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INHIBITORY EFFECT OF EUGENOL ON NON-ENZYMATIC LIPID PEROXIDATION IN RAT LIVER MITOCHONDRIA

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Abstract—The anti-peroxidative activity of eugenol on Fe^{2+}–ascorbate- and Fe^{2+}–H_2O_2-induced lipid peroxidation was studied using rat liver mitochondria. Eugenol inhibited thiobarbituric acid reactive substance (TBARS) formation induced by both the systems in addition to oxygen uptake and mitochondrial swelling induced by Fe^{2+}–ascorbate. Time course studies on TBARS formation indicated the ability of eugenol to inhibit initiation and propagation reactions. There was no measurable chemical modification of eugenol during the course of mitochondrial peroxidation by both the systems. Mitochondrial peroxidation by Fe^{2+}–H_2O_2 was inhibited by hydroxyl radical (OH) scavengers like mannitol, benzoate, formate and dimethyl sulfoxide apart from eugenol. The OH scavenging ability of eugenol was evident from its inhibitory effect on OH-mediated deoxyribose degradation. The second-order rate constant for the reaction of OH with eugenol was about 4.8×10^{10} M^{-1} sec^{-1}. Eugenol reduced Fe^{3+} ions and Fe^{3+} chelated to citrate or ADP but it did not exhibit pro-oxidant activity in OH-mediated deoxyribose degradation. Incubation of mitochondria with eugenol resulted in the uptake of small but significant quantities of eugenol which inhibited subsequent lipid peroxidation by acting as a chain breaking antioxidant.

Peroxidation of cell membrane lipids results in membrane destabilization and changes in transport characteristics which, if left uninhibited, eventually lead to cell death. The deleterious effects of several environmental toxins, carcinogens and drugs are associated with lipid peroxidation due to their conversion to radicals which, in turn, generate oxygen radicals [1]. Administration of antioxidants greatly reduces or eliminates the biochemical and pathological changes brought about by lipid peroxidation [2]. Although synthetic antioxidants are in common use, there is a growing trend in consumer preference for natural ingredients in view of toxicity concerns [3].

Eugenol (4-allyl-2-methoxyphenol), a naturally occurring phenolic compound, is a major component of clove oil and is also present in oils of cinnamon, basil and nutmeg. It is used mainly as a flavouring agent at a level of up to 0.01% in foods such as baked products, beverages, sweets and frozen dairy products [4]. Eugenol is reported to show high antibacterial activity at a level of 500 ppm or less [5]. In view of its analgesic and anesthetic properties, eugenol is also used along with zinc oxide as a component of several dental materials such as dental cements, impression pastes and surgical pastes [6]. It is also used for disinfecting root canals. In traditional medicine eugenol has been used in the treatment of flatulent colic, chronic diarrhoea and other gastrointestinal disorders [7, 8]. The joint FAO/WHO Expert Committee on Food Additives (1982) established an acceptable daily intake of eugenol of up to 2.5 mg/kg body weight for humans [9]. The daily per capita consumption is estimated to be 0.6 mg although the intakes are bound to show wide variations [4]. Eugenol at a level of 0.3–1.25% in the diets did not show any adverse toxic effects on prolonged feeding to rats for 103 weeks [10]. It is considered non-mutagenic, non-carcinogenic and generally recognised as safe (GRAS) by Food and Drug Administration [4, 11]. However, reports on the cytotoxic effects of eugenol on polymorphonuclear leukocytes, fibroblasts and dental pulp tissue are also available [12, 13].

Among the spices, cloves were found to exhibit high antioxidant activity using the hemoglobin peroxidation test for screening [14]. Clove essential oil inhibited the oxidation of cotton seed oil [15]. Eugenol inhibited Cu^{2+}–H_2O_2-catalysed lipid peroxidation in human erythrocyte membranes [16]. A commonly used model procedure for studying non-enzymatic lipid peroxidation involves the use of mitochondria as lipid substrate [17] and Fe^{2+}–ascorbate or Fe^{2+}–H_2O_2 as peroxidation inducers. Using these models, the present study describes the characteristics of antiperoxidative effects of eugenol.

MATERIALS AND METHODS

Chemicals. Eugenol, α-tocopherol, butylated hydroxytoluene (BHT†), 2-thiobarbituric acid, 1,1,3-3-tetraethoxypropane, bovine erythrocyte superoxide dismutase (SOD) (EC 1.15.1.1) and thymol free bovine liver catalase (EC 1.11.1.6) were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). All other chemicals were of the highest purity available (analytical grade).

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† Abbreviations: TBARS, thiobarbituric acid reactive substance; MDA, malonaldehyde; BHT, butylated hydroxy toluene (2,6-di-tert-butyl-4-methylphenol); SOD, superoxide dismutase.

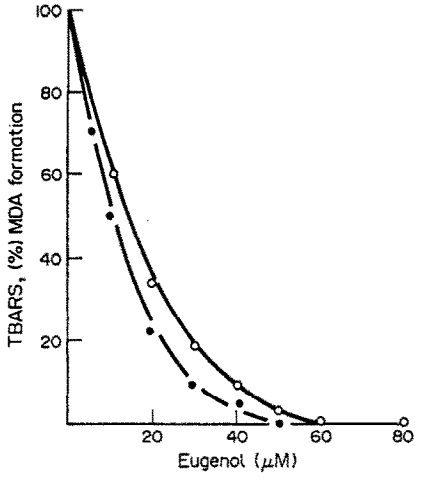

Fig. 1. Effect of eugenol on (●) Fe^{2+}-ascorbate- and (○) Fe^{2+}-H_2O_2-dependent peroxidation in liver mitochondria. The reaction mixture (2 mL) contained mitochondria (1 mg protein) and 10 μM $FeSO_4$, 200 μM ascorbate and 0.125 M KCl–0.2 M Tris–HCl buffer, pH 7.4, for the Fe^{2+}-ascorbate system or 100 μM H_2O_2, 200 μM Fe^{2+} and 0.15 M NaCl, pH 7.0, for the Fe^{2+}-H_2O_2 system. Eugenol was added at the indicated concentrations. The reaction mixture was incubated at 37° for 60 min. The amount of TBARS formed without eugenol was taken as 100%.

Preparation of mitochondria. Liver mitochondria were prepared in 1.15% KCl medium according to Johnson and Lardy [18]. Use of sucrose was avoided in view of its interference with malonaldehyde (MDA) estimation [19]. The mitochondria were suspended in a medium containing 0.125 M KCl or 0.15 M NaCl. Protein content was determined by the method of Lowry *et al.* [20].

Preparation of eugenol solution. Eugenol was dissolved in 70% alcohol and stored at 0°. Fresh solutions were prepared every week. For deoxyribose degradation studies, eugenol (up to 1 mM) was dissolved in 0.05 N NaOH and the pH was adjusted to 7.4 with 0.1 N HCl. Organic solvents were not used for preparing eugenol solution since they interfere with hydroxyl radical (OH) determination.

Induction and assay of peroxidation. Lipid peroxidation was induced in mitochondria by Fe^{2+}-ascorbate [21] or Fe^{2+}-H_2O_2 [22]. Briefly, mitochondria (500 μg mitochondrial protein/mL incubation mixture) were incubated with either Fe^{2+}-ascorbate system (10 μM $FeSO_4$, 200 μM ascorbate and 0.125 M KCl–0.02 M Tris–HCl buffer, pH 7.4) or Fe^{2+}-H_2O_2 system (200 μM $FeSO_4$, 100 μM H_2O_2 and 0.15 M NaCl, pH 7.0) for a period of 60 min at 37°. Inhibitors were added at indicated concentrations 2 min prior to the addition of peroxidation inducers. Peroxidation was terminated by adding 20% trichloroacetic acid (0.5 mL/mL incubation mixture). Thiobarbituric acid reactive substance (TBARS) was determined according to Wilbur *et al.* [23]. Acid hydrolysed 1,1,3,3-tetraethoxypropane was used as an authentic standard for MDA. Although it is not specific for MDA, the assay of TBARS is used as a reliable index of lipid peroxidation.

Oxygen uptake. Oxygen utilization was measured using a Gilson oxygraph fitted with a Clark-type electrode. The reaction mixtures were incubated at 25° and eugenol was added 2 min before the induction of peroxidation. The electrode system was calibrated using the oxygen-consuming reaction of phenylhydrazine-HCl and ferricyanide according to Mishra and Fridovich [24].

Mitochondrial swelling. Mitochondrial swelling was measured at room temperature by continuous recording of the change in absorbance at 520 nm using Shimadzu spectrophotometer [25]. Stock mitochondria (600 μg protein) were diluted to 3 mL in 0.125 M KCl, 0.02 M Tris–HCl buffer, pH 7.4. The initial absorbance at 520 nm was 0.8–0.9. The induction of swelling was brought about by 10 μM Fe^{2+} and 200 μM asborbate. The mitochondria were exposed to inhibitors such as α-tocopherol and eugenol for 2 min before the addition of swelling

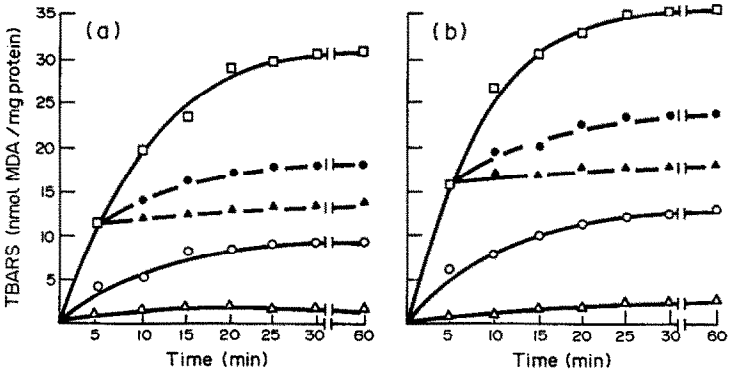

Fig. 2. Effect of eugenol on the time course of (a) Fe^{2+}-ascorbate- and (b) Fe^{2+}-H_2O_2-dependent peroxidation in liver mitochondria. The assay system and incubation conditions were as described in Fig. 1. (□) Without eugenol; (○) 20 μM and (△) 50 μM eugenol added at zero time; (●) 20 μM and (▲) 50 μM eugenol added after 5 min of incubation.

Table 1. IC_{50} values for free radical scavengers in lipid peroxidation system

Inhibitor	IC_{50} (μM)	
	Fe^{2+}–ASC-dependent lipid peroxidation	Fe^{2+}–H_2O_2-dependent lipid peroxidation
Eugenol	10.0	14.0
α-Tocopherol	54.0	68.2
BHT	1.15	1.25

Peroxidation was measured as described in Materials and Methods.
Values are means of three individual experiments.
IC_{50}, amount required for 50% inhibition of peroxidation.
ASC, ascorbic acid.

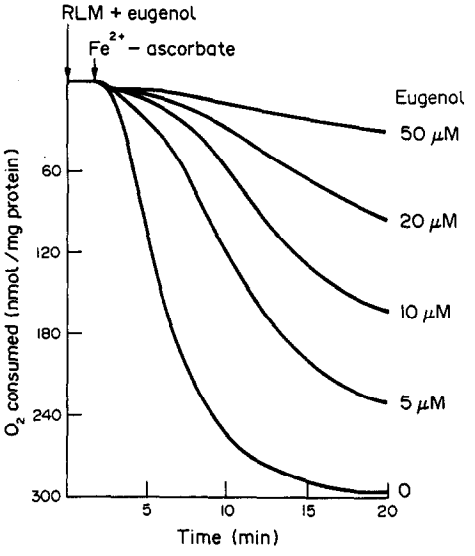

Fig. 3. Effect of eugenol on Fe^{2+}–ascorbate-dependent oxygen uptake by rat liver mitochondria (RLM). The reaction mixture (1.7 mL) contained mitochondria (1 mg protein), 10 μM $FeSO_4$, 200 μM ascorbate and indicated concentrations of eugenol in 0.125 M KCl–0.02 M Tris–HCl buffer, pH 7.4. Incubation and measurement of oxygen uptake are described in Materials and Methods.

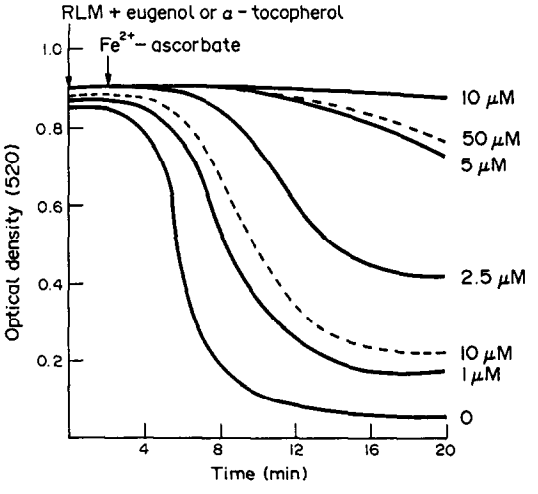

Fig. 4. Inhibitory effect of eugenol and α-tocopherol on Fe^{2+}–ascorbate-induced swelling of rat liver mitochondria (RLM). The assay system contained mitochondria (200 μg protein/mL), 10 μM $FeSO_4$, 200 μM ascorbate and 0.125 M KCl–0.02 M Tris–HCl buffer, pH 7.4. The final volume was 3 mL. Mitochondrial swelling was determined as described in Materials and Methods. Eugenol (—) or α-tocopherol (---) was added 2 min before the addition of swelling inducers at concentrations indicated on each trace.

agents. The reference cuvette contained all additions except mitochondria.

Eugenol estimation using HPLC. Hexane extracted eugenol was injected on to Shodex C-18 reverse-phase column (4.6 × 150 mm, particle size 5 μm). The HPLC system consisted of a Shimadzu model SC1-6A system controller, LC-6A pumps and SPD-6AV U.V. spectrophotometer connected to a C-R3A recorder. The wavelength was set at 280 nm. Eugenol was eluted using an isocratic mobile phase of methanol/water (1:1, v/v) at a flow rate of 1 mL/min. The retention time of eugenol under these conditions was 16 min.

Deoxyribose degradation. Deoxyribose was incubated at 37° for 60 min with the hydroxyl radical generating system containing 50 μm $FeCl_3$, 1 mM H_2O_2 and 100 μM ascorbic acid in 1 mL of 10 mM KH_2PO_4–KOH buffer, pH 7.4. Products of deoxyribose degradation were measured by the thiobarbituric acid test [26].

Chemical changes in eugenol during incubation with mitochondria. Incubation mixtures containing eugenol and mitochondria in Fe^{2+}–ascorbate or Fe^{2+}–H_2O_2 peroxidation systems were extracted three times with 3 mL lots of hexane and the combined extracts were carefully evaporated under N_2 at 25° avoiding over-exposure to the gas. The residue was dissolved in 70% alcohol immediately and eugenol was quantitated by HPLC.

Studies on eugenol uptake by mitochondria. Mitochondria (50 mg mitochondrial protein) were incubated for 10 min at 25° with varying concentrations of eugenol (25–250 μM) in a final volume

Table 2. Effect of SOD, catalase and hydroxyl radical scavengers on Fe^{2+}–H_2O_2-dependent peroxidation of mitochondria

Additions	Concentration	TBARS (nmol/mg protein)	Per cent inhibition
None	—	34	—
Catalase	100 U	4.2	87.6
SOD	25 U	32.8	3.5
Mannitol	20 mM	3.4	90.0
Benzoate	20 mM	7.5	77.4
Formate	20 mM	4.4	87.6
Dimethyl sulfoxide	20 mM	10.0	70.6

The assay mixture (2 mL) contained mitochondria (1 mg protein) 200 μM Fe^{2+}, 100 μM H_2O_2, 0.15 M NaCl pH 7.0 and various scavengers. All solutions were carefully adjusted to pH 7.0 just before use. Determination of TBARS is described in Materials and Methods.

Values are means of three individual experiments.

of 10 mL of 0.125 M KCl–0.02 M Tris–HCl buffer, pH 7.4, and rapidly centrifuged at 20,000 *g* for 5 min at 4°. The mitochondrial pellet was washed with cold buffer four to five times by repeated pelleting at 20,000 *g* and finally suspended in the buffer to give 1 mg mitochondrial protein/mL. The eugenol content of washed mitochondria was assayed by HPLC following extraction into hexane. The peroxidation in eugenol pre-treated mitochondria was assessed by incubating at 37° for 60 min with various concentrations of $FeSO_4$ (12.5–100 μM) or 10 μM Fe^{2+}–200 μM ascorbate. The TBARS was determined as described earlier.

RESULTS

Effect of eugenol on Fe^{2+}–ascorbate-induced lipid peroxidation in liver mitochondria

Fe^{2+}–ascorbate-dependent non-enzymatic lipid peroxidation in liver mitochondria was assessed by estimating TBARS formation, oxygen uptake and peroxidation-induced swelling. The formation of TBARS was inhibited by eugenol in a concentration-dependent manner (Fig. 1). Lipid peroxidation increased with time and reached a plateau at 20 min as shown in Fig. 2a. When added at the start, 20 μM eugenol showed 75% inhibition while 50 μM eugenol inhibited completely. Addition of eugenol 5 min after the induction of peroxidation also inhibited further progress of the reaction. The inhibitory activity of eugenol was about 5-fold higher than that of α-tocopherol and about one-tenth that of BHT (Table 1). Addition of Fe^{2+}–ascorbate to mitochondria resulted in a rapid oxygen uptake which was inhibited in eugenol in a concentration-dependent manner (Fig. 3). Fe^{2+}–ascorbate also induced rapid swelling in mitochondria suspended in isotonic medium after a lag period of 4 min (Fig. 4). Pre-incubation of mitochondria with increasing concentrations of eugenol resulted in progressive inhibition of swelling and at 10 μM eugenol concentration the inhibition of swelling was complete. The inhibitory effect of eugenol on mitochondrial swelling was about 8-fold higher than that of α-tocopherol.

Effect of eugenol on Fe^{2+}–H_2O_2-induced lipid peroxidation in rat liver mitochondria

Eugenol inhibited the TBARS formation by rat liver mitochondria in the presence of Fe^{2+}–H_2O_2. At a 50 μM concentration of eugenol the peroxidation was completely inhibited (Fig. 1). The anti-peroxidative activity of eugenol was five times that of α-tocopherol and one-eleventh that of BHT in the Fe^{2+}–H_2O_2-supported peroxidation system (Table 1). The effect of other agents on this peroxidation system is shown in Table 2. Catalase, mannitol, benzoate, formate and dimethyl sulfoxide inhibited the reaction indicating the involvement of H_2O_2 and hydroxyl radicals. The superoxide radical scavenger SOD had no effect. The time course of peroxidation and the effect of eugenol are shown in Fig. 2b. The pattern of inhibition was similar to that observed with the Fe^{2+}–ascorbate system as in Fig. 2a.

Effect of eugenol on $\dot{O}H$ radical mediated deoxyribose degradation

There was very little degradation of deoxyribose by Fe^{3+} ions, Fe^{3+}–ADP and Fe^{3+}–citrate in the presence of H_2O_2 (Table 3). However, Fe^{3+}–EDTA promoted considerable degradation. Ascorbic acid, when added to these mixtures, greatly stimulated degradation due to its ability to reduce Fe^{3+} ions and Fe^{3+}–chelates. Eugenol was found to reduce Fe^{3+}, Fe^{3+}–ADP, Fe^{3+}–citrate but not Fe^{3+}–EDTA (using potassium thiocyanate and bathophenanthroline colour tests). However, eugenol failed to stimulate deoxyribose degradation when added to the above mixtures. On the other hand, it inhibited the degradation promoted by Fe^{3+}–EDTA–H_2O_2.

Deoxyribose degradation by $\dot{O}H$ radicals [27] generated in a site specific manner (without EDTA) or in bulk solution (with EDTA) was inhibited by eugenol (Fig. 5). The concentration of eugenol required for inhibition was considerably high when compared to inhibition of mitochondrial peroxidation systems.

Table 3. Effect of eugenol and ascorbate on extent of deoxyribose degradation by Fe^{3+} or Fe^{3+} chelates

Additions	Extent of deoxyribose degradation A_{532}
Fe^{3+}	0.062 ± 0.005
Fe^{3+} + ascorbate	0.467 ± 0.012
Fe^{3+} + eugenol	0.061 ± 0.006
Fe^{3+}–EDTA	0.292 ± 0.015
Fe^{3+}–EDTA + ascorbate	1.091 ± 0.034
Fe^{3+}–EDTA + eugenol	0.086 ± 0.012*
Fe^{3+}–ADP	0.065 ± 0.006
Fe^{3+}–ADP + ascorbate	0.501 ± 0.018
Fe^{3+}–ADP + eugenol	0.066 ± 0.001
Fe^{3+}–citrate	0.072 ± 0.004
Fe^{3+}–citrate + ascorbate	0.625 ± 0.020
Fe^{3+}–citrate + eugenol	0.073 ± 0.002

The reaction mixture contained 2.8 mM deoxyribose and 1.0 mM H_2O_2 in 10 mM KH_2PO_4–KOH buffer, pH 7.4, in a total volume of 1 mL. Other additions were as follows: 50 μM $FeCl_3$, 100 μM iron chelators (EDTA, ADP, citrate), 100 μM ascorbate and 100 μM eugenol. The reaction mixtures were incubated at 37° for 60 min. Determination of TBARS was as indicated in Materials and Methods. Values are means ± SE for four tests.

* $P < 0.05$ compared to Fe^{3+}–EDTA.

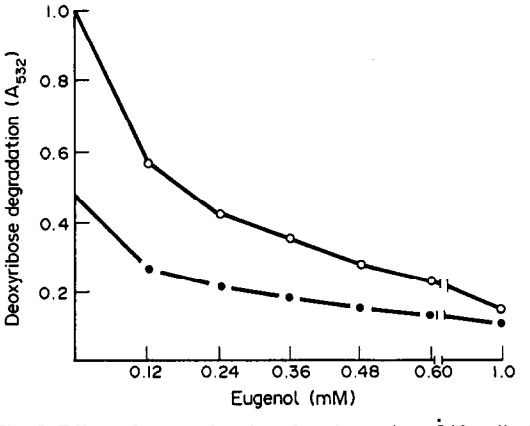

Fig. 5. Effect of eugenol on iron ion-dependent ȮH radical generation measured by deoxyribose degradation. The reaction mixture (1 mL) containing 2.8 mM deoxyribose, 1 mM H_2O_2, 50 μM $FeCl_3$, 100 μM ascorbic acid and where indicated, 100 μM EDTA in 10 mM KH_2PO_4–KOH buffer, pH 7.4, were incubated at 37° for 60 min. Iron salts were mixed with EDTA before addition. Reactions were started with ascorbic acid (●) without EDTA or (○) with EDTA.

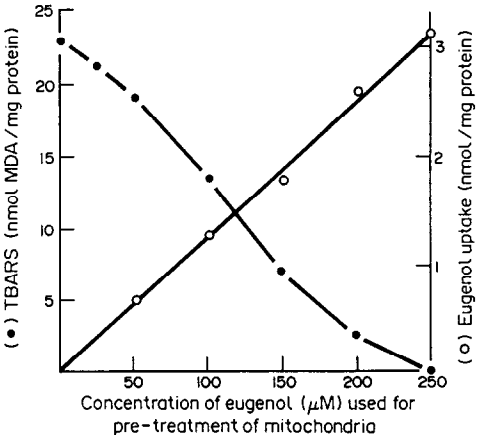

Fig. 6. Eugenol uptake by mitochondria and its effect on Fe^{2+}–ascorbate-induced lipid peroxidation. Methodology for pre-incubation of mitochondria with eugenol and measurement of eugenol uptake are mentioned in Materials and Methods. Mitochondria, pre-treated with varying concentrations of eugenol, were used for Fe^{2+}–ascorbate-induced peroxidation (formation of TBARS). Contents of assay mixtures and conditions were as described in Fig. 1.

Chemical transformation of eugenol during incubation with mitochondria

Conversion of eugenol to products like quinones or polymers should result in the disappearance of eugenol from the reaction mixture during the course of the reaction. Incubation of eugenol for 1 hr with Fe^{2+}–ascorbate or Fe^{2+}–H_2O_2 and mitochondria did not result in its disappearance. Most of the eugenol was recovered unchanged (86% and above) and the recovery from incubations with and without peroxidation inducers was almost same (results not shown).

Eugenol uptake by mitochondria

The eugenol uptake by mitochondria increased linearly with increasing concentration of eugenol in the medium (Fig. 6). This uptake did not reach

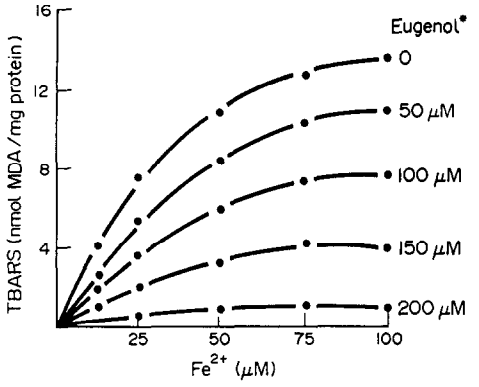

Fig. 7. Fe^{2+}-induced peroxidation in eugenol pre-treated mitochondria. Methodology for pre-incubation of mitochondria with eugenol was as mentioned in Materials and Methods. Eugenol*, indicated concentrations of eugenol used for pre-treatment of mitochondria. Reaction mixture (2 mL) contained eugenol pre-treated mitochondria (1 mg protein), indicated concentrations of Fe^{2+} and 0.125 M KCl–0.02 M Tris–HCl buffer, pH 7.4. The reaction was carried out at 37° for 60 min. Determination of TBARS is described in Materials and Methods.

considerable controversy exists in the available reports. Aust and co-workers [22, 28, 29] have proposed the requirement of a specific Fe^{2+}–Fe^{3+}–O_2 complex or a 1:1 ratio of Fe^{2+} to Fe^{3+} for initiation of peroxidation, whereas Aruoma *et al.* [30] could replace Fe^{3+} with Pb^{2+} or Al^{3+} negating the proposal for the Fe^{3+} requirement. The near total inhibition of peroxidation by 50 μM eugenol when present from the start of the reaction (Fig. 2a) indicates the possibility of inhibition of initiation apart from its ability to suppress propagation. However, we cannot presume complete absence of lipid hydroperoxides in the mitochondrial preparation, thereby raising the possibility of propagation directly on addition of Fe^{2+} ions. The inhibition exerted by eugenol after 5 min of progression of peroxidation indicates its effect on the propagation sequence (Fig. 2a and b).

It is becoming increasingly evident that ȮH radicals are always detectable in Fe^{2+}–H_2O_2-dependent peroxidative systems but these radicals are not essential for peroxidation [22]. In the present study using mitochondria, the ȮH radical scavengers inhibited Fe^{2+}–H_2O_2-supported peroxidation (Table 2), clearly indicating the participation of the ȮH radical. Nagashima [16] suggested the suppression of ȮH radical formation by eugenol in Cu^{2+}–H_2O_2-supported lipid peroxidation. Interestingly, this peroxidation was inhibited by iodide, but not by

LOOH Lipid hydroperoxide	+	M^{n+} Metal ion	⟶	LO· Alkoxyl radical	+	$OH^- + M^{(n+1)+}$
LOOH	+	$M^{(n+1)+}$		LOO· Peroxyl radical	+	$H^+ + M^{n+}$
LO· or LOO·	+	LH Lipid	⟶	LOH	or	LOOH + L· Lipid radical

L·	+	O_2	⟶	LOO·		
LO· LOO· L·	+	AH Eugenol	⟶	LOH LOOH LH	+	A· Eugenol radical

saturation even at a concentration of 1 mM eugenol. Pretreatment of mitochondria with eugenol resulted in the inhibition of Fe^{2+}–ascorbate- or Fe^{2+}-supported peroxidation depending on the amount of eugenol taken up (Figs 6 and 7).

DISCUSSION

The results of the present study show the inhibitory effect of eugenol on lipid peroxidation by scavenging radical intermediates. Eugenol may inhibit lipid peroxidation at the level of initiation, propagation or both. Little is known about the chemistry of initiation of peroxidation by Fe^{2+}–ascorbate and other usual ȮH radical quenchers like formate, mannitol, benzoate, histidine or ethanol. In view of this, the effect of eugenol on deoxyribose degradation by the ȮH radical generating system was studied. Despite its ability to reduce Fe^{3+}, Fe^{3+}–ADP and Fe^{3+}–citrate, eugenol did not stimulate deoxyribose degradation indicating the absence of pro-oxidant effect (Table 3) attributed to certain other plant phenols [31]. Thus, the radical quenching ability of eugenol appears to outweigh the pro-oxidant activity expected to result from its ability to reduce iron. The second-order rate constant for reaction with ȮH radicals calculated according to Halliwell *et al.* [32] was about $4.8 \times 10^{10}\,M^{-1}\,sec^{-1}$ (Fig. 5), which explains the powerful radical scavenging effect of eugenol. The inhibition observed in the absence of EDTA (site specific deoxyribose degradation) does not appear to be due to complexation of deoxyribose bound iron by eugenol since the inhibition was not complete even at high concentrations of eugenol (Fig. 5).

The small amounts of eugenol, incorporated into the membrane due to its hydrophobic nature, appear

to play a major role in inhibiting lipid peroxidation by radical quenching. The eugenol retained in mitochondria (3 nmol approx.) is unlikely to chelate the excessive amount (25–100 μM) of Fe^{2+} in the incubation mixture discounting chelation as a possible mechanism of inhibition. A similar conclusion was drawn by Nagashima [16] since a 2-fold excess of eugenol could not completely inhibit Cu^{2+}–H_2O_2-mediated peroxidation. Halliwell and Gutteridge [33] emphasized the importance of site-specific generation of oxygen radicals at metal binding sites in membranes. Eugenol can reach these sites in view of its hydrophobic nature and interfere with peroxidation by propagation radicals generated at these sites. Such mechanism does not require large quantities of eugenol for inhibition. Eugenol did not undergo any perceptible chemical change during the course of peroxidation with either of the systems employed. Formation of a phenoxyl radical and reconversion to eugenol appear to be major reactions involved in quenching the radical intermediates. In view of the consumer preference for natural antioxidants eugenol appears to be a very useful antioxidant.

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