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Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species

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Abstract

Caffeine (1,3,7-trimethyl xanthine), an ingredient of coffee, has been investigated for its potential antioxidant activity against oxidative damage to rat liver microsomes. Such damage was induced by three reactive oxygen species of cardinal importance in causing membrane damage in vivo namely hydroxyl radical ($^{\cdot}OH$), peroxyl radical ($ROO^{\cdot}$) and singlet oxygen ($^1O_2$). The results obtained showed that caffeine was an effective inhibitor of lipid peroxidation, at millimolar concentrations, against all the three reactive species. The extent of inhibition was high against peroxidation induced by $^{\cdot}OH$, medium against $^1O_2$ and low against $ROO^{\cdot}$. In general, the antioxidant ability of caffeine was similar to that of the established biological antioxidant glutathione and significantly higher than ascorbic acid. Investigations into the possible mechanisms involved in the observed antioxidant effect reveal that the quenching of these reactive species by caffeine may be one of the possible factor responsible. The rate constant of caffeine with $^{\cdot}OH$ was $7.3 \cdot 10^9$ $M^{-1} s^{-1}$ and with $^1O_2$ it was $2.9 \cdot 10^7$ $M^{-1} s^{-1}$. Considering their potential for damage, half-life estimates and generation in biological systems, the ability of caffeine to inhibit oxidative damage induced by these reactive species in membranes suggest one more positive attribute of caffeine, whose daily intake as coffee may be considerable in most populations.

Keywords: Caffeine; Free radical; Singlet oxygen; Lipid peroxidation; Antioxidant; (Rat liver microsome)

1. Introduction

Caffeine or 1,3,7-trimethyl xanthine is a component of the widely used beverage coffee and is used as adjuvant analgesic in combination with drugs like acetaminophen, aspirin and ibuprofen [1,2]. Though it has been shown to have several pharmacologically useful effects, it is largely regarded as a sensitizer of damage or radiosensitizer by virtue of its reported inhibition of DNA repair in certain cell types [3]. However, several recent studies suggest that if caffeine is present during the time of occurrence of damage induced by radiation or other agents, it can afford significant protection. For instance in human TK6 lymphoblastoid cells, 2 mM caffeine either before or up to 3 h after irradiation almost completely prevented radiation-induced apoptosis [4]. Much earlier, caffeine had been shown to exert protective effect against oxic component of radiation damage in cell culture systems (Chinese hamster ovary cells) [5], plant systems using seeds [6,7], and in aqueous suspensions of bacterial spores [8,9]. It was later shown that the radioprotective effect of caffeine could be due to its ability to scavenge potentially damaging $^{\cdot}OH$ and electrons [8–10].

A number of biochemical reactions in our body generate reactive oxygen species (ROS) mainly comprising free radicals and excited states, capable of damaging crucial biomolecules. If they are not effectively neutralised by the antioxidant defense system in the tissues, oxidative stress results [11,12]. Polyunsaturated fatty acids have become a central area of interest in the biochemistry of oxidative stress caused by ROS [11]. These fatty acids are mainly present in the phospholipids of biological membranes and their unspecific oxidation by ROS known as lipid peroxidation, is a radical mediated pathway and a highly damaging event. It generates toxic byproducts such as 4-hydroxynonanal known to be capable of altering other biomolecules in the cell [13,14]. Among the various reactive species which may induce this reaction in vivo, peroxyl radical ($ROO^{\cdot}$), the hydroxyl radical ($^{\cdot}OH$) and singlet oxygen ($^1O_2$) are of cardinal importance [15].

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One of the areas which has attracted a great deal of attention is the possible role of antioxidants in the control of oxidative damage [16]. Caffeine is consumed by millions either as an ingredient of coffee or as an analgesic adjuvant. Though there are reports on the free radical scavenging ability of caffeine [10,17], there are no detailed studies on the effect of caffeine on oxidative membrane damage induced by ROS. The present study examines the effect of caffeine in vitro on lipid peroxidation in rat liver microsomes induced by the three major reactive species of interest in biological systems, namely $^{\cdot}OH$, $ROO^{\cdot}$ and $^1O_2$, besides the possible mechanism(s) involved in terms of its ability to react with these species.

2. Materials and methods

2.1. Chemicals

Ascorbic acid, caffeine, ethylene diamine tetraacetic acid (EDTA), tetraethoxypropane, 2-thiobarbituric acid, Tris, methylene blue, Rose Bengal and reduced glutathione were purchased from Sigma Chemical Co., USA. Sodium azide was from BDH, UK. Chelex-100 was from Bio-Rad Laboratories. Deuterium oxide (99.8%) was obtained from the Heavy Water Division of our Research Centre. Other chemicals and reagents used in our studies were of analytical grade from reputed manufacturers. The peroxyl radical generating compound, azobis(2-amidopropane)dihydrochloride (AAPH), was a gift from Prof. Lester Packer, University of California.

2.2. Preparation of microsomes

Three-month-old female Wistar rats weighing approximately 270 ± 30 g were used for the preparation of microsomes. After fasting overnight, rats were killed by cervical dislocation, livers removed and homogenised in 0.25 M sucrose containing 1 mM EDTA. After centrifugation at 10 000 × *g* for 10 min, the supernatant obtained was recentrifuged to remove the remaining contaminants. This supernatant was then centrifuged in a Beckman L8-80 ultracentrifuge at 105 000 × *g* for 1 h to sediment microsomes. The pellet of microsomes was washed three times with 0.05 M sodium phosphate buffer (pH 7.4), previously treated with Chelex-100 for several hours to remove the contaminating trace metals (buffer A) or 0.05 mM Tris-HCl (pH 7.4) (Buffer B), resuspended in the same buffer at a concentration of 10 mg protein/ml and distributed into aliquots, which were first frozen in liquid nitrogen. After so freezing, these were stored in a deep freeze maintained at −20°C.

2.3. Exposure of microsomes to reactive oxygen species

For examining the protective effect of caffeine against lipid peroxidation induced by $^{\cdot}OH$, microsomes were subjected to γ-radiation in N_2O-saturated buffer A. For examining the effect on oxic radiation damage, the buffer was saturated with oxygen. For irradiation, the microsomes (final concentration 0.5 mg protein/ml) with or without caffeine were placed in a sealed tube in a Gamma cell (Chemistry Division, Bhabha Atomic research Centre, ^{60}Co source). The dose rate as determined by standard Fricke dosimeter was found to be 540 Gy/h. Lipid peroxidation induced by peroxyl radical initiator AAPH, was carried out by incubating 10 mM AAPH with microsomes in Buffer B at 37°C for 30 min with or without caffeine. The peroxidative products were estimated [18]. For exposing microsomes to $^1O_2$, this reactive species was generated in the gas phase using surface-separated sensitizer system [19]. For this Rose Bengal was immobilized on a glass plate using a thin layer of araldite and placed over 50 mM sodium phosphate buffer in D_2O, pD 7.4 (= pH 7.0) (Buffer C) containing microsomes (with or without caffeine) maintained at 37°C at a distance of 2 mm. Oxygen was passed through this gap and the plate was exposed to a light source of 100 W tungsten lamp. The rate of $^1O_2$ generation as well as the rate constant with caffeine was calculated using the histidine destruction assay [19] and the Stern-Volmer plot.

The system for exposing microsomes to photo-sensitization was ingenious and physiologically more relevant. In principle it was similar to that described earlier [20], with minor modifications as specified below. The microsomes (final concentration 0.5 mg protein/ml) were suspended in buffer A or C and kept in a 'trap' maintained at 37°C, with or without the sensitizer (methylene blue, 50 μM) and continuous and constant bubbling of O_2. The light source used was a 300 W projector lamp (tungsten), sufficient to induce significant peroxidation. The distance between light source and the trap was 15 cm. The light intensity, as measured by potassium ferrioxalate at 665 nm, in which methylene blue absorbs, was 0.11 mJ/cm^2/s. The duration of exposure was 5, 15, 30 and 60 min. However, photosensitization was carried at 30 min in order to avoid complications arising from peroxidative damage to microsomes when exposed to longer than 30 min exposure. After photosensitization, the thiobarbituric acid reactive substances (TBARS) formed were estimated as malonaldehyde equivalents, using tetraethoxypropane as standard [21]. Assays of conjugated dienes and lipid hydroperoxides were carried out by the methods of Buege and Aust [22]. Values represented in the present studies are mean ± S.E. of four experiments. Data have been analysed using Student's *t*-test. In all the figures, wherever necessary the level of significance as compared to respective controls are symbolized as follows: $^{*} = P < 0.05$; $^{++} = P < 0.001$.

2.4. Pulse radiolysis

To study the reaction of caffeine with $^{\cdot}OH$ and $ROO^{\cdot}$ pulse radiolysis technique was employed. 7 MeV electrons

from linear accelerator of pulse widths 50 ns were used and the transients were detected by kinetic spectrophotometry. Typical maximum doses with 50 ns pulses were 15 Gy [23]. The dosimetry was performed with air saturated 0.01 mol dm^{-3} KSCN solution with a G ∈ (500 nm) value of $2.23 \cdot 10^{-4}$ $M^2 J^{-1}$ for $(SCN)_2^-$ transient species. The kinetic spectrophotometric detection system covered the wavelength range from 300 to 700 nm. Cells with optical pathlength of 1 cm were used for these measurements. For pulse radiolysis measurements the absorbed dose was kept to a minimum to avoid decomposition of the test compound and the samples were changed after every pulse to minimise losses due to sample decomposition. The reactions of caffeine with 'OH were carried out using N_2O saturated aqueous solution where e_{aq}^- is quantitatively converted to 'OH. ($N_2O + e_{aq}^- \rightarrow$ 'OH + OH^- + N_2).

Lipid radical (L') was generated on reaction of H'/'OH radicals with linoleic acid (LH) in N_2O saturated solutions ($2 \cdot 10^{-4}$ M). LOO' radical was generated in N_2O/O_2 saturated solutions.

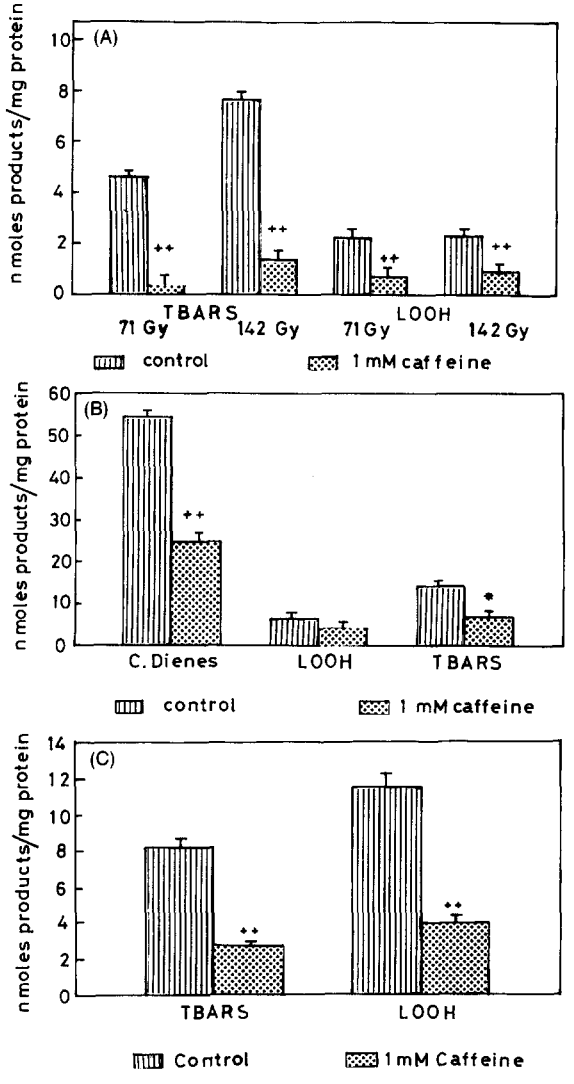

Fig. 1. Effect of caffeine on (A) 'OH-induced; (B) ROO'-induced and (C) $^1O_2$-induced lipid peroxidation in rat liver microsomes.

Table 1
Inhibition of lipid peroxidation induced by reactive oxygen species by caffeine, glutathione and ascorbic acid in rat liver microsomes

Inhibitor	Percent inhibition of peroxidation induced by		
	OH	ROO'	O_2
Against formation of TBARS			
Caffeine	82.6±6.8	42.7±4.8	50.5±2.9
Glutathione	67.5±3.0	10.9±0.6 $^{++}$	64.3±0.5 *
Ascorbic acid	29.7±1.7 $^{++}$	7.2±0.5 $^{++}$	0.0 $^{++}$
Against formation of LOOH			
Caffeine	60.5±5.0	40.5±2.2	53.5±4.5
Glutathione	71.7±5.2	18.8±0.8 $^{++}$	35.2±3.5 *
Ascorbic acid	21.5±1.1 $^{++}$	0.0 $^{++}$	13.0±0.9 $^{++}$

Values represent mean±S.E. of four experiments. Generation of reactive species were carried out as described in Section 2 and the concentration of antioxidants used was 1 mM. * $P < 0.05$, $^{++}$ $P < 0.001$, as compared to caffeine.

3. Results

The results show that reactive oxygen species like 'OH, ROO' and $^1O_2$ induced significant lipid peroxidation in rat liver microsomes as determined by the formation of TBARS, LOOH and conjugated dienes (Fig. 1). Increase in the dose of radiation from 71 to 142 Gy enhanced the formation of TBARS but not LOOH. Caffeine (1 mM) effectively inhibited peroxidation induced by 'OH radicals (Fig. 1A). The protective effect of caffeine was much larger for TBARS than for LOOH. Caffeine was also effective in inhibiting conjugated dienes ($P < 0.001$) and to a lesser extent the TBARS ($P < 0.05$) (Fig. 1B). Caffeine effect on LOOH induced by peroxyl radical was however not evident.

Table 1 presents data on the antioxidant abilities of caffeine as compared to two of the standard biological antioxidants namely glutathione and ascorbic acid, at the concentration of 1 mM. When formation of TBARS was used as the indicator of peroxidation, caffeine was more effective against peroxidation induced by 'OH and ROO', followed by glutathione and ascorbic acid. Glutathione was more effective against $^1O_2$-induced peroxidation than caffeine and ascorbic acid did not show any inhibition. The pattern of inhibition, however, was slightly different when LOOH was used as the indicator of oxidative damage. Glutathione was similar to caffeine against 'OH-induced damage while caffeine was more effective against peroxidation induced by ROO' and $^1O_2$. Ascorbic acid was the least effective in both the cases.

Fig. 2 shows the dose-dependent effect of caffeine on lipid peroxidation induced by ROS, as assessed by TBARS. Fig. 2A reveals that caffeine inhibits the 'OH-induced peroxidation significantly at or higher than 0.4 mM con-

centration. Significant ($P < 0.05$) inhibition against ROO·-induced peroxidation also occurred at and above 0.4 mM (Fig. 2B). Caffeine in the range of 0.4 to 4 mM exerts a dose-dependent inhibition of the ·OH-mediated but not of the ROO·-mediated formation of TBARS (Fig. 2A and B). Caffeine shows a 'biphasic effect' on peroxidation induced by $^1O_2$. Low concentrations (up to 0.4 mM) enhanced the formation of TBARS, whereas the higher concentrations (1 and 4 mM) inhibited peroxidation. The 'biphasic' effect of caffeine consistently occurred in several repeated experiments, but the cause of the influence of concentration is at present not clear. In this context, it is pointed out that Kesavan and Ahmed [24] had observed that caffeine acts as a radioprotective, radiosensitizer or neither in the presence of oxygen depending upon its concentration. It has been shown that caffeine competes with oxygen for electrons at a reaction rate constant $\sim 1.5 \cdot 10^{10}\ M^{-1}\ s^{-1}$ [8]. Under these circumstances more work is necessary to elucidate the cause of 'biphasic effect'.

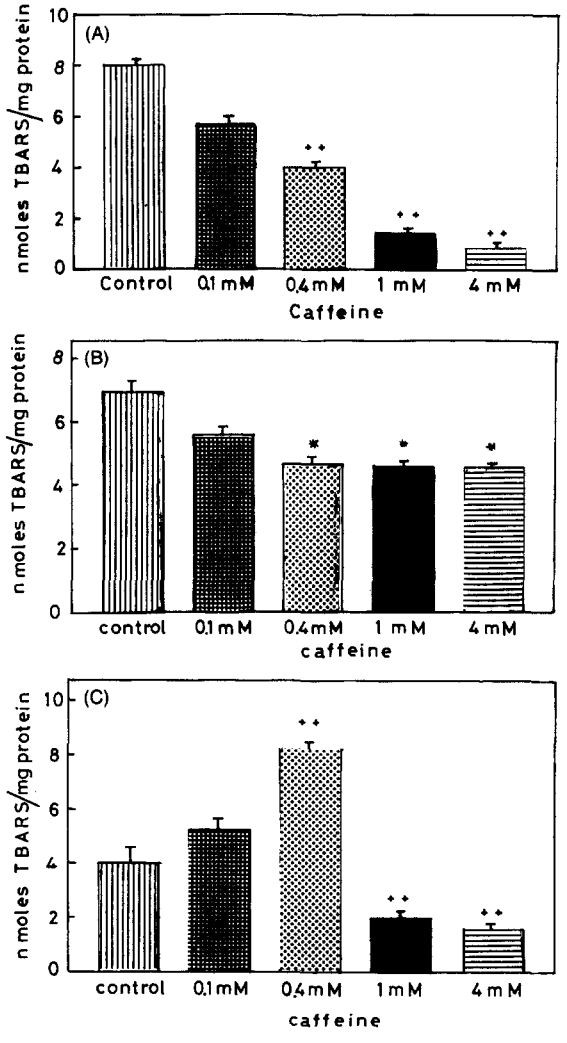

Fig. 2. Dose-dependent effect of caffeine on (A) ·OH-induced; (B) ROO·-induced and (C) $^1O_2$-induced lipid peroxidation as assessed by TBARS.

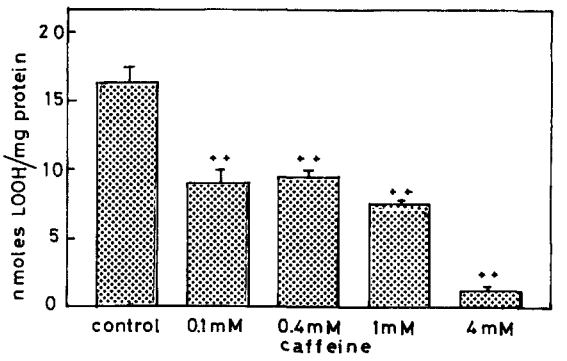

Fig. 3. Dose-dependent effect of caffeine on $^1O_2$-induced lipid peroxidation as assessed by lipid hydroperoxides.

Since lipid hydroperoxides are the specific products of peroxidation formed solely due to the addition of $^1O_2$ [25], we have also examined the dose-dependence of caffeine against the formation of LOOH. The results (Fig. 3) show that the caffeine significantly ($P < 0.001$) inhibits the formation of LOOH. It is noted that at 4 mM the inhibition is dramatic. Since TBARS are formed as breakdown products of LOOH, lower concentrations of caffeine may selectively enhance this phase of peroxidation. Similar biphasic effect of ascorbic acid has been shown against lipid peroxidation [13,16].

Fig. 4 presents data on the inhibitory effect of caffeine on the three products of peroxidation induced by ROO· as a function of time. The inhibition was higher against the formation of conjugated dienes and LOOH in the initial stages, and lower at the later stages of inhibition. With the formation of TBARS the pattern was slightly different, higher inhibitions being associated with the later half of the incubation period. The extent of inhibition observed at 2 h of incubation was significantly higher with TBARS than with other two products of peroxidation. The time course of $^1O_2$-induced lipid peroxidation and its inhibition by caffeine is shown in Fig. 5A and B. The formation of LOOH was more gradual than that of TBARS. The pattern of inhibition was linear with LOOH formation. The inhibition against the formation of TBARS was lower up to 30 min and then increased at a rapid rate.

γ-Radiation, in the presence of oxygen, is known to produce several other reactive species besides ·OH, capable of inducing peroxidation [26]. These include $HO_2^{\cdot}$ and $O_2^{\cdot -}$. Fig. 6A shows that the yield of peroxidation products did not increase significantly in the presence of oxygen (in comparison with data shown in Fig. 1A). Unlike the observation in Fig. 1A, it turned out that caffeine is a poor inhibitor of peroxidation induced by $HO_2^{\cdot}$ and $O_2^{\cdot -}$. Data presented in Fig. 6B show that deuteration of the buffer greatly enhanced peroxidation induced by γ-radiation. As compared with peroxidation in H_2O-based buffer at 70 Gy, a much lower dose of 25 Gy could produce significantly more peroxidation. The pattern of inhibition by caffeine,

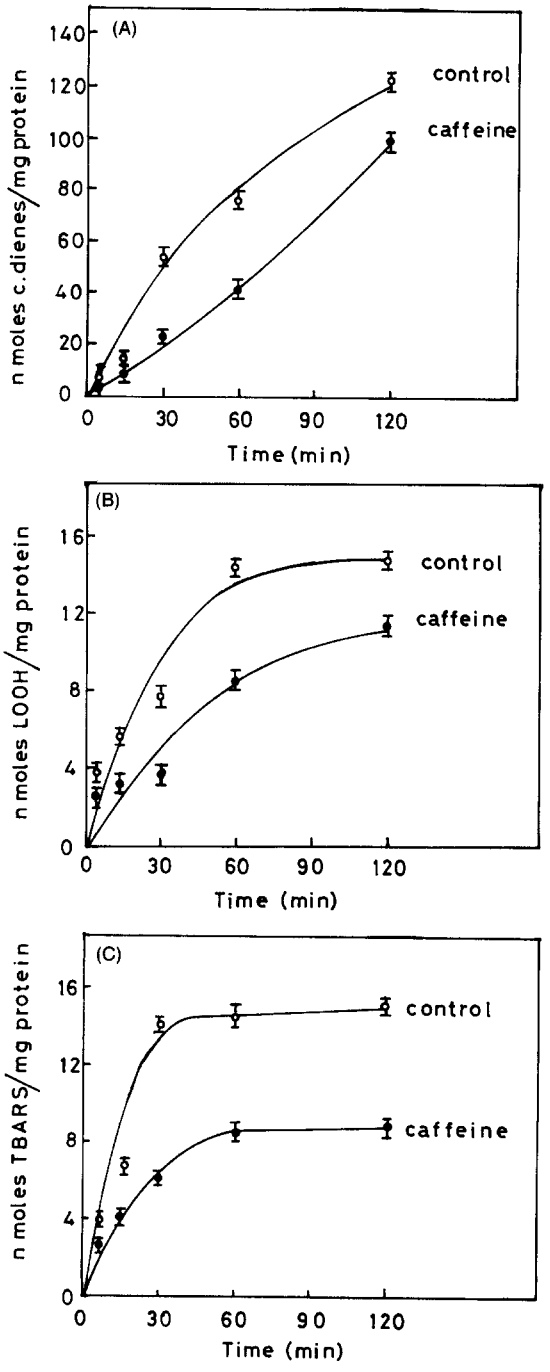

Fig. 4. Time-dependent effect of caffeine on ROO·-induced lipid peroxidation. (A) Conjugated dienes; (B) lipid hydroperoxides; and (C) TBARS. Caffeine was used at a concentration of 1 mM.

however, was almost similar to that of radiation induced peroxidation in H_2O-based buffer (Fig. 6A).

Photosensitization by methylene blue plus light-induced peroxidation in membranes which has been shown predominantly to be due to $^1O_2$ [20,25]. Caffeine inhibited the $^1O_2$-induced LOOH ($P < 0.001$), but not the TBARS (Fig. 7).

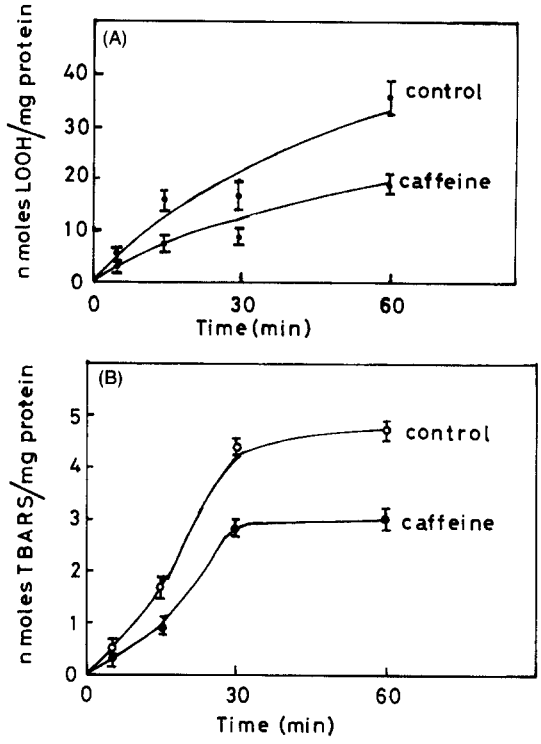

Fig. 5. Time-dependent effect of caffeine on $^1O_2$-induced lipid peroxidation. (A) lipid hydroperoxides; and (B) TBARS. The concentration of caffeine was 1 mM.

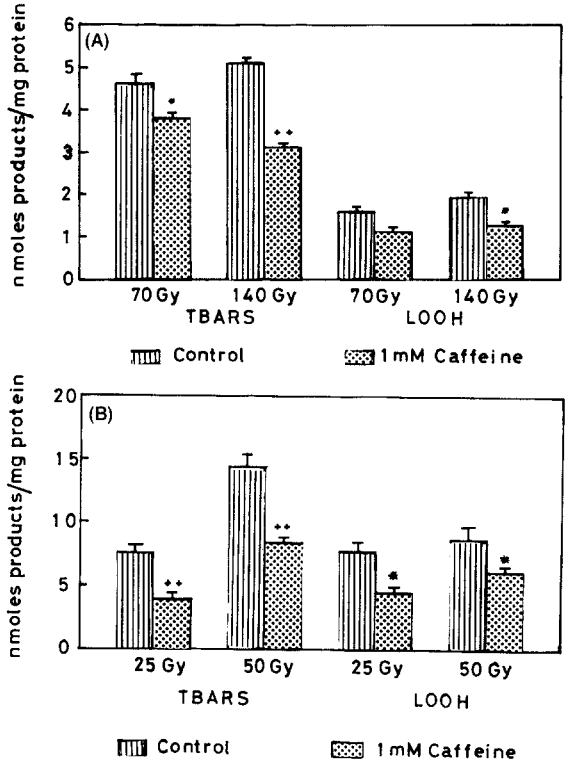

Fig. 6. Effect of caffeine on the oxic radiation induced lipid peroxidation in H_2O-based (A) and deuterated (B) buffers.

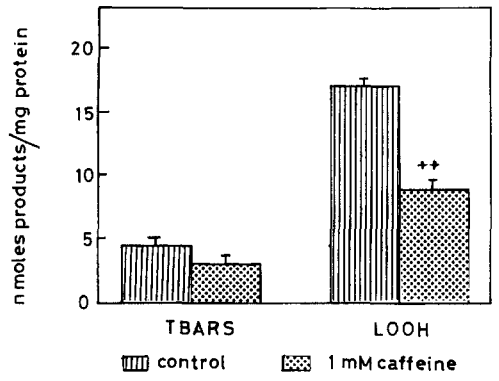

Fig. 7. Effect of caffeine on lipid peroxidation induced by photosensitization.

The rate constant for the reaction of 'OH with caffeine was determined by formation kinetic studies by monitoring the growth of the transient band at $\lambda_{max} = 340$ nm as a function of caffeine concentration. The first order rate (k_{obs}) was found to increase with caffeine concentration $(0.5–3.0) \cdot 10^{-4}$ M, and the bimolecular rate constant determined from the slope of linear plot of k_{obs} vs. caffeine concentration was $7.3 \cdot 10^{9}$ $M^{-1} s^{-1}$ (Fig. 8). The reaction of caffeine with lipid peroxyl radical (linoleic acid radical) was also determined by pulse radiolysis. Their reaction with caffeine was studied on monitoring the absorption changes in 300–700 nm region, containing linoleic acid ($1 \cdot 10^{-4}$ M) and low concentrations of caffeine ($3.0 \cdot 10^{-6}$ M). Under these conditions, H'/'OH radicals would initially react with linoleic acid and the radicals L'/LOO' generated would then react with caffeine. The absence of any transient absorption in 300–700 nm region suggest that either L'/LOO' radicals have very low reactivity with caffeine or the transient species formed on reaction of L'/LOO' with caffeine do not absorb in 300–700 nm region. Studies with high concentration of linoleic acid could not be carried out due to solubility limitation.

Singlet oxygen quenching constant of caffeine was determined by plotting concentration (0.1 to 1 mM) against

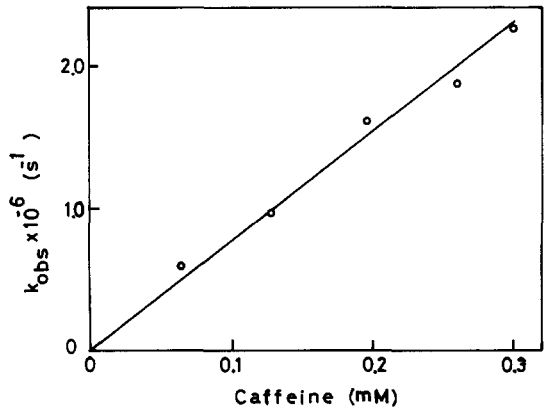

Fig. 8. Plot for determining rate constant of caffeine with hydroxyl radical.

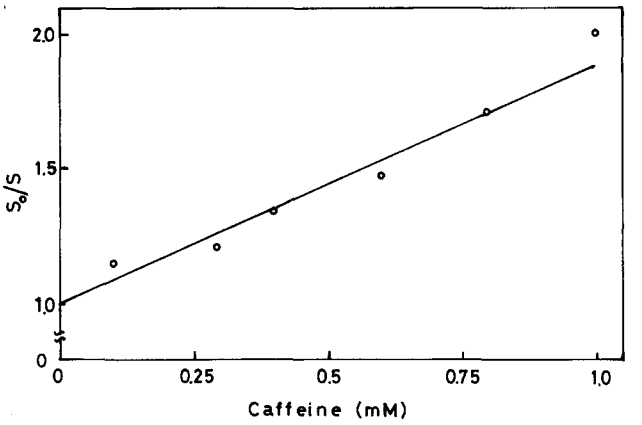

Fig. 9. Stern-Volmer plot for determining singlet oxygen quenching constant of caffeine.

S_0/S as Stern-Volmer plot (Fig. 9) [27]. The rate constant was estimated to be $2.9 \cdot 10^{7}$ $M^{-1} s^{-1}$.

4. Discussion

Earlier studies have shown that caffeine has many pharmacological properties. It is an adjuvant capable of enhancing the analgesic effect of aspirin [1], acetaminophen and other drugs [2]. It also has been shown to be metabolized by the hepatic microsomal cytochrome *P*-450 system and has been proposed as a metabolic probe to study liver function in humans, particularly to determine the levels of expression of *P*-450 1A2 [28]. Some of the earlier studies dealing with subcutaneous administration of caffeine showed that it is capable of inducing fatty liver and certain forms of oxidative damage such as unstimulated lipid peroxidation at certain doses [29]. There are also reports of free radicals observed in roasted but not in green coffee beans [30,31]. Instant or brewed coffee also was reported to generate $^1O_2$ [32]. It is likely that caffeine and/or other molecules present in coffee seeds or coffee itself, act as 'free radical sinks', capturing the reactive species generated during heating. However, our present studies as well as of certain others [10] clearly show that caffeine, at millimolar concentrations, is a potent antioxidant capable of preventing lipid peroxidation induced in vitro by the cardinal reactive oxygen species, 'OH, ROO' and $^1O_2$.

Hydroxyl radical has been shown to be generated in various biological systems by metal-driven Fenton reaction or by Haber-Weiss reaction [13,33]. They are most reactive species, initiating the peroxidation of the biomembranes [13,33]. The lipid radicals (L'), thus generated, would initiate chain reaction in the presence of oxygen giving rise to LOOH, which breaks down to aldehyde. Recent studies show that peroxyl radical (ROO') is also important in initiating peroxidation [34]. It induces peroxidation by participating in the chain branching step.

Singlet oxygen is another reactive species whose ability to induce molecular damage in biological systems has been substantiated in recent years [23,35,36]. It is generated by photosensitization, besides enzymatic and non-enzymatic pathways. Recent studies also has shown that it is the major oxidant inducing damage in Haber-Weiss reaction involving H_2O_2 and $O_2^{\cdot-}$ [37]. It can directly add to lipid to form lipid hydroperoxides.

$$LH + {}^1O_2 \rightarrow LOOH \quad (1)$$

Conjugated diene ($L^{\cdot}$) is the initial product of peroxidation, to which oxygen is added to form lipid hydroperoxide. This further breaks down to form more stable aldehydes that react with thiobarbituric acid to form TBARS. Our study showed that caffeine inhibited the formation of these products to varying extents in both $ROO^{\cdot}$ and $^1O_2$-induced systems as a function of time.

The reactivity of caffeine with $^{\cdot}OH$ was extremely high, with the rate constant of $7.3 \cdot 10^9$ M^{-1} s^{-1}. This is close to the value of $6.9 \cdot 10^9$ M^{-1} s^{-1} determined by competition kinetic studies [8]. The reactivity with $^1O_2$ was about 2 orders of magnitude less ($2.9 \cdot 10^7$ M^{-1} s^{-1}). This value is an order of magnitude higher than that of glutathione [27]. Though the reactivity of caffeine with $^1O_2$ is considerably lesser than that with $^{\cdot}OH$, the protection afforded will still be significant since the half life of $^{\cdot}OH$ in biological systems is in nanoseconds and hence distance travelled to effect damage is considerably less than $^1O_2$ half- life in μseconds.

The extent of inhibition by caffeine against peroxidation induced by $^{\cdot}OH$ was significantly more than that induced by $^1O_2$. The differential abilities to quench these reactive species may at least partly account for this. Though we were not able to observe quenching of $ROO^{\cdot}$ by caffeine, it did show significant inhibition of peroxidation induced by this reactive species. One possible explanation for this is that both caffeine and oxygen may compete for $L^{\cdot}$ formed by the reaction of $LOO^{\cdot}$ with LH. $LOO^{\cdot}$ essentially propagates the peroxidation reaction in the presence of oxygen i.e. it enhances oxygen dependent damage. Similar effect of caffeine on oxygen-dependent effect of radiation has been observed [10].

Our results show that ability of caffeine to inhibit lipid peroxidation is similar to glutathione and significantly greater than that of ascorbic acid. The biochemical importance of vitamin C or ascorbic acid is primarily related to its reducing potential which makes it an efficient free radical scavenger. It has a rate constant of $> 10^9$ M^{-1} s^{-1} with $^{\cdot}OH$, $2 \cdot 10^8$ M^{-1} s^{-1} with water soluble $ROO^{\cdot}$ and 10^7 M^{-1} s^{-1} with $^1O_2$ [12]. It can protect lipids and membranes from oxidative damage by effective scavenging of lipid peroxidation-initiating radicals [12,38]. The antioxidant effect of ascorbic acid, however, depends on its concentration and presence of other antioxidants such as α-tocopherol/glutathione, and transient metal ions like Fe^{2+} [16]. Glutathione is an important water-soluble antioxidant present intracellularly in the millimolar concentration range. It reacts directly with free radicals and can protect cells from $^1O_2$, $^{\cdot}OH$ and $O_2^{\cdot-}$. Because glutathione is a substrate for the hydroperoxide-reducing enzyme glutathione peroxidase, its absence may lead to the accumulation of lipid hydroperoxides. However, dietary glutathione is poorly absorbed in humans. Caffeine, on the other hand, is absorbed fairly well and metabolized [28].

Caffeine has been shown to increase ratings of alertness, energy, and motivation for work, especially more so in conditions of mental fatigue such as that noticed during sleep deprivation [39]. Recent studies also suggest that caffeine has increased selective attention or alertness in fatigued subjects [40]. 'The free radical flux theory of sleep' proposes that cerebral free radicals accumulate during wakefulness and sleep functions essentially as an antioxidant for the brain [41]. Hence the observed antioxidant ability of caffeine may be, at least in part, responsible for rejuvenating effect of coffee, especially in fatigued subjects.

In conclusion, our present study shows that caffeine has significant antioxidant abilities in protecting membranes against oxidative damage induced by three of the major reactive oxygen species of biological significance, and that its ability to quench these species may account for the observed antioxidant ability.

References

[1] Castaneda-Hernandez, G., Castillo-Mendez, M.S., Lopez-Munoz, F.J., Granados-Soto, V. and Flores-Murrieta, F.J. (1994) Can. J. Physiol. Pharmacol. 72, 1127–1131.

[2] Migliardi, J.R., Armellino, J.J., Friedman, M., Gillings, D.B. and Beaver, W.T. (1994) Clin. Pharmacol. Ther. 56, 576–586.

[3] Selby, C.P. and Sancer, A. (1990) Proc. Natl. Acad. Sci. USA 87, 3522–3525.

[4] Zhen, W. and Vaughan, T.M. (1995) Radiat. Res. 141, 170–175.

[5] Kesavan, P.C. and Natarajan, A.T. (1985) Mutat. Res. 143, 61–68.

[6] Kesavan, P.C., Trasi, S. and Ahmad, A. (1973) Int. J. Radiat. Biol. 24, 581–587.

[7] Kesavan, P.C., Sharma, G.J. and Afzal, S.M.J. (1978) Radiat. Res. 75, 18–30.

[8] Kesavan, P.C. and Powers, E.L. (1985) Int. J. Radiat. Biol. 48, 223–233.

[9] Raghu, B. and Kesavan, P.C. (1986) Indian J. Exp. Biol. 24, 742–746.

[10] Kesavan, P.C. (1992) Current Sci. 62, 791–797.

[11] Sies, H. (1986) Angew. Chem. Int. Ed. Engl. 25, 1058–1071.

[12] Briviba, K. and Sies, H. (1994) in *Natural Antioxidants in Health and Disease*, (Frei, B., ed.), pp. 107–128, Academic Press, Orlando, FL.

[13] Buettner, G.R. (1993) Arch. Biochem. Biophys. 300, 535–543.

[14] Park, J.W. and Floyd, R.A. (1992) Free Radical Biol. Med. 12, 245–250.

[15] Paillous, N. and Fery-Forgues, S. (1994) Biochemie 76, 355–368.

[16] Ong, A.S.H. and Packer, L. (eds.) (1992) *Lipid Soluble Antioxidants*, Birkaüser Verlag, Basel.

[17] Rao, R.R. Aravindakumar, C.T., Rao, B.S.M., Mohan, H. and Mittal, J.P. (1995) J. Chem. Soc. Faraday Trans. 91, 615–621.

[18] Kamat, J.P. and Devasagayam, T.P.A. (1995) Neurosci. Lett. 195, 179–182.

[19] Blazek, E.R., Peak, J.G. and Peak, M.J. (1989) Photochem. Photobiol. 49, 607–613.
[20] Briviba, K., Devasagayam, T.P.A., Sies, H. and Steenken, S. (1993) Chem. Res. Toxicol. 6, 548–553.
[21] Pushpendran, C.K., Subramanian, M. and Devasagayam, T.P.A. (1994) Mech. Ageing Dev. 73, 197–208.
[22] Buege, J.A. and Aust, S.D. (1978) Methods Enzymol. 52, 302–310.
[23] Das, T.N. and Priyadarsini, K.I. (1994) J. Phys. Chem. 98, 5272–5278.
[24] Kesavan, P.C. and Ahmad, A. (1976) Int. J. Radiat. Biol. 29, 395–398.
[25] Girotti, A.W. (1990) Photochem. Photobiol. 51, 497–509.
[26] Buxton, G.V. (1987) in *Radiation Chemistry, Principles and Applications* (Rodgers, M.A.J., ed.), pp. 321–348, VCH Publishers, New York.
[27] Devasagayam, T.P.A., Sundquist, A.R., Di Mascio, P., Kaiser, S. and Sies, H. (1991) J. Photochem. Photobiol. B: Biol. 9, 105–116.
[28] Sanz, F., López-deBriñas, Rodriguez, J and Manaut, F. (1994) Quant. Struct-Act. Relat. 13, 281–284.
[29] Dianzani, M.U., Muzio, G., Biocca, M.E. and Canuto, R.A. (1991) Int. J. Tiss. Reac. XIII, 79–85.
[30] Troup, G.J., Hutton, D.R., Pilbrow, J.R., Hunter, C.R., Smith, B.R. and Bryant, B.J. (1988) Med. J. Aust. 148, 537–538.
[31] Gonis, J., Hewitt, D.G., Troup, G.J., Hutton, D.R. and Hunter, C.R. (1995) Free Radical Res. 23, 393.
[32] Kato, T., Hiramoto, K. and Kikugawa (1994) Mutat. Res. 306, 9–17.
[33] Halliwell, B. and Gutteridge, J.M.C. (1990) Methods Enzymol. 186, 1–85.
[34] Aikens, J. and Dix, T.A. (1993) Arch. Biochem. Biophys. 305, 516–525.
[35] Devasagayam, T.P.A., Steenken, S., Obendorf, M.S.W., Schulz, W.A. and Sies, H. (1991) Biochemistry 30, 6283–6289.
[36] Sies, H. (1993) Mutat. Res. 209, 183–191.
[37] Khan, A.U. and Kasha, M. (1994) Proc. Natl. Acad. Sci. USA 91, 12365–12367.
[38] Frei, B., England, L. and Ames, B.N. (1989) Proc. Natl. Acad. Sci. USA 86, 6377–6381.
[39] Sawyer, D.A., Julia, H.L. and Turin, A.C. (1982) J. Behav. Med. 5, 415–439.
[40] Lorist, M.M., Snel, J., Kok, A. and Mulder, G. (1994) Psychophysiology 31, 525–534.
[41] Reimund, E. (1994) Med. Hypotheses 43, 231–233.