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Free Radical Reactions and Antioxidant Activities of Sesamol: Pulse Radiolytic and Biochemical Studies

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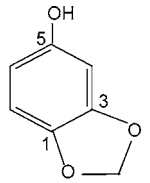
Sesamol (from *Sesamum indicum*) is a dietary compound, which is soluble in aqueous as well as lipid phases. Free radical scavenging reactions of sesamol, 5-hydroxy-1,3-benzodioxole, have been studied using a nanosecond pulse radiolysis technique. Sesamol efficiently scavenges hydroxyl, one-electron oxidizing, organo-haloperoxyl, lipid peroxyl, and tryptophanyl radicals. Its antioxidant activity has also been evaluated with cyclic voltammetry. In biochemical studies, it has been found to inhibit lipid peroxidation, hydroxyl radical-induced deoxyribose degradation, and DNA cleavage. These antioxidant and free radical scavenging activities of sesamol have been reported in the paper.

KEYWORDS: Sesamol; dietary antioxidant; pulse radiolysis; lipid peroxidation; deoxyribose degradation; DNA cleavage

INTRODUCTION

Various antioxidants present in the human diet are of great interest because of their possible protective actions against free radicals produced in oxidative stress coupled with their nontoxicity (*1, 2*). The free radicals produced endogenously are known to play a significant role in the pathogenesis of certain human diseases and aging (*3*−*7*) along with normal biological functions. The compounds present in fruits, nuts, tea, spices, edible oils, etc. have attracted considerable attention in recent years due to their antioxidant activities (*2*). Sesame seed (*Sesamum indicum*) as well as its oil are used for human consumption worldwide in various forms. Sesame seed oil contains sesamolin and sesaminol lignans among other compounds. Sesamol (SOH), 5-hydroxy-1,3-benzodioxole or 3,4-methylenedioxyphenol, is a constituent of sesame seed oil. SOH is also formed by thermal reduction of sesamolin. SOH has been found to be a good antioxidant in lard, vegetable oils, and hydrogenated oil samples on the basis of oil stability (*8*). Recently, the antiphotooxidative activity of SOH for oil has been reported to be due to the scavenging of singlet oxygen (*9*).

SOH has a phenolic and a benzodioxole group in its molecular structure. The phenolic group of the molecule is generally responsible for the antioxidant activity of many natural products and vitamins (*2, 10*−*13*). On the other hand, benzodioxole derivatives are widely distributed in nature and have been shown to possess antitumor, antioxidant, radioprotector, antifungal, antibacterial, and many other biological activities (*14*−*17*). These activities have been attributed to the effect on various enzymes as well as scavenging of oxidizing free radicals.



Sesamol (SOH)

SOH is a unique phenolic compound due its solubility in the aqueous as well as the oil phase and thermal stability. It has a benzodioxole group, which is known to scavenge hydroxyl radical to produce 1,2-dihydroxybenzene (*18*). Thus, even the product of the hydroxyl radical scavenging reaction is a possible antioxidant. SOH is reported to have antioxidant activity in UV- or Fe(II)-induced lipid oxidation (*19*) and antimutagenic activity against oxygen species in *Salmonella typhimurium* (*20*). Continuous or pulsed UV exposure to aqueous solution of SOH is known to generate sesamolyl, benzoquinone anion, cyclohexadienyl type radicals, and dimer radicals (*21, 22*). SOH has also been found to scavenge stable 2,2-diphenyl-1-picrylhydrazyl free radicals (*23*) and imidazoquinoxaline type radicals (*24*). It scavenges the hydroxyl radical generated by the Fenton reagent to inhibit the formation of single strand DNA breaks (*25*). Although it has been shown by using indirect techniques that SOH scavenges various free radicals, the kinetics and mechanisms of the concerned reactions of SOH and the reactions of the SOH transients produced in these reactions are not known.

Various studies mentioned above, related to SOH, clearly indicate its reaction with the hydroxyl, lipid peroxyl, and other

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physiologically produced free radicals. The efficacy of any compound to act as an antioxidant depends on its capacity to scavenge free radicals and reactivity of the antioxidant-derived radical/s with other relevant biomolecules. Therefore, reactions of SOH with various oxidizing free radicals have been studied using the pulse radiolysis technique (*26*). UV−visible absorption of the transients has been monitored to have direct insight into these fast reactions. The Fenton reaction has been used to study the effect of SOH on hydroxyl radical-induced lipid peroxidation, deoxyribose degradation, and DNA damage.

MATERIALS AND METHODS

Pulse Radiolysis Studies. The pulse radiolysis system using a 7 MeV linear electron accelerator has been described elsewhere (*27*). The dosimetry was carried out using an air-saturated aqueous solution containing 5×10^{-2} mol dm^{-3} KSCN (Gå $= 2.6 \times 10^{-4}$ m^2 J^{-1} at 475 nm (*28*). The kinetic spectrophotometric detection system covered the wavelength range from 250 to 800 nm. The optical path length of the cell was 1.0 cm. The width of the electron pulse was 50 ns. SOH (>98%) from Fluka was used as received. Deoxyribose and thiobarbituric acid were from Sigma (United States). All other chemicals were of analytical reagent grade. Nanopure water (conductivity, 0.06 μS cm^{-1}) was used for preparing solutions. The pH values were adjusted by adding 2×10^{-3} mol dm^{-3} phosphate buffer or KOH. High purity (>99.9%) N_2 and N_2O from British Oxygen Company (India) were used as per requirement. The bimolecular rate constants were calculated by plotting pseudo-first-order rates of formation of the solute radical or decay of the reacting radical (e.g., $Br_2^{\bullet-}$) against the solute concentration. The uncertainty in the measurement of bimolecular rate constant is ±10%. Water radiolysis and the reactions used to generate secondary radicals are shown below.

$$H_2O \rightsquigarrow e_{aq}^- (0.28), H^\bullet (0.06), {}^\bullet OH (0.28), H_2, \text{and } H_2O_2 \quad (1)$$

$$e_{aq}^- + N_2O + H_2O \rightarrow {}^\bullet OH + OH^- + N_2 \quad (2)$$

$${}^\bullet OH \overset{pK_a=11.8}{\rightleftharpoons} O^{\bullet-} + H^+ \quad (3)$$

$${}^\bullet OH + N_3^- (Br^-) \rightarrow OH^- + N_3^\bullet (Br^\bullet) \quad (4)$$

$$Br^- + Br^\bullet \rightarrow Br_2^{\bullet-} \quad (5)$$

$${}^\bullet OH + \textit{tert}\text{-}C_4H_9OH \rightarrow \textit{tert}\text{-}{}^\bullet C_4H_8OH + H_2O \quad (6)$$

$$e_{aq}^- + CH_mX_n \rightarrow {}^\bullet CH_mX_{(n-1)} + X^- \; [m = 0, 1, 2; n = (4 - m)] \quad (7)$$

$${}^\bullet CH_mX_{(n-1)} + O_2 \rightarrow CH_mX_{(n-1)}O_2^\bullet \quad (8)$$

The number in parentheses is radiation chemical yield (G), which is defined as micromoles of the transient produced on absorbing one Gray (Gy) radiation dose (1 Gy = 1 Joule/1 kg).

Cyclic Voltammetry. The cyclic voltammetric experiment was carried out using an Autolab Electrochemical System (Eco Chemie, Netherlands) equipped with PGSTAT 20 and driven by GPES software. The working electrode was a highly polished glassy carbon electrode (GCE) with a diameter of 2 mm, and a platinum wire was used as a counter electrode. The reference electrode was saturated calomel electrode (SCE) with a salt bridge containing 3 mol dm^{-3} KCl solution. The GCE surface was polished with an aqueous slurry of alumina powder before each measurement. Solutions were deaerated by bubbling N_2 for 10 min prior to recording the cyclic voltammogram.

Preparation of Rat Brain Homogenate. Albino Charles−Foster rats (180−200 g) of either sex were used for the study. Prior to decapitation and removal of the brain, the animals were anesthetized with ether and perfused transcardially with ice-cold normal saline to prevent contamination of the brain tissue with blood. The tissue was weighed, and the homogenate was prepared in 150×10^{-3} mol dm^{-3} KCl and centrifuged at 800*g* for 10 min. The supernatant was immediately used for the study of in vitro lipid peroxidation (*29*).

Lipid Peroxidation Study. The incubation mixture contained, in a final volume of 1 mL, brain homogenate (500 μL), KCl (150×10^{-3} mol dm^{-3}), and ethanol (10 μL) or test compound dissolved in ethanol. Peroxidation was initiated by adding, to give the final concentrations stated, $FeSO_4$ (200×10^{-6} mol dm^{-3})/$FeCl_3$ (200×10^{-6} mol dm^{-3}) or CuOOH (100×10^{-6} mol dm^{-3}). This mixture was incubated for 20 min at 37 °C, the reaction was stopped by adding 2 mL of ice-cold 0.25 N HCl containing 15% trichloroacetic acid, 0.38% thiobarbituric acid, and 0.05% butylated hydroxytoluene (BHT). Then the mixture was heated at 80 °C for 15 min, samples were cooled and centrifuged at 1000*g* for 10 min, and the absorbance of the supernatant was measured at 532 nm. The amount of lipid peroxidation was determined using the molar extinction coefficient of 1.56×10^5 mol^{-1} dm^3 cm^{-1} and expressed as thiobarbituric acid reactive substances (TBARS). Another identical experiment was performed in the absence of any inducing agents to find out the amount of TBARS due to spontaneous peroxidation, and the value was subtracted from TBARS values obtained in the presence of the inducing agents (*30*). The percent inhibition of TBARS formed was calculated by comparing with vehicle only control experiments. Iron solutions were prepared fresh and used immediately. Because most buffers trap the hydroxyl radical or interfere with iron, the reactions were carried out unbuffered in 150×10^{-3} mol dm^{-3} KCl. Results are the means of experiments conducted in triplicate.

Hydroxyl Radical-Induced Deoxyribose Degradation Assay. The reaction mixture containing SOH ($0.1-60 \times 10^{-6}$ mol dm^{-3}), deoxyribose (2.8×10^{-3} mol dm^{-3}), phoshate buffer (pH 7.4, 20×10^{-3} mol dm^{-3}), ferric chloride (100×10^{-6} mol dm^{-3}), ethylenediaminetetraacetic acid (EDTA) (104×10^{-6} mol dm^{-3}), and hydrogen peroxide (1×10^{-3} mol dm^{-3}) in a final volume of 1 mL was incubated at 25 °C for 1 h. One milliliter of thiobarbituric acid and 1 mL of trichloroacetic acid were added to this solution and placed in a boiling water bath for 15 min. The reaction mixture was cooled, and the absorbance was measured at 532 nm (*31*). Fresh solutions of $FeCl_3$ and SOH were prepared and used.

Reduction of Ferric Ions. The reaction mixture containing SOH ($25-200 \times 10^{-6}$ mol dm^{-3}), *o*-phenanthroline (0.25 mg/mL), $FeCl_3$ (50×10^{-6} mol dm^{-3}), and phosphate buffer (pH 7.4, 20×10^{-3} mol dm^{-3}) in a final volume of 2 mL was incubated at 25 °C for 10 min. The absorbance was measured at 510 nm (*29*). In another experiment, ascorbic acid (0.2×10^{-3} mol dm^{-3}) was added instead of SOH to reduce all of the Fe(III) and the absorbance obtained was considered as equivalent of 100% reduction of Fe(III).

DNA Nicking Assay. All experiments were conducted in phosphate buffer (pH 7.4, 50×10^{-3} mol dm^{-3}). To assess the nonspecific scavenging activity of SOH toward the hydroxyl radical, 2 μL of SOH (different concentrations), EDTA (30×10^{-3} mol dm^{-3}), phosphate buffer (pH 7.4, 50×10^{-3} mol dm^{-3}), H_2O_2 (30×10^{-3} mol dm^{-3}), $FeSO_4$ (16×10^{-3} mol dm^{-3}), and plasmid DNA (PGEM-7ZF, 0.1 mg/mL) was mixed in a 500 μL microcentrifuge tube. The molar ratio of $FeSO_4$/EDTA was kept at 0.53. The final volume of the reaction mixture was brought to 12 μL with deionized distilled water and incubated for 1 h at 37 °C. Following incubation, 2 μL of loading dye (6XESB-20XTEA buffer, glycerol, bromophenol blue, xylene cyanole, and water) was added and 12 μL of this mixture was loaded onto an agarose gel well and the gel was run using 0.5XTBE running buffer (Tris, boric acid, and EDTA). DNA bands were visualized and recorded in a gel documentation system (Vilber Loumat).

RESULTS AND DISCUSSION

The observed pK_a of 8.75 of SOH has been attributed to deprotonation of the only ionizable phenolic group present in SOH at the 5-position. Reactions of SOH with various free radicals have been studied and compared to assign the absorption maxima to various transient free radicals produced. These studies are used to understand its reaction with oxidizing radicals and mode of antioxidant action.

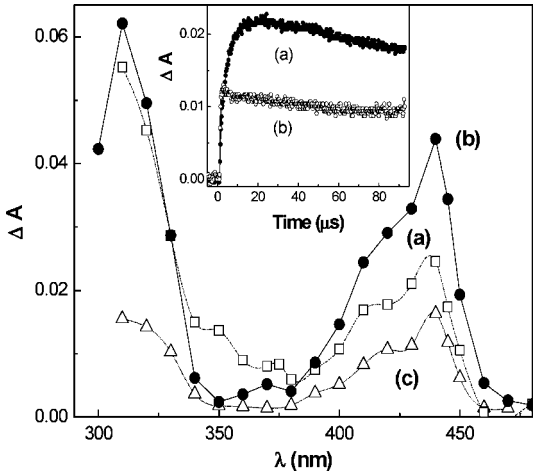
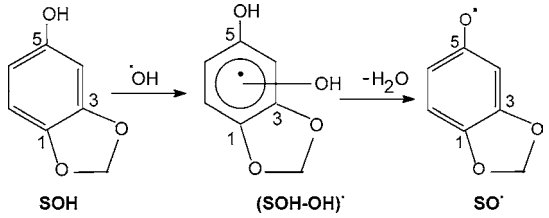


Figure 1. Transient absorption spectrum obtained for the reaction of SOH with (**a**) •OH (dose = 14.3 Gy) at 20 μs, (**b**) N_3• (dose = 14.5 Gy) at 5 μs, and (**c**) CCl_3O_2• (dose = 13.1 Gy) at 20 μs; after the electron pulse. Inset: kinetic traces at (**a**) 440 and (**b**) 350 nm for reaction with •OH (dose = 14.3 Gy).

Scheme 1



Reaction with the Hydroxyl, Azidyl, and Dibromide Anion Radicals. The hydroxyl radical (•OH) is one of the most damaging oxidizing radicals and is produced in physiological systems (*4*, *5*). Its reactions with various biomolecules are diffusion-controlled and are well-compiled (*32*). SOH (4.4×10^{-4} mol dm^{-3}) has been found to react with •OH at pH 6.8 to give transient/s with absorption maxima at 350 and 440 nm (**Figure 1**, curve a). The hydroxyl radical scavenging rate constant has been measured at 350 nm ($k = 1.1 \times 10^{10}$ mol^{-1} dm^3 s^{-1}). The transient absorption at 440 nm, however, grows with first-order kinetics ($k = 2 \times 10^5$ s^{-1}). The difference in rates of formation of transient absorptions at 350 and 440 nm are evident from the inset in **Figure 1**. The absorptions at 350 and 440 nm can be ascribed to the formation of a SOH−hydroxyl radical adduct (SOH−OH)• and sesamolyl radical (SO•), respectively. A clear transformation of the solute−radical adduct into phenoxyl radical is generally not observed in the aqueous medium, but a slow first-order formation of 440 nm absorption in the present case supports the proposed reaction mechanism (**Scheme 1**). Because •OH is known to react by addition, H-abstraction, and electron transfer reactions, the reaction of SOH with one-electron oxidants has been studied to characterize the one-electron oxidized radical unambiguously and study its kinetics. The azidyl radical (N_3•) is known to cause only selective one-electron oxidation of an organic molecule, so the solute radical produced by it can be assigned to their one-electron oxidized species (*33*). N_3• has been found to react with SOH (2×10^{-4} mol dm^{-3}), at pH 6.8, to produce a transient absorption spectrum with a maximum at 440 nm with a measured rate constant of 4×10^9 mol^{-1} dm^3 s^{-1} (**Figure 1**, curve b).

SOH has also been found to scavenge another one-electron oxidant, $Br_2^{\bullet-}$, at a measured rate of 7×10^8 mol^{-1} dm^3 s^{-1} as measured by observing the decay of $Br_2^{\bullet-}$ at 360 nm. Although $Br_2^{\bullet-}$ is a stronger oxidant (*34*), the measured rate is less than that with N_3•. This is due to the measurement of the decay rate of oxidant ($Br_2^{\bullet-}$) rather than the formation of the solute radical and is bound to have more error.

The oxidizable group in SOH is the phenolic group. Phenols react with oxidants to produce a phenoxyl radical with an absorption ~430 nm. Therefore, the absorption at 440 nm can be assigned to the one-electron oxidized sesamolyl (SO•) radical, which is also suggested earlier in the photochemical reaction of SOH (*23*, *24*). The transient absorption corresponding to SO• formed by •OH as well as N_3• decayed with second-order kinetics ($2k = 9.1 \times 10^8$ mol^{-1} dm^3 s^{-1}) as is the case with phenoxyl radicals.

Reaction with the Trichloromethyl Peroxyl Radical. The reactions of organohalo peroxyl radical with various solutes are well-reported (*34*). These studies are of importance because the toxicity of organohalogens, which are used in solvents and pesticides, is known to be due to the formation of organohaloperoxyl radicals (*34*). The reduction potential of the trichloromethyl peroxyl peroxyl (CCl_3O_2•) radical, 1.5 V (*35*), is more as compared to those of the physiologically relevant peroxyl radicals. However, CCl_3O_2• radical has been used as a representative one for its inherent simplicity in performing the experiments and generation in the toxicity of CCl_4.

SOH (5×10^{-4} mol dm^{-3}) has been found to react with CCl_3O_2• at pH 6.8 to produce a transient absorption maximum at 440 nm with a rate constant of 3.7×10^8 mol^{-1} dm^3 s^{-1} (**Figure 1**, curve c). SOH has also been found to react with $CHCl_2O_2$• to produce SO• with a rate constant of 7.2×10^7 mol^{-1} dm^3 s^{-1} as measured at 440 nm.

The reaction of SOH with various oxidizing radicals has been found to generate a phenoxyl radical of SOH (440 nm) in amounts ranging from 44% with •OH to 80% with CCl_3O_2•. The reaction of SOH with N_3• is assumed to produce the one-electron oxidized SO• only. The rate constant values for the reactions of SOH with oxidizing radicals have been found to follow the order of their reduction potentials. The benzodioxole derivatives are reported to undergo demethylation in their reaction with the hydroxyl radical (*18*) to give 1,2-dihydroxy benzene derivatives as products, and the sesamolyl radical is known to decay to produce dimers (*22*) along with other products. Therefore, reaction of SOH with oxidizing radicals produces more than one transient, which makes it difficult to ascribe decay kinetics and mechanism of the produced transients explicitly.

Reduction Potential of SO•/SOH Couple. The reduction potential of the oxidized species of the antioxidant is generally measured to compare it with other known antioxidants. Cyclic voltammetry can be used to determine the redox potential and the total antioxidant capacity of natural products, biological samples, etc. (*36*). The potential at which the anodic current is maximum and the area under the current−voltage curve are a measure of the antioxidant potential and capacity, respectively. The reduction potential of SO•/SOH couple has been measured using cyclic voltammetry and differential cyclic voltammetry. It is known that methoxy and hydroxy substitution at ortho-, meta-, and para-positions reduce the bond dissociation energy of the O−H bond of phenol (*10*) making it a better reductant. This concept suggests a lower bond dissociation energy of the O−H bond of natural phenolic compounds, as compared to phenols, depending on their substituents.

The oxidation potential of SOH at pH 7 has been found to be +0.525 V vs SCE, i.e., +0.766 V vs NHE (**Figure 2**, curve

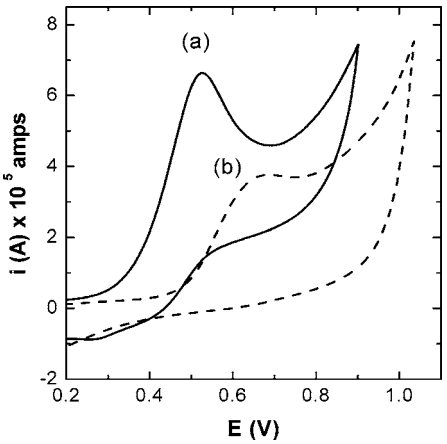


Figure 2. Cyclic voltamogram of (**a**) SOH and (**b**) *p*-coumaric acid in normal mode with SCE as the reference electrode.

a) in normal mode. The cyclic voltamogramm of *p*-coumaric acid (a phenolic acid) has been also shown (**Figure 2**, curve b) along with it for comparison. The oxidation potential of SOH (0.766 V) has been found to be more than those for trolox (*37a*) and ascorbic acid (*37a*); comparable to ferulic acid (*37b*) and lesser than those for *p*-coumaric acid (*37b*) and curcumin (*37c*). This suggests that the electron donating ability of SOH is more than those for, at least, *p*-coumaric acid and curcumin in aqueous solutions. This electron donating capaciy is sufficient to scavenge hydroxyl, peroxyl, lipid peroxyl, and trptophanyl radicals and consequently produce a less reactive sesamolyl radical, which in turn is scavenged by ascorbic acid. In addition to free radical scavenging capacity, solubility in aqueous as well as lipid phases, stability at high temperatures, and nontoxicity make it an efficient antioxidant in various chemical environments as compared to other phenolic antioxidants.

Reaction of the Tryptophanyl Radical with SOH. Tryptophanyl radical ($Trp^{\bullet}$) is first generated in the reaction of proteins with oxidizing radicals. This is followed by charge transfer from tyrosine to $Trp^{\bullet}$ to produce phenoxyl radical of tyrosine ($TyrO^{\bullet}$) before producing protein−protein cross-linking or damage (*38*, *39*). The repair of the tryptophanyl radical ($Trp^{\bullet}$) by SOH has been attempted to study its antioxidant effect against protein damage. The concentrations of tryptophan and SOH were selected optimally to observe the reaction of $Trp^{\bullet}$ with SOH. $Trp^{\bullet}$ was generated by reacting tryptophan with $N_3^{\bullet}$. In the reaction of $Trp^{\bullet}$ with SOH, a simultaneous decrease in the absorption of $Trp^{\bullet}$ at 510 nm and an increase in the absorption of $SO^{\bullet}$ at 440 nm suggest a repair of $Trp^{\bullet}$ by charge transfer from SOH (**Figure 3**).

After 500 μs, the absorption due to $Trp^{\bullet}$ at 510 nm becomes negligible but that due to $SO^{\bullet}$ is still present. The rate constant of the repair of $Trp^{\bullet}$ by SOH at pH 6.8 has been measured to be 6.7×10^7 mol^{-1} dm^3 s^{-1} at 510 nm. This shows that SOH can protect the proteins against their oxidative damage thereby acting as an antioxidant.

Reaction of $SO^{\bullet}$ with Vitamin C. Vitamin C ($AscH_2$, pK_a = 4.1, 11.8) is a well-known physiological antioxidant, which either repairs or scavenges oxidizing radicals. Vitamin C and its one-electron oxidized radical exist as $AscH^-$ and $Asc^{\bullet-}$, respectively, at neutral pH (*40*). The transient absorption spectrum for the reaction of $CCl_3O_2^{\bullet}$ with SOH and $AscH^-$ (both 1×10^{-3} mol dm^{-3} each) at pH 6.8 showed $Asc^{\bullet-}$ (360 nm) and $SO^{\bullet}$ (440 nm) at 5 μs after the initiation of the reaction (**Figure 4**).

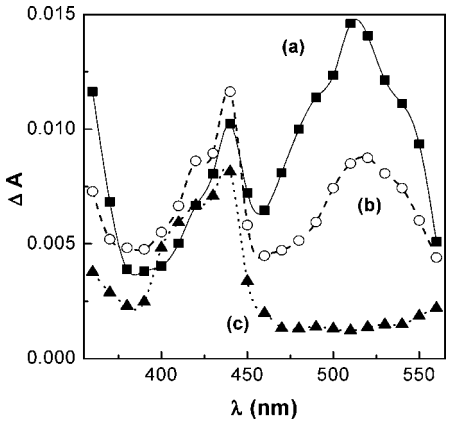


Figure 3. Transient absorption spectrum obtained from N_2O-saturated aqueous solution containing SOH (5.0×10^{-5} mol dm^{-3}), tryptophan (1.0×10^{-3} mol dm^{-3}), and NaN_3 (1.0×10^{-1} mol dm^{-3}) at pH 6.8; (**a**) 10, (**b**) 100, and (**c**) 500 μs after the electron pulse. Dose = 14.3 Gy.

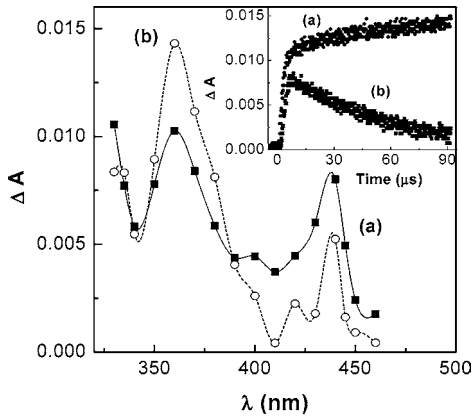


Figure 4. Transient absorption spectrum obtained from an aerated aqueous solution containing SOH (1.0×10^{-3} mol dm^{-3}), ascorbic acid (1.0×10^{-3} mol dm^{-3}), *tert*-butyl alcohol (2.5 mol dm^{-3}), and CCl_4 (1.0×10^{-2} mol dm^{-3}) at pH 6.8; (**a**) 5 and (**b**) 80 μs after the electron pulse. Dose = 13.5 Gy. Inset: kinetic traces under identical conditions at (**a**) 360 and (**b**) 440 nm.

The concentrations of SOH and $AscH^-$ have been selected to observe the reaction of $SO^{\bullet}$ with $AscH^-$. The formation of $Asc^{\bullet-}$ and $SO^{\bullet}$ has been followed by charge transfer from $AscH^-$ to $SO^{\bullet}$ as observed by the transient absorption spectrum at 80 μs as well as traces at 360 and 440 nm (**Figure 4** and inset). The rate constant measured at pH 6.8 for this charge transfer was 2.2×10^7 mol^{-1} dm^3 s^{-1} at 440 nm. This suggests that sesamolyl radical ($SO^{\bullet}$) is repaired by vitamin C ($AscH^-$) at neutral pH. This agrees well with the fact that the couple of higher reduction potential values is reduced as the reduction potential of the $SO^{\bullet}$/SOH couple (+0.766 V) found by cyclic voltammetry is higher than that of the reported value of the $Asc^{\bullet-}$/$AscH^-$ couple (+0.33 V) (*37a*). Various measured rate constants and transient absorption maxima are reported in **Table 1**.

Inhibition of Lipid Peroxidation. SOH is known to protect sesame oil against oxidative deterioration and has been found to efficiently scavenge the lipid peroxyl radical. Therefore, inhibition of lipid peroxidation by SOH in biomimetic systems has been studied. Ferric-, ferrous-, and cumene hydroperoxide-induced lipid (rat brain homogenate) peroxidation has been studied as described in the Materials and Methods.

Lipid peroxidation in rat brain homogenate was stimulated by the addition of Fe(III) (200×10^{-6} mol dm^{-3})/Fe(II) (200

Table 1. Rate Constants and Transient Characteristics in the Reaction of SOH with Various Radicals

radical	pH	λ (nm)	k_f (mol^{-1} dm^3 s^{-1})
$^{\bullet}OH$	6.8	350, 440	1.1×10^{10}
$N_3^{\bullet}$	6.8	440	4.0×10^9
$Br_2^{\bullet -}$	6.8	360	7×10^{8a}
$CCl_3O_2^{\bullet}$	6.8	440	3.7×10^8
$CHCl_2O_2^{\bullet}$	6.8	440	7.2×10^7
Trp$^{\bullet}$	6.8	510	6.7×10^{7a}
$LO_2^{\bullet}$	11	440	3×10^8
$SO^{\bullet} + AscH^-$	6.8	440	2.2×10^{7a}

[a] From decay kinetics.

Table 2. Inhibition of Fe(III)-, Fe(II)-, and Cumene Hydroperoxide-Induced Lipid Peroxidation by SOH

$[SOH] \times 10^6$ (mol dm^{-3})	absorbance ± SE	TBARS ± SE (nmol/mL)	% protection
	Fe(III)		
control (without $FeCl_3$)	0.257 ± 0.004	1.65 ± 0.02	
control (with $FeCl_3$)	1.263 ± 0.02	8.09 ± 0.12	
90	0.264 ± 0.01	1.69 ± 0.06	99.3
60	0.664 ± 0.007	4.26 ± 0.08	59.49
30	0.948 ± 0.002	6.08 ± 0.04	31.3
15	1.022 ± 0.005	6.55 ± 0.06	23.9
10	1.122 ± 0.002	7.19 ± 0.04	14.0
7.5	1.145 ± 0.005	7.34 ± 0.05	11.7
	Fe(II)		
control (without $FeSO_4$)	0.142 ± 0.01	0.912 ± 0.06	
control (with $FeSO_4$)	0.481 ± 0.02	3.08 ± 0.04	
90	0.244 ± 0.01	1.56 ± 0.009	69.91
	cumene hydroperoxide		
control (without CuOOH)	0.228 ± 0.002	1.46 ± 0.03	
control (with CuOOH)	0.482 ± 0.02	3.09 ± 0.1	
90	0.335 ± 0.01	2.15 ± 0.09	57.87

$\times 10^{-6}$ mol dm^{-3}) or CuOOH (100×10^{-6} mol dm^{-3}), and the data are given in **Table 2**.

Ferric ions have been found to be more effective as compared to ferrous ions or CuOOH in stimulating peroxidation. The amount of TBARS formed at the end of 20 min incubation was 8.09, 3.08, and 3.09 nmoles/mL of the tissue homogenate when stimulated by Fe(III), Fe(II), and CuOOH, respectively.

It has been found that 90 μM concentration of SOH inhibited Fe(III)-, Fe(II)-, and cumene hydroperoxide-induced lipid peroxidation by ~99, ~70, and ~58%, respectively (**Table 2**). The effect of SOH on Fe(III)-induced lipid peroxidation measured by calculating the percentage protection of lipid as presented by columns (with ordinate on left) and formation of TBARS as presented by a line graph (with ordinate on right) is shown in **Figure 5a**.

This shows the concentration-dependent antioxidant activity of SOH against Fe(III)-induced lipid peroxidation. A similar behavior of SOH is also observed against lipid peroxidation induced by Fe(II) and cumene hydroperoxide (figures not shown). In unstimulated control experiments, the amount of TBARS formed was 1.65 nmol/mL. However, in the absence of the inducing agents, TBARS formed was negligible. In all of these experiments, BHT was added after incubation but before heating. This prevents the formation of additional TBARS during heating due to the breakdown of the lipid hydroperoxide (*30*). Control experiments showed that none of the test compounds affected the measurement of TBARS (omission of the brain homogenate from the reaction mixture abolished chromogen formation). Furthermore, none of the compounds interfered with the TBA test since they did not alter the color

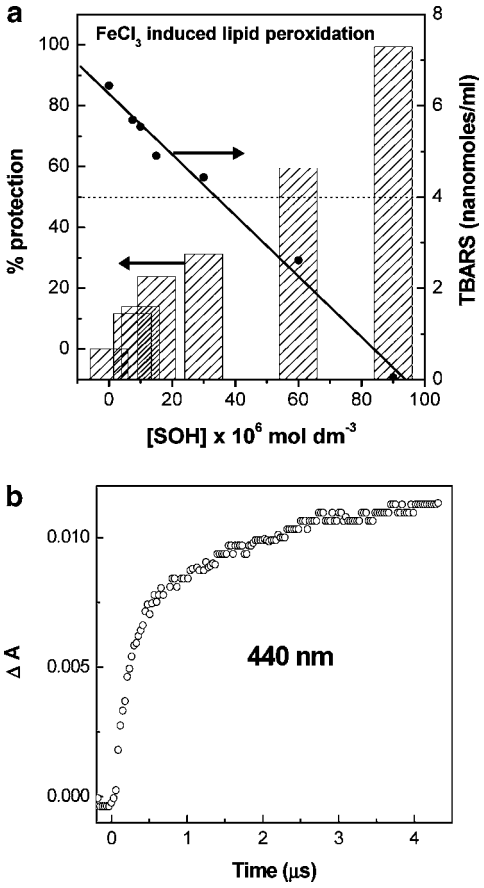


Figure 5. (**a**) Effect of SOH on Fe(III)-induced lipid peroxidation and TBARS formation. (**b**) Kinetic trace observed on pulse irradiation of aqueous solution containing linoleic acid (2.0×10^{-2} mol dm^{-3}) and SOH (2.0×10^{-4} mol dm^{-3}) at pH 11 and saturated with $N_2O/O_2 = 3$; dose = 14.5 Gy.

development if they were added at the end of the incubation but before heating (*41*).

Cumene hydroperoxide stimulates lipid peroxidation through the free radicals originating from the homolytic or the hetrolytic fission of the O−O bond of the hydroperoxides at the level of cytochrome P450. In this case, the lipid peroxidation is completely independent of iron ions and takes place even in the presence of relatively high concentrations of chelating agents such as EDTA (*42*). Fe(II) stimulates lipid peroxidation by generation of the hydroxyl radical whereas Fe(III) stimulates lipid peroxidation by the intermediacy of perferryl or ferryl species. Our studies (discussed in later section) have shown that the ferric ions also undergo reduction in the presence of SOH. Thus, the total amount of the ferric ions available for the stimulation may be partly reduced by SOH to the ferrous ions, which are less effective in stimulating lipid peroxidation. A similar explanation (*43*) was also suggested to explain the greater effectiveness of curcumin in inhibiting Fe(III)-induced lipid peroxidation as compared to that by the Fe(II) system.

Reaction with Lipid Peroxyl Radicals. Lipids (LH) are constituents of the cell membrane and are also known to generate oxidizing radicals upon reacting with physiologically produced oxidizing radicals. Thus, the produced lipid peroxyl radical ($LOO^{\bullet}$) can initiate a chain reaction to cause further damage to the cell membrane and bioconstituents. This vicious cycle of oxidation can only be terminated by lipid soluble antioxidants.

$$LH + {}^{\bullet}OH \rightarrow L^{\bullet} + H_2O \quad (9)$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad (10)$$

$$LOO^{\bullet} + LH/SOH \rightarrow LOOH + L^{\bullet}/SO^{\bullet} \quad (11)$$

The reaction of $LOO^{\bullet}$ radical with SOH has been studied to investigate its antioxidant action in the lipid phase. $LOO^{\bullet}$ has been generated in situ by radiolysis of N_2O and O_2 saturated (3:1) aqueous solution of linoleic acid (2×10^{-2} mol dm^{-3}) at pH 11. Scavenging of $LOO^{\bullet}$ has been studied by the formation of $SO^{\bullet}$ at 440 nm (**Figure 5b**). The $LOO^{\bullet}$ scavenging rate constant (3×10^8 mol^{-1} dm^3 s^{-1}) at pH 11 is high due to the fact that anionic forms of phenols, SOH with pK_a = 8.75, transfer electrons faster than their protonated counterparts. Thus, it acts as a chain breaking antioxidant in the lipid phase. The solubility of SOH in the lipid phase also makes it a potent antioxidant as compared to other phenols of specific solubilities. These studies suggest that SOH inhibits lipid peroxidation in chemical as well as biochemical systems.

Inhibition of Deoxyribose and Plasmid DNA Degradation. The nonsite specific $^{\bullet}OH$ scavenging activity of SOH was assessed by employing both chemical (deoxyribose) and biological (DNA nicking) in vitro assays. **Table 3** lists data on the effect of SOH on the degradation of deoxyribose by the hydroxyl radical. SOH has been found to inhibit degradation of deoxyribose in a concentration-dependent manner (**Table 3**) with 50% protection at $\sim 10 \times 10^{-6}$ mol dm^{-3}. However, the protection has not been found to increase significantly above 53% at and above $\sim 40 \times 10^{-6}$ mol dm^{-3} concentration of SOH. In pulse radiolysis experiments, SOH has not been found to scavenge deoxyribose radical, which was produced with $^{\bullet}OH$. The direct scavenging of $^{\bullet}OH$ radical by SOH at 30×10^{-6} mol dm^{-3} is $\sim$2.5%, which is not sufficient to show high (>50%) deoxyribose protection. Therefore, reduction of Fe(III) into Fe(II) and sequestration of intermediates (ferryl, perferryl, etc.) produced in Fenton reactions seem to play a major role in the deoxyribose protection in the present case.

The efficiency of SOH to protect DNA against oxidative damage by hydroxyl radical produced by Fenton reactants in an aqueous medium was also studied. The effect of different concentrations of SOH was studied on PGEM-7ZF plasmid DNA damage (**Figure 6**). It has not been found to affect the plasmid DNA in the ground state and showed differential antioxidant activity for DNA against oxidative damage. No protection of the plasmid DNA is observed at low SOH concentration (5×10^{-4} mol dm^{-3}), but at a higher SOH concentration (2.5×10^{-3} mol dm^{-3}), protection is almost 50% (**Figure 6**). $^{\bullet}OH$ generated by Fenton reactants attacks DNA guanosine residues, resulting in strand breaks and transformation from the native supercoiled (i.e., lane 1) to the linear form (i.e., lane 2). SOH alone did not break DNA (lanes 3−5 in **Figure 6**), at all of the concentrations tested. SOH at a concentration of 5×10^{-4} mol dm^{-3}, in the presence of Fenton reactants, failed to prevent nonsite specific $^{\bullet}OH$-induced oxidative damage to DNA (lane 6 in **Figure 6**) but, at 1.5×10^{-3} and 2.5×10^{-3} mol dm^{-3}, protected DNA in the supercoiled and nicked circular forms, without allowing complete breakage to the degraded form by Fenton agents (lanes 7 and 8 in **Figure 6**).

Reaction with Fe(III). Ferrous [Fe(II)] and ferric [Fe(III)] salts have been used in Fenton reactions and are known to be involved in various free radical reactions occurring in physiological conditions. A study of the reactions of SOH with Fe(II) and Fe(III) has been attempted. SOH has not been found to react with Fe(II) at pH 7. However, it reduces Fe(III) into Fe(II) in a concentration-dependent manner at pH 7.4 with $\sim$50% reduction at $\sim 1.45 \times 10^{-4}$ mol dm^{-3} (**Table 4**). In this study, reduction of Fe(III) into Fe(II) by ascorbate is taken as 100%.

Table 3. Inhibition of Hydroxyl Radical-Induced Deoxyribose Degradation by SOH

[SOH] × 10^6 (mol dm^{-3})	absorbance ± SE	TBARS ± SE (nmol/mL)	% protection
blank	0.12 ± 0.004	0.77 ± 0.02	
control	1.53 ± 0.02	9.8 ± 0.12	
60	0.77 ± 0.007	4.99 ± 0.05	53.32
40	0.78 ± 0.02	5.01 ± 0.10	53.06
20	0.79 ± 0.01	5.09 ± 0.04	52.21
10	0.81 ± 0.005	5.23 ± 0.04	50.65
8	0.85 ± 0.01	5.46 ± 0.06	48.12
4	1.0 ± 0.01	6.41 ± 0.08	37.55
0.1	1.32 ± 0.005	8.49 ± 0.02	14.58

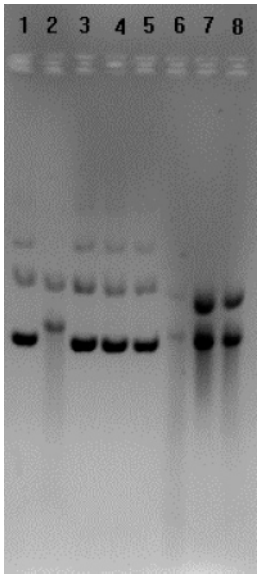


Figure 6. Modulation of nonsite specific DNA strands cleavage by SOH. Lane 1, original DNA; lane 2, DNA (0.2 μg) + EDTA (30 m mol dm^{-3}) + H_2O_2 (30×10^{-3} mol dm^{-3}) + $FeSO_4$ (16×10^{-3} mol dm^{-3}) + phosphate buffer (50×10^{-3} mol dm^{-3}, pH 7.5); lanes 3–5, DNA (0.2 μg) + SOH at concentrations of 0.5, 1.5, and 2.5×10^{-3} mol dm^{-3}, respectively. Lanes 6–8, DNA (0.2 μg) + SOH at concentrations of 0.5, 1.5, and 2.5 $\times 10^{-3}$ mol dm^{-3}, respectively, + EDTA (30×10^{-3} mol dm^{-3}) + H_2O_2 (30×10^{-3} mol dm^{-3}) + $FeSO_4$ (16×10^{-3} mol dm^{-3}) + phosphate buffer (50×10^{-3} mol dm^{-3}, pH 7.5). Form I = supercoiled DNA; form II = nicked circular DNA.

Table 4. Reduction of Fe(III) to Fe(II) by SOH

[SOH] × 10^6 (mol dm^{-3})	absorbance ± SE	% reduction
blank	0.006 ± 0.00	
ascorbic acid	1.59 ± 0.02	100
25	0.38 ± 0.002	24.16
50	0.62 ± 0.006	39.27
100	0.65 ± 0.01	41.22
150	0.88 ± 0.001	55.31
200	0.90 ± 0.007	56.7

Conclusions. SOH, a unique dietary phenolic compound, is stable in physiological pH range and soluble in both the aqueous and the lipid phase with a pK_a at 8.75. It scavenges various primary and secondary oxidizing radicals, including physiologically produced hydroxyl, tryptophanyl, and lipid peroxyl

radicals. One-electron oxidized radical of SOH is scavenged by physiologically present antioxidant, vitamin C. Cyclic voltammetry suggests that its electron donating capacity is better or comparable to other natural antioxidants. SOH inhibits lipid peroxidation, deoxyribose degradation, and DNA degradation in in vitro biomimetic model systems.

It can be inferred that the free radical scavenging activities of SOH along with its nontoxicity, solubility in aqueous as well as lipid phases, and stability at high temperatures during cooking make it a potent antioxidant in physiological conditions and in edible oils.

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