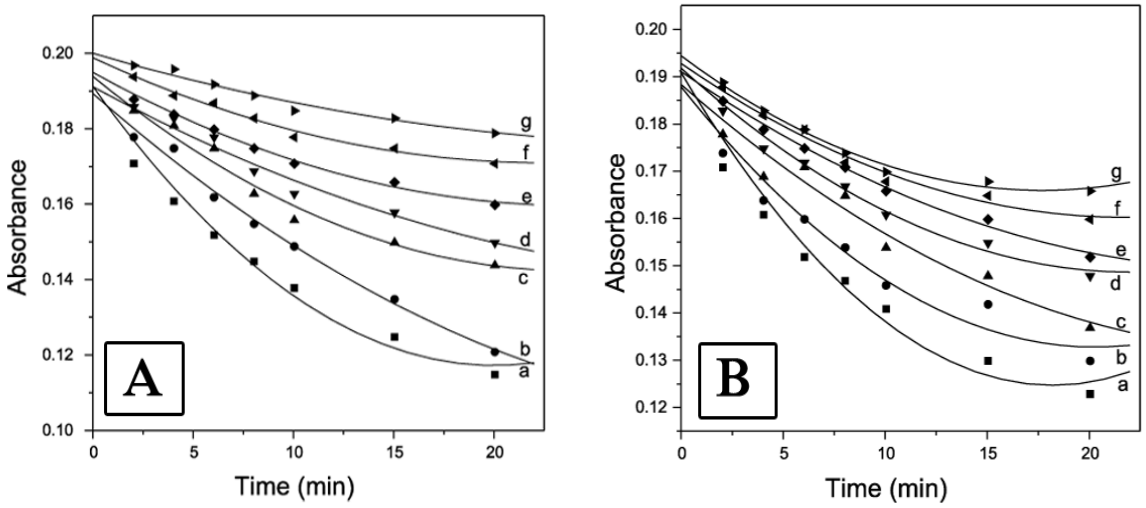


Fig. 1 - Absorption spectra of photooxidation of CGA in the presence of *tert*-butyl hydroperoxide and pyrimidine nucleosides (A-Thymidine, B-Uridine and C-cytidine) at different irradiation times.[CGA] = 1 × 10^{-5}mol dm^{-3}, [*t*-BuOOH] = 5 × 10^{-3}mol dm^{-3}, [nucleoside] = 5 ×10^{-5} mol dm^{-3}, Light Intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, temperature = 298 K.

Table 1 - Effect of varying [thymidine] on the rate and quantum yield of photooxidation of CGA in the presence of *t*-BuOOH in *t*-BuOH-water (1:4 v/v) medium.[CGA] = 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

$10^5 \times$ [thymidine] (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	6.7251	0.004473	0.004375	0.9398	0.004759	93.98	2.22
8.0	6.6416	0.004417	0.004223	0.9070	0.004864	90.70	4.59
10.0	6.5998	0.004389	0.004127	0.8864	0.004952	88.64	6.36
50.0	4.9284	0.003278	0.002838	0.6095	0.005383	60.95	15.6
80.0	4.2774	0.002845	0.002299	0.4938	0.005761	49.38	23.7
100.0	3.9682	0.002639	0.002041	0.4383	0.006021	43.83	29.3

The rate constant for the reaction of *t*-$BuO^{\bullet}$ with nucleosides have been calculated by the CGA competition method, which is very similar to the one chosen by Akhalaq *et al*,[41] 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 CGA and varying amounts of nucleosides in presence of *t*-BuOOH and *t*-BuOH were irradiated for two minutes and the decrease in absorbance of CGA was measured. The decrease in absorbance of CGA reflects the amount of *t*-$BuO^{\bullet}$ radicals that has reacted with CGA. From the known rate constant[40] of the reaction of CGA with *t*-$BuO^{\bullet}$ radicalunder similar experimental conditions of the present work ($k_{chlorogenic\ acid}$ = 3.20× 10^9 dm^3 $mol^{-1}s^{-1}$), the rate constant of *t*-$BuO^{\bullet}$ radical reaction with nucleosides ($k_{nucleoside}$) can be calculated. From competition kinetics, the rate constant of *t*-$BuO^{\bullet}$radical reaction with thymidine, uridine and cytidine has been calculated to be 4.23 × 10^7, 6.52 × 10^7 and 3.75 × 10^7 dm^3mol^{-1} s^{-1} respectively.



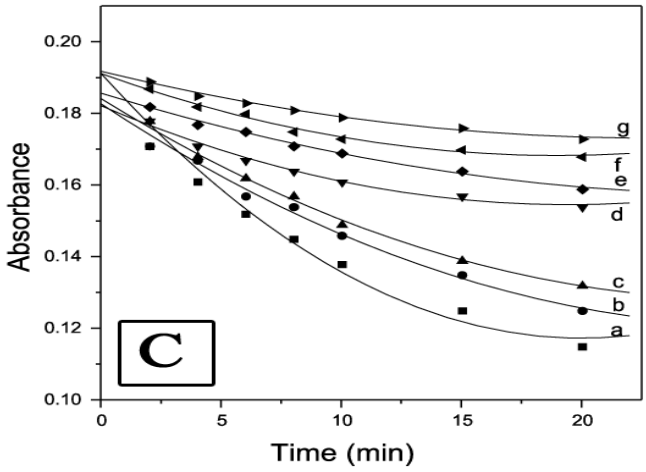


Fig. 2 - Effect of varying concentration of nucleoside on the photooxidation of CGA (1.0 ×10^{-5} mol dm^{-3}) in the presence of *t*-BuOOH (5.0 × 10^{-3}mol dm^{-3}) at 298 K. [nucleoside] = (a) 0.0, (b) 5.0 × 10^{-5}mol dm^{-3}, (c) 8.0 × 10^{-5}mol dm^{-3}, (d) 1.0 × 10^{-4}mol dm^{-3}, (e) 5.0 × 10^{-4}mol dm^{-3}, (f) 8.0 × 10^{-4}mol dm^{-3}, (g) 1.0 × 10^{-3}mol dm^{-3}

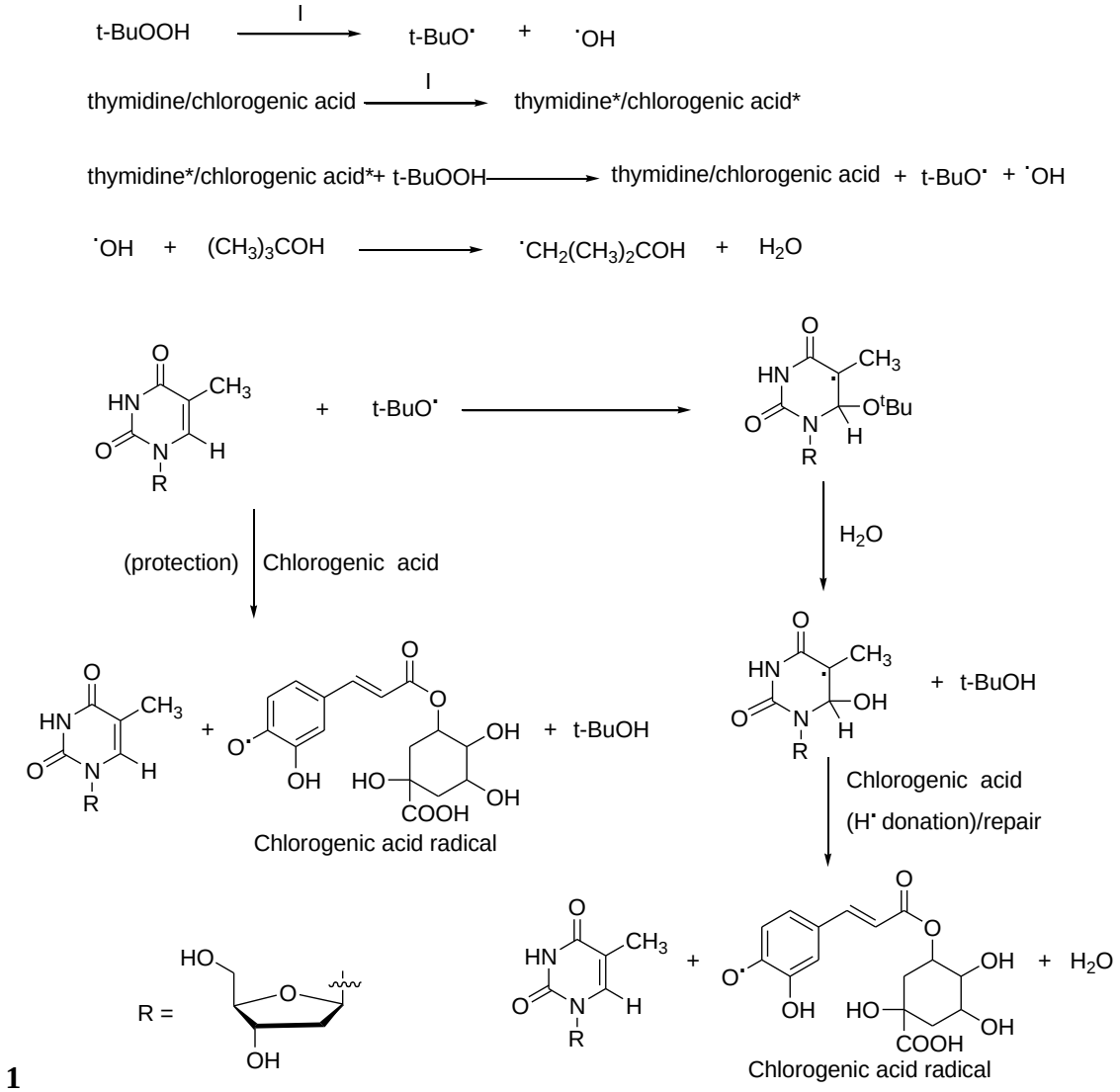
Table 2 - Effect of varying [uridine] on the rate and quantum yield of photooxidation of CGA in the presence of *t*-BuOOH in *t*-BuOH-water (1:4 v/v) medium.[CGA] = 1.0 × 10^{-5} mol dm^{-3}, [*t*-BuOOH] = 5.0 × 10^{-3} mol dm^{-3},Light Intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, Temperature = 298 K

10^5 × [uridine] (mol dm^{-3})	10^9× 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	6.5164	0.004333	0.004216	0.9058	0.004785	90.58	2.76
8.0	6.3408	0.004217	0.003991	0.8573	0.004919	85.73	5.65
10.0	6.2824	0.004178	0.003854	0.8278	0.005047	82.78	8.41
50.0	4.0433	0.002688	0.002282	0.4902	0.005483	49.02	17.8
80.0	3.4419	0.002289	0.001748	0.3754	0.006097	37.54	30.9
100.0	3.2164	0.002139	0.001512	0.3247	0.006588	32.47	41.5

The calculated quantum yield (ϕ_{cal}) values and experimental quantum yield values (ϕ_{expt}) at different nucleoside concentrations presented in Tables 1-3 were calculated using the method reported earlier[40].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 CGA molecules is consumed in the system than expected and the most likely route for this is H atom donation by CGA to nucleoside radicals. In Tables1-3, are presented the fraction of *t*-BuO$^{\bullet}$radicals scavenged (*p*) by CGA at different concentrations of nucleosides. These values refer to the measure of protection offered to nucleoside due to scavenging of *t*-BuO$^{\bullet}$ radicals by CGA. Using the ϕ_{exptl} values, ϕ' values have been calculated and are presented in Tables 1-3 which

represents the experimentally found quantum yield values if no scavenging of nucleoside radicals by CGA occurs. In the absence of any "repair" of nucleoside radicals by CGA, the ϕ'values should all be equal to ϕ^{o}_{expt}. The observed increase in ϕ' with increasing nucleoside concentration clearly indicated that repair of nucleoside radicals does occur. The extent of repair of nucleoside radicals formed due to reaction with *t*-BuO$^{\bullet}$ radicals by CGA was calculated and presented in Tables 1-3 for the nucleosides thymidine, uridine and cytidine respectively. This shows that CGA acts not only as an efficient scavenger of *t*-BuO$^{\bullet}$ radicals, but also as an agent for the repair of nucleoside radicals. The repair reaction of CGA is explained in terms of the H donation as shown in scheme 1 taking thymidine as representative molecule.

Scheme



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The results obtained in the present study indicated that nucleoside radicals*viz.*, thymidine, uridine and cytidine were efficiently repaired by CGA to the extent of ~29 %, ~41 % and ~18 % respectively at about 10μM of CGA concentration. This type of repair reactions by CGA have been reported in the oxidation of nucleobases by *t*-BuO$^{\bullet}$ radicals,[42,43] clearly indicating that CGA repairs the transient oxidizing radicals of nucleosides very efficiently similar to caffeic acid.[44] The protection of nucleoside and repair of nucleoside radicals by CGA are summarized in the following scheme.

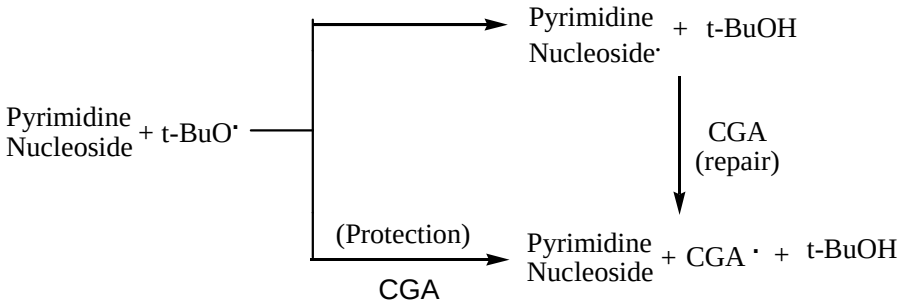

Table 3 - Effect of varying [cytidine] on the rate and quantum yield of photooxidation of CGA in the presence of *t*-BuOOH in *t*-BuOH-water (1:4 v/v) medium.[CGA] = 1.0 × 10^{-5} mol dm^{-3}, [*t*-BuOOH] = 5.0 × 10^{-3} mol dm^{-3},Light Intensity = 2.7168 × 10^{15} quanta s^{-1}, λ_{max} = 328 nm, pH ~ 7.5, Temperature = 298 K

10^5 × [cytidine] (mol dm^{-3})	10^9× 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	6.6416	0.004417	0.004399	0.9448	0.004675	94.48	0.40
8.0	6.5163	0.004334	0.004258	0.9145	0.004739	91.45	1.78
10.0	6.4323	0.004278	0.004168	0.8953	0.004778	89.53	2.63
50.0	4.7201	0.003139	0.002939	0.6312	0.004973	63.12	6.82
80.0	4.0434	0.002689	0.002406	0.5168	0.005204	51.68	11.8
100.0	3.8262	0.002545	0.002147	0.4611	0.005519	46.11	18.5

Pyrimidine nucleosides reacts with *t*-BuO$^{\bullet}$ radicals to form *tert*-butoxyl adduct radical, which on hydrolysis gives oxidizing C_6-OH adduct radical. This oxidizing adduct radical captures an electron from CGA and repaired by dehydroxylation to give the parent molecule nucleoside and CGA radical. It is known that antioxidants such as CGA can repair the transient oxidizing radicals efficiently.[32,45] It has been proposed that *t*-BuO$^{\bullet}$ radicals attacks pyrimidine base to give both reducing and oxidizing radicals. The C_5-yl radical formed by attacking at C_6 are oxidizing while radicals produced by addition to C_5 forming C_6-yl radicals are reducing in nature.The *t*-BuO$^{\bullet}$ radicals were found to attack nucleoside predominantly at C_6 position leading to the formation of C_6-OH adduct type of product. In the oxidation of

pyrimidine nucleosides by $^{\bullet}OH$ radicals it has been proposed that the base moiety is preferentially oxidized over the sugar moiety.[46]

CONCLUSIONS

The results in Tables 1-3 indicate that when [CGA] is 1.0×10^{-5} mol dm^{-3} and [nucleoside] is 1.0×10^{-3} mol dm^{-3}, the percentage repair obtained is 29%, 41% and 18% for thymidine, uridine and cytidine respectively. The rate of oxidation of nucleosides *viz.,* uridine and cytidine by *t*-$BuO^{\bullet}$ radicals are found to be very close to the corresponding nucleobases *viz.,* uracil,[42] (31%) and cytosine (21%) indicating that the attacking moiety could be the same for nucleobases and nucleosides. This supports the contention that the base moiety is preferentially attacked by *t*-$BuO^{\bullet}$ radical during the oxidation of nucleosides. Thus, the data on the percentage repair obtained indicates the formation of similar transient oxidizing radicals on pyrimidine nucleosides and pyrimidine bases. The results also point out that the radicals produced on reaction of nucleosides with *t*-$BuO^{\bullet}$ radicals might be reducing in nature to a large extent and hence, CGA could not repair such transient radicals produced in these systems.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest associated with this publication.

REFERENCES

1. Halliwell B, Gutteridge J. In Free Radicals in Biology and Medicine, third ed. Oxford University Press, Midsomer Norton, Avon, England. 1999.
2. Simpson JA, Narita S, Geiseg S, Gebicki S, Gebicki JM, Dean RT. (Long lived reactive species on free-radical-damaged proteins). Biochem J, 1992; 282: 621-624.
3. Schaich KM, Yang MH. (Factors affecting DNA damage caused by lipid hydroperoxides and aldehydes). Free Radic Biol Med, 1996; 20: 225-236.
4. Von Sonntag C, The Chemical Basis of Radiation Biology, Taylor & Francis, London.1987.

5. Von Sonntag C, Teoule R. (Radiation-induced DNA damage and its repair). Int J Radiat Biol Relat Stud Phys Chem Mol, 1987; 51: 573-589.
6. Dizdaroglu M.(Oxidative damage to DNA in mammalian chromatin). Mutat Res, 1992; 275: 331-342.
7. Adinarayana M, Bothe E, Schulte-Frohlinde.(Hydroxyl radical-induced strand break formation in single-stranded polynucleotides and single-stranded DNA in aqueous solutionas measured by light scattering and by conductivity). Int J Radiat Biol, 1988; 54: 723-737.
8. Catterall H, Davies MJ, Gilbert BC.(An EPR study of the transfer of radical-induced damage from the base to sugar in nucleic acid components: Relevance to the occurrence of strand-breakage). J Chem Soc Perkin Trans., 1992; 2: 1379-1385.
9. Steenken S. (Purine bases, nucleosides, and nucleotides: aqueous solution redox chemistry and transformation reactions of their radical cations and e– and OH adducts). Chem Rev, 1989; 89: 503-520.
10. Pryor WA.(Why is the hydroxyl radical the only radical that commonly adds to DNA?) Free Radic Biol Med, 1988; 4: 219-223.
11. Havron A, Sperling J, Elad D. (Reactivity and selectivity in light-induced free radical reactions of 2-propanol with purine and pyrimidine mononucleotides and dinucleoside monophosphates). Nucleic Acid Res, 1976; 3: 1715-1725.
12. Dizdaroglu M, Laval J, Boiteux S. (Substrate specificity of the Escherichia coli endonuclease III: excision of thymine- and cytosine-derived lesions in DNA produced by radiation-generated free radicals). Biochemistry, 1993; 32: 12105-12111.
13. Melvin T, Botchway S, Parke AW, O'Neill P. (Induction of strand breaks in single-stranded polyribonucleotides and DNA by photoionization: one electron oxidized nucleobase radicals as precursors). J Am Chem Soc, 1996; 118: 10031-10036.
14. Hartley JA, Gibson NW, Kilkenny A, Yuspa SH. (Association of DNA strand breaks with accelerated terminal differentiation in mouse epidermal cells exposed to tumor promoters). Cancer Res, 1985; 45: 4864-4870.
15. Birboim HC. (DNA strand breaks in human leukocytes induced by superoxide anion, hydrogen peroxide and tumor promoters are repaired slowly compared to breaks induced by ionizing radiation). Carcinogenesis. 1986; 7: 1511-1517.
16. Fujita T, Fujimoto Y. (Formation and removal of active oxygen species and lipid peroxides in biological systems). Folia Pharmakol Jpn, 1992; 99: 381-389.

17. Hazlewood Davies MJ. (EPR Spin-trapping studies of the reaction of radicals derived from hydroperoxide tumour-promoters with nucleic acids and their components). J Chem Soc Perkin Trans., 1995; 2: 895-901.
18. Pullman B, Pullman A. Quantum Biochem, Wiley-Interscience, New York. 1963.
19. Teissedre PL, Franke, EN, Waterhouse AL, Peleg H, German JB. (Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines). J Sci Food Agric, 1996; 70: 55-61.
20. Tovel J, Cillard J, Cillard P. (Antioxidant activity of flavonoids and reactivity with peroxy radical). Phytochem, 1986; 25: 383-385.
21. Samotyja U, Malecka M. (Effects of blackcurrant seeds and rosemary extracts on oxidative stability of bulk and emulsified lipid substrates). Food Chem, 2007; 104: 317-323.
22. Harborne JB, Williams CA. (Advances in flavonoid research since 1992). Phytochem, 2000; 55: 481-504.
23. Ferrari CKB, Torres EAFS. (Biochemical pharmacology of functional foods and prevention of chronic diseases of aging). Biomed Pharmacother, 2003; 57: 251-260.
24. Bravo L..Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance.Nutr. Rev., 1998; 56: 317-333.
25. Lafay S, Gil-Izquierdo A, Manach C, Morand C, Besson C, Scalbert A. (Chlorogenic Acid is absorbed in its intact form in the stomach of rats). J Nutr, 2006; 136: 1192-1197.
26. Foley S, Navaratnam S, McGarvey DY, Land EJ, Truscotti G, Rice-Evans CA. (Singlet oxygen quenching and the redox properties of hydroxycinnamic acids). Free Radic Biol Med, 1999; 26: 1202-1208.
27. Nardini M, D'Aquino M, Tomassi G, Gentili V, Di Felice M, Scaccini C. (Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives). Free Radic Biol Med, 1995; 19: 541-552.
28. Shibata H, Sakamoto Y, Oka M, Kono Y. (Natural antioxidant, chlorogenic acid, protects against DNA breakage caused by monochloramine). Biosci Biotechnol Biochem, 1999; 63: 1295-1297.
29. Kadoma Y, Fujisawa S. (A comparative study of the radical-scavenging activity of the phenolcarboxylic acids caffeic acid, p-coumaric acid, chlorogenic acid and ferulic acid, with or without 2-mercaptoethanol, a thiol, using the induction period method). Molecules, 2008; 13: 2488-2499.

30. Moyers SB, Kumar NB. (Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for phase II trials). Nutr Rev, 2004; 62: 204-211.
31. Yen WJ, Wang BS, Chang LW, Duh PD. (Antioxidant properties of roasted coffee residues). J Agric Food Chem, 2005; 53: 2658-2663.
32. Yamasaki H, Grace SC. (EPR detection of phytophenoxyl radicals stabilized by zinc ions: evidence for the redox coupling of plant phenolics with ascorbate in the H2O2-peroxidase system). FEBS Lett, 1998; 422: 377-380.
33. Roussyn I, Briviba K, Masumoto H, Sies H. (Selenium containing compounds protect DNA from single-strand breaks caused by peroxynitrite). Arch Biochem Biophys, 1996; 330: 216-218.
34. Grace SC, Salgo MG, Pryor WA. (Scavenging of peroxynitrite by a phenolic/peroxidase system prevents oxidative damage to DNA). FEBS Letts, 1998; 426: 24-28.
35. Namazian M, Zare HR. (Electrochemistry of chlorogenic acid: Experimental and theoretical studies). Electrochim Acta, 2005; 50: 4350-4355.
36. Howard JA, Ingold KU. (Absolute rate constants for hydrocarbon autoxidation. VI. Alkyl aromatic and olefinic hydrocarbons). Can J Chem, 1967; 45: 793-802.
37. Ravi Kumar M, Adinarayana M. (Oxidation of caffeine by phosphate radical anion in aqueous solution under anoxic conditions). Proc Indian Acad Sci, 2000; 112: 551-557.
38. Bors W, Michel, Saran M. (Inhibition of the bleaching of the carotenoid crocin. A rapid test for quantifying antioxidant activity). Biochem Biophy Acta, 1984; 796: 312-319.
39. Asmus KD, Mockel H, Henglein A. (Pulse radiolytic study of the site of hydroxyl radical attack on the aliphatic alcohols in aqueous solution). J Phys Chem, 1973; 77: 1218-1221.
40. Vijayalakshmi G, Adinarayana M, Jayaprakash Rao P. (Kinetics of oxidation of adenosine by tert-butoxyl radicals: Protection and repair by Chlorogenic acid). Indian J Biochem Biophys, 2009; 46: 389-394.
41. Akhalaq MS, Al-Baghdad S, Von Sonntag C. (On the attack of hydroxyl radicals on polyhydric alcohols and sugars and the reduction of the so-formed radicals by 1,4-dithiothreitol). Carbohydrate Res, 1987; 164: 71-83.
42. Vijayalakshmi G, Adinarayana M, Jayaprakash Rao P. (A kinetic and mechanistic approach to protection and repair of *tert*-butoxyl radicals induced uracil radicals by chlorogenic acid). Intl J Pharma Sci Res, 2015; 6(1): 277-285.
43. Vijayalakshm, G, Adinarayana M, Jayaprakash Rao P. (A kinetic and mechanistic approach to protection and repair of *tert*-butoxyl radicals induced adenine radicals by chlorogenic acid). J Chem Pharma Res, 2013; 5(10): 246-253.

44. Charitha L, Adinarayan, M. (Kinetics of Oxidation of Adenosine by *tert*-butoxyl Radical: Protection and Repair by Caffeic Acid). Int J Chem Kinetics, 2005; 37: 515-521.
45. Kalyanaraman B. (Characterization of *o*-semiquinone Radicals in Biological Systems). Meth Enzymol, 1990; 186: 333-343.
46. Davies MJ, Gilbert BC, Hazlewood C, Polack NP. (EPR spin trapping studies of radical damage to DNA). J Chem Soc Perkin Trans 2, 1995; 13-21.