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INHIBITION OF MICROSOMAL LIPID PEROXIDATION AND MONOOXYGENASE ACTIVITIES BY EUGENOL

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Previously we reported that eugenol (4-allyl-2-methoxyphenol) inhibits non-enzymatic peroxidation in liver mitochondria (E. Nagababu and N. Lakshmaiah, 1992, Biochemical Pharmacology. 43, 2393–2400). In the present study, we examined the effect of eugenol on microsomal mixed function oxidase mediated peroxidation using Fe^{+3}-ADP-NADPH, carbon tetrachloride (CCL_4)-NADPH and cumene hydroperoxide (CumOOH) systems. In the presence of eugenol the formation of thiobarbituric acid reactive substances (TBARS) was decreased in all the systems (IC_{50} values: 14 μM for Fe^{+3}-ADP-NADPH, 4.0 μM for CCl_4-NADPH and 15 μM for CumOOH). Oxygen uptake was also inhibited to a similar extent with Fe^{+3}-ADP-NADPH and CumOOH systems. A comparative evaluation with other antioxidants showed that in Fe^{+3}-ADP-NADPH and CumOOH systems, the antioxidant efficacy was in the order: butylated hydroxytoluene (BHT) > eugenol > α-tocopherol, while in CCl_4-NADPH system the order was α-tocopherol > BHT > eugenol. Time course of inhibition by eugenol indicated interference in initiation as well as propagation of peroxidation. Eugenol did not inhibit cytochrome P-450 reductase activity but it inhibited P–450 – linked monooxygenase activites such as aminopyrine-N-demethylase, N-nitrosodimethylamine demethylase, benzo(a)pyrene hydroxylase and ethoxyresorufin-O-deethylase to different extents. However, CumOOH supported monooxygenases (aminopyrine-N-demethylase and benzo(a)pyrene hydroxylase) required much higher concentrations of eugenol for inhibition. The concentration of eugenol required to inhibit monooxygenase activities was more than that required to inhibit peroxidation in all the systems. Eugenol elicited type I changes in the spectrum of microsomal cytochrome P-450. These results suggest that the inhibitory effect of eugenol on lipid peroxidation is predominantly due to its free radical quenching ability. Eugenol significantly protected against the degradation of cytochrome P-450 during lipid peroxidation with all the systems tested. These findings suggest that eugenol has the potential to be used as a therapeutic antioxidant. Further evaluation may throw more light on this aspect.

KEY WORDS: Eugenol, peroxidation, cytochrome P-450, free radicals, monooxygenases, antioxidants.

Abbreviations used: ADP: adenosine 5′-diphosphate, CumOOH: cumene hydroperoxide, Fe^{2+}: ferrous sulphate, Fe^{3+}: ferric chloride, CCl_4: carbon tetrachloride, TBARS: thiobarbituric acid reactive substances, MDA: malonaldehyde, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, HRP: horse radish peroxidase, NDMA: N-nitrosodimethylamine, DMSO: dimethyl sulfoxide.

INTRODUCTION

Recently, reactive oxygen species (ROS) have been implicated in aging and several human diseases.[1] It has been frequently suggested that the increased ROS and lipid peroxidation are responsible for the toxic actions of a wide range of compounds including paraquat, ethanol, alloxan and carbon tetrachloride.[2,3] Several lines of

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evidence suggest that lipid peroxidation is promoted by the mixed function oxidases of liver microsomes which normally catalyse the oxidation of various exogenous (xenobiotics), and endogenous (steroids and fatty acids etc.) substrates.[4,5] The oxidation of nonpolar inert substances by monooxygenases requires the donation of electrons from NADPH which produce partially reduced oxygen species.[6] Leakage of such species (ROS), can occur causing damage to the surrounding tissue membranes. The NADPH-stimulated lipid peroxidation in microsomes has been studied extensively.[7,8] Metabolism of xenobiotics by microsomal systems can directly form free radical metabolites which may lead to tissue injury.[2,9]

Eugenol (4-allyl-2-methoxyphenol) is the active principle of cloves and is extensively used as a flavouring and pharmaceutical agent. In view of its analgesic and anesthetic properties, eugenol is used in several dental materials.[10] In Indian traditional medicine eugenol is used in the treatment of flatulent colic, chronic diarrhoea and other gastrointestinal disorders.[11,12] The acceptable daily intake of eugenol for humans is upto 2.5 mg/kg body weight.[13] It is considered non-mutagenic, non-carcinogenic and generally recognised as safe (GRAS) by the FAO.[12,14] Pharmacokinetic and metabolic studies on eugenol in humans by Fisher *et al.*[15] revealed high first-pass conjugation, rapid elimination, minimal epoxidation and short residence of epoxide ((metabolite of eugenol) in the body which help reduce the toxicity. However, Thompson *et al.*[16,17,18] reported that a quinone methide like compound toxic to hepatocytes was formed on incubation of eugenol with microsomes or HRP. The relevance of such findings to eugenol metabolism *in vivo* is not known.

We have previously shown that eugenol effectively inhibits non-enzymatic lipid peroxidation induced by Fe^{2+}-ascorbate and H_2O_2-Fe^{2+} systems in liver mitochondria.[19] We have also shown that eugenol does not exhibit pro-oxidant activity in this assay system in spite of its ability to reduce iron. Hepatic microsomal fractions have been shown to be particularly sensitive to peroxidation and as such, have been extensively used to investigate the antioxidant activity of many compounds. The present study was carried out to examine: (1) the effects of eugenol on lipid peroxidation in rat liver microsomes using Fe^{+3}-ADP-NADPH, CumOOH and CCl_4-NADPH systems as peroxidation inducers, (2) the effect of eugenol on NADPH and CumOOH supported monooxygenase activities, (3) comparision of antioxidant activity of eugenol with known antioxidants.

MATERIALS AND METHODS

Materials

Eugenol, dl-α-tocopherol, ADP, NADPH, NADP, CumOOH, 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid, aminopyrine, benzo(a)pyrene, N-nitrosodimethylamine, ethoxyresorufin, resorufin, dl-isocitric acid, isocitrate dehydrogenase (EC 1.1.1.42), and glucose-6-phosphate dehydrogenase (EC 1.1.1.49), were obtained from Sigma chemicals. All other chemicals were of the highest purity available (Analytical grade).

Preparation of Microsomes

Liver microsomes were prepared from overnight fasted NIN Wistar strain rats in 1.15% KCl medium according to Ernster (20). The microsomes were suspended in

1.15% KCl to give a protein content of 10 mg/ml. Protein content was determined by the method of Lowry *et al.*[21]

Preparation of Solutions

α-Tocopherol and BHT were dissolved in absolute alcohol while eugenol was dissolved in 70% alcohol for use in peroxidation studies. For monooxygenase studies eugenol was dissolved in DMSO. CumOOH was suspended in Tris-HCl buffer pH 7.4 and sonicated before use. The final concentrations of solvents in incubation mixtures were less than 1% (v/v) and the solvent controls without antioxidants were always included.

Induction and Assay of Lipid Peroxidation

Lipid peroxidation was induced in microsomes by Fe^{+3}-ADP-NADPH[20], CCl_4-NADPH[22] and CumOOH[23] systems. The contents of incubation mixtures, reaction conditions and period of incubation are specified in the legends. Inhibitors were added 2 min prior to the addition of peroxidation inducers. The extent of lipid peroxidation was assayed by estimating TBARS formation and oxygen uptake.[19] Following incubation for specified periods, the peroxidation was terminated by adding 20% TCA (0.5 ml/ml incubation mixture). 2-Thiobarbituric acid (TBA) was added subsequently (1 ml of 0.67% (w/v) solution/ml incubation mixture) and heated for 10 min. at 100°. After centrifugation the absorbance of the supernatant was read at 532 nm to measure the thiobarbituric acid reactive substances (TBARS). Acid hydrolyzed 1,1,3,3-tetraethoxypropane was used as authentic standard for malonaldehyde (MDA). Addition of eugenol to MDA standards, or peroxidized microsomes just before the addition of TCA did not affect the colour yield with TBA reagent. The oxygen utilization was monitored at 25°C using a Gilson Oxygraph fitted with Clark-type electrode (Model 5/6 H).

NADPH Cytochrome P-450 Reductase

This enzyme was estimated by cytochrome c reduction method according to Masters *et al.*[24]

NADPH Supported Monooxygenases

Aminopyrine-N-demethylase was determined according to Mazel.[25], NDMA demethylase according to Kawanishi *et al.*[26], benzo(a)pyrene hydroxylase according to Nebert and Gelboin[27], and ethoxyresorufin-O-deethylase according to Burke *et al.*[28]

CumOOH Supported Monooxygenases

In these assays CumOOH was used instead of NADPH at a concentration of 1.5 mM for aminopyrine-N-demethylase and 75 μm for benzo(a)pyrene hydroxylase.

Spectral Studies

A Hitachi spectrophotometer (Model 220S) was used to study the difference spectra of cytochrome P-450 between 350 and 510 nm in presence of eugenol.

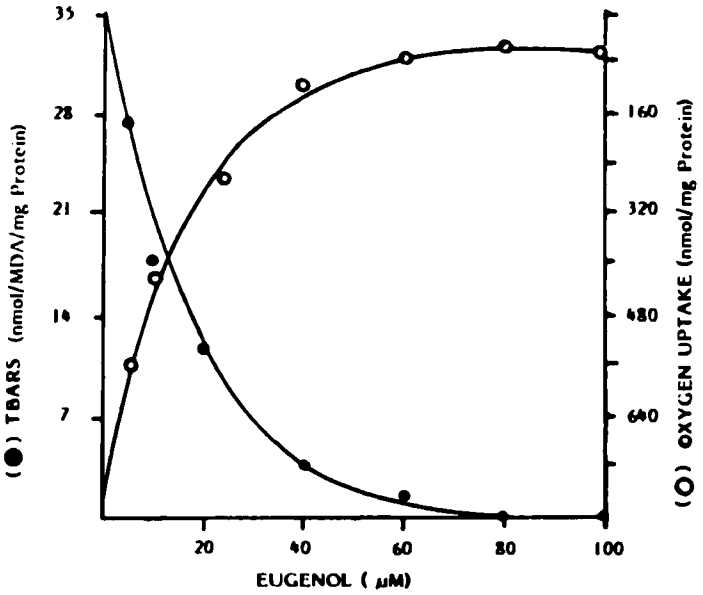

FIGURE 1 Effect of eugenol on Fe^{+3}-ADP-NADPH mediated lipid peroxidation: Microsomes (1 mg protein) were incubated with 1 mM ADP, 12 μM $FeCl_3$ (mixed before use) 0.18 mM NADPH and indicated concentrations of eugenol in 0.15 M KCl - 0.025 M Tris-HCl buffer, pH 7.4, at 37°C for 60 min in a total volume of 1 ml (for TBARS estimation) or 1.7 ml (for oxygen uptake).

Estimation of Cytochrome P-450 Content

The spectrum of the CO adduct of dithionite-reduced hemoprotein was recorded in Hitachi Spectrophotometer to determine the content of cytochrome P-450.[29]

RESULTS

ADP-Fe-$^{+3}$-NADPH Mediated Peroxidation

Studies have shown that cytochrome P-450 reductase and cytochrome P-450 are involved in the NADPH linked, Fe^{+3}-ADP activated microsomal lipid peroxidation.[30,31] Addition of eugenol to this system resulted in the inhibition of peroxidation as assessed by TBARS formation and oxygen uptake. A linear relationship existed between TBARS formation and oxygen uptake. Approximately 1 nmole of TBARS (MDA equivalents) formed for 21 nmoles of oxygen consumed (Fig. 1). As shown In Fig. 2, lipid peroxidation increased with time and reached a plateau after 20 min. With 20 μM eugenol added at the start of the reaction, the peroxidation was observed after a lag period of 5 min and was inhibited to an extent of 75% of the control. With 50 μM eugenol the lag period was 20 min and inhibition was 95%. Addition of eugenol 5 min after the induction of peroxidation also inhibited further progress of the reaction (Fig. 2). The inhibitory effect of eugenol on peroxidation was about 7 fold higher than that of α-tocopherol and 8 fold less than that of BHT (Table 1).

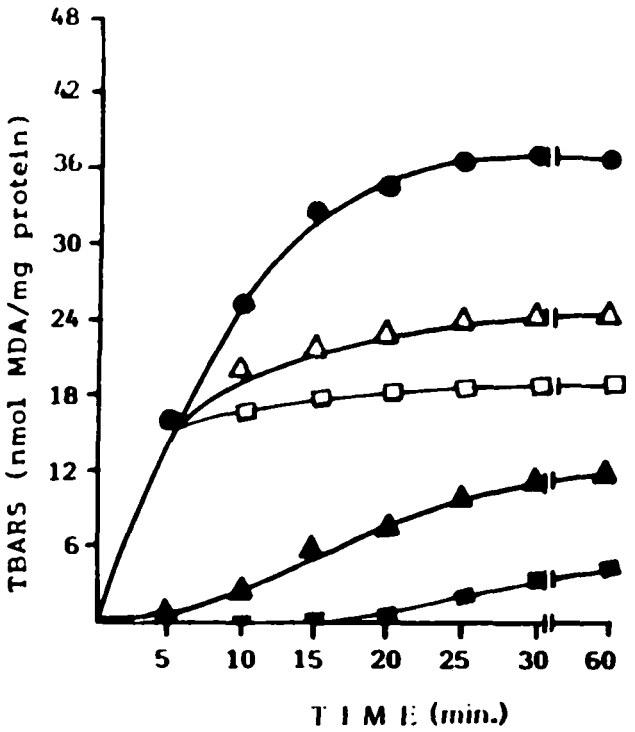

FIGURE 2 Effect of eugenol on time course of Fe^{+3}-ADP-NADPH mediated peroxidation. The assay system and incubation were as described in Fig. 1. (●) without eugenol; (▲) 20 μM and (■) 50 μM eugenol added at zero time; (△) 20 μM and (□) 50 μM eugenol added after 5 min of incubation.

TABLE 1
Comparison of antioxidant activity of eugenol with known antioxidants

Inhibitors	IC_{50} (μM)		
	Fe^{+3}-ADP-NADPH	CumOOH	CCl_4 ± NADPH
Eugenol	14.0 ± 1.6	15 ± 3.2	4.0 ± 0.18
α-tocopherol	100 ± 9.0	225 ± 18.0	1.2 ● 0.10
BHT	1.8 ± 0.14	8.2 ● 1.2	4.0 ± 0.18

Peroxidation was measured as described in materials and methods. Values are means ● SEM for three individual experiments each performed in duplicate.

CCl_4-NADPH Mediated Peroxidation

Carbon tetrachloride is cleaved by the cytochrome P-450 system giving rise to trichloromethyl radical ($\dot{C}Cl_3$) which combines with O_2 to form trichloromethyl peroxyl radical ($CCl_3O\dot{O}$),[32] which initiates lipid peroxidation.

Addition of EDTA to this system suppresses the NADPH triggered, iron mediated lipid peroxidation permitting specifically the estimation of $\dot{C}Cl_3$ and $CCl_3O\dot{O}$ radical mediated peroxidation. Eugenol inhibited this peroxidation in a concentration dependent manner, decreasing the extent and initial rates of TBARS formation

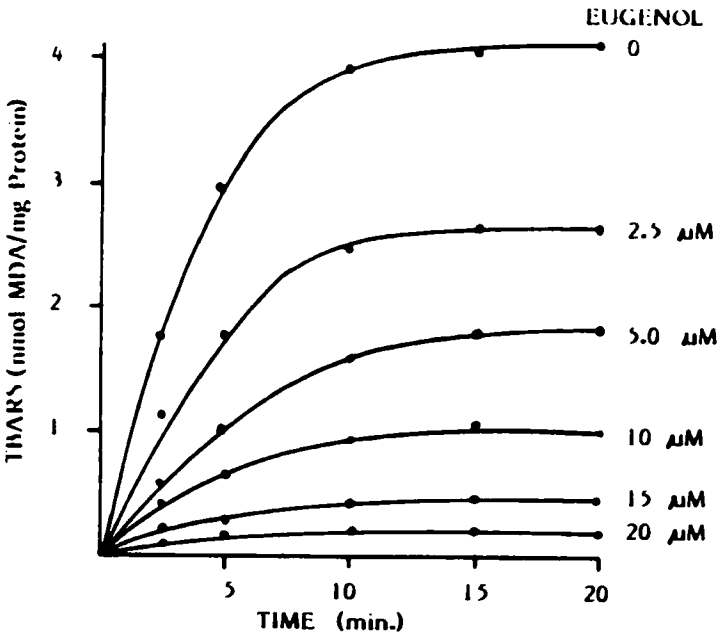

FIGURE 3 Effect of eugenol on time course of CCl_4 induced lipid peroxidation: Microsomes (1 mg protein) were incubated at 37°C with 5 mM CCl_4, 0.1 mM EDTA, a NADPH generating system (1 mM NADPH, 10 mM glucose-6-phosphate and 2 units of glucose-6-phosphate dehydrogenase) and indicated concentrations of eugenol in 0.1 M potassium phosphate buffer pH 7.4.

(Fig. 3). At 20 μM eugenol the inhibition was complete. The inhibitory effect of eugenol was about half that of BHT and one third that of α-tocopherol (Table-1).

CumOOH Mediated Peroxidation

Organic hydroperoxides undergo homolytic cleavage in presence of P-450 and generate alkoxyl/aroxyl radicals which initiate microsomal lipid peroxidation.[22,33]

$$\text{CumOOH} + \text{P-450}\,(Fe^{3+}) \xrightarrow[\text{cleavage}]{\text{Homolytic}} \underset{\text{(Cumyloxyl radical)}}{\text{CumO}^{\cdot}} + \text{P-450}\,(Fe^{4+}) - \text{OH}$$

Incubation of microsomes with CumOOH caused a significant increase in TBARS formation which was inhibited by eugenol in a dose dependent manner (Fig. 4). CumOOH promoted oxygen uptake by microsomes, the extent and initial rates of which decreased with increasing concentration of eugenol. Eugenol inhibited oxygen uptake when added during the progress of the peroxidation also (Fig. 5). The inhibitory effect of eugenol was about 2 times less than that of BHT and 15 times more than that of α-tocopherol.

Effect of Eugenol on Cytochrome P-450 Reductase and Microsomal Monooxygenase Activities

In view of the inhibitory effects of eugenol on various microsomal peroxidation systems tested, it was of interest to study the effect of eugenol on P-450 reductase

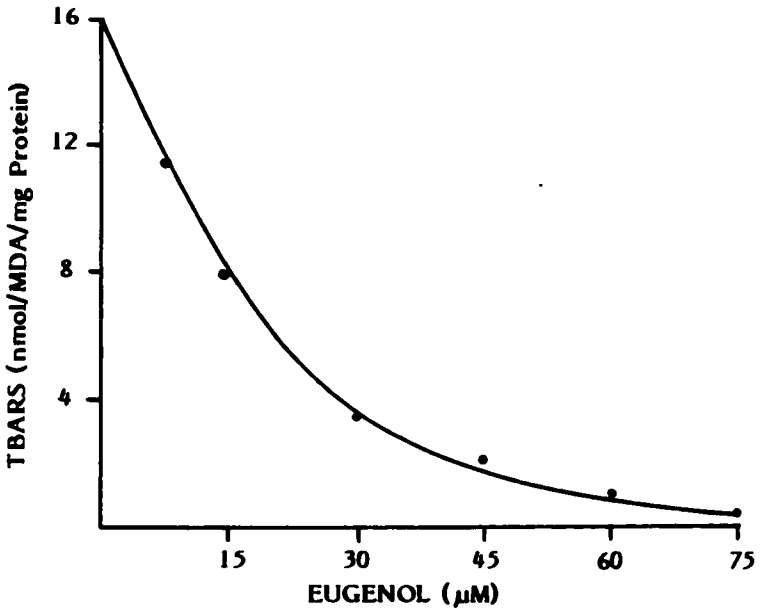

FIGURE 4 Effect of eugenol on CumOOH mediated peroxidation: Microsomes (1 mg protein/ml) were incubated with 125 μM CumOOH, 10 mM $MgCl_2$ and indicated concentrations of eugenol in 0.15 M KCl - 0.05 M Tris HCl buffer pH 7.5 at 37°C for 30 min in a total volume of 1 ml.

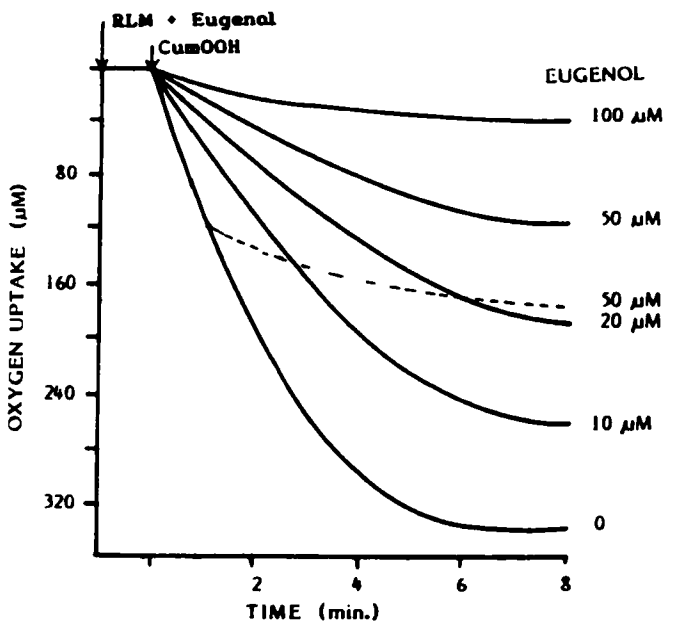

FIGURE 5 Effect of eugenol on CumOOH induced oxygen uptake: The assay system was described in Fig. 4 except that total incubation mixture was 1.7 ml and oxygen uptake was monitored at 25°C (----). Eugenol added during the progress of reaction. RLM: Rat liver microsomes.

and P-450-monooxygenase activities *per se*. Eugenol had no effect on transfer of electrons from NADPH to oxidized cytochrome c (nmole cytochrome c reduced/mg protein/min: control = 36 ± 5.2; with 20 μM eugenol 34.7 ± 4.9; with 100 μM eugenol 38.2 ± 7.4). Eugenol itself could not reduce cytochrome c. Eugenol inhibited monooxygenase activities supported by both NADPH/O_2 and CumOOH to varying degrees. Among the NADPH/O_2 supported enzymes the inhibition of NDMA - demethylase was maximal followed by ethoxyresorufin-O-deethylase, aminopyrine-N-demethylase and benzo(a)pyrene hydroxylase. The CumOOH supported benzo(a)pyrene hydroxylase and aminopyrine-N-demethylase were inhibited at considerably higher levels of eugenol (Table 2).

Effect of Eugenol on the Spectra of Microsomal P-450

Eugenol elicited type I spectral changes in microsomal cytochromes P-450 with an absorption peak at 385 nm and trough at 422 nm (Fig. 6).

Effect of Eugenol on the Stability of Heme Component of Cytochrome P-450 with Different Peroxidation Inducing Systems

Incubation of microsomes with the three peroxidation inducing systems caused substantial decrease in the CO-binding capacity of dithionite reduced microsomes. Eugenol significantly inhibited such decreases (Table 3).

DISCUSSION

Using liver mitochondria as substrate we have shown earlier that eugenol inhibits non-enzymatic peroxidation. The current study demonstrates that eugenol is also an effective inhibitor of microsomal lipid peroxidation mediated by NADPH cytochrome P-450 reductase and cytochrome P-450. Controversies still exist regarding the mechanistic details of NADPH-Fe^{+3}-ADP mediated peroxidation in microsomes. Earlier studies[34,35] proposed the involvement of reductase in the reduction of iron, since the direct addition of ferrous form of iron resulted in microsomal lipid peroxidation even in the absence of NADPH. Aust *et al.*[36] have shown that Fe^{+3}-ADP is not reduced by reductase either directly or through O_2^- generation. Vegh *et al.*[37] demonstrated direct reduction of Fe^{+3}-ADP to Fe^{2+}-ADP in rat liver microsomes and suggested that membrane integrity is essential for full expression of reductase activity. However, studies by Morehouse and Aust[30] using reconstituted microsomal lipid peroxidation systems have demonstrated the involvement of reductase and P-450 in ADP-Fe^{3+} reduction. Sevanian *et al.*[31] confirmed the observations of Morehouse and Aust and suggested a mechanism for ADP-Fe^{+3}-NADPH mediated peroxidation, which is similar to cytochrome P-450 - linked monooxygenase reactions.

From our results it appears that eugenol does not inhibit cytochrome P-450 reductase as judged by cytochrome c reduction. However, cytochrome P-450 itself appears to be the component with which eugenol interacts in view of the following considerations: (a) the monooxygenase activities of cytochrome P-450 were inhibited by eugenol to a considerable degree (Table 2). (b) eugenol brought about type I spectral changes when incubated with microsomes. (c) microsomes metabolize eugenol to a quinone methide like compound.[17] In addition eugenol could interact with various

TABLE 2
Effect of eugenol on monooxygenase activities

	NADPH supported (nmol/mg protein/min)				CumOOH supported (nmol/mg protein/min)				
	Eugenol (μM)				Eugenol (μM)				
	0	20	50	100	0	100	250	500	1 mM
Aminopyrine-N-demethylase	3.62 ± 0.230	2.30 ± 0.205 (36%)	1.37 ± 0.125 (62%)	1.12 ± 0.146 (69%)	4.85 ± 0.312	3.25 ± 0.265 (23%)	2.76 ± 0.201 (43%)	2.03 ± 0.186 (58%)	1.35 ± 0.212 (72%)
NDMA demethylase	1.12 ± 0.138	0.58 ± 0.064 (51%)	0.20 ± 0.038 (82%)	0.15 ± 0.041 (87%)	-	-	-	-	-
Benzo(a)pyrene hydroxylase	0.62 ± 0.051	0.48 ± 0.042 (25%)	0.35 ± 0.025 (45%)	0.28 ± 0.036 (56%)	1.21 ± 0.142	1.05 ± 0.10 (13%)	0.85 ± 0.125 (30%)	0.59 ± 0.11 (51%)	0.42 ± 0.007 (65%)
Ethoxyresorufin -O-deethylase	0.52 ± 0.035	0.35 ± 0.021 (33%)	0.18 ± 0.009 (65%)	0.11 ± 0.006 (79%)	-	-	-	-	-

Enzyme activities were determined after incubation of various substrates with liver microsomes and either NADPH generating system or CumOOH. Values in parentheses are given as the percentage of controls (without eugenol). Values are mean ± SEM for three separate experiments, each performed in duplicate.

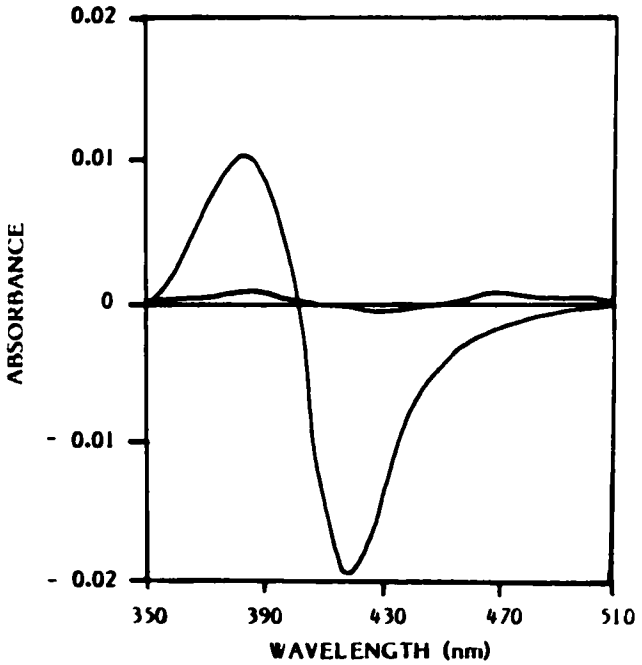

FIGURE 6 Effect of eugenol on the spectrum of microsomal cytochrome P-450: microsomes (2 mg/ml) were suspended in 0.1 M phosphate buffer (pH 7.4) and equal aliquots taken in 2 cuvettes. A base-line of equal light absorbance between 350–510 nm was recorded. A difference spectrum was recorded 2 min after addition of 0.5 mm eugenol to sample cuvette and equal volume of solvent to reference cuvette.

TABLE 3
Effect of eugenol on the stability of heme component of cytochrome P-450 with different peroxidation inducing systems

Peroxidation	Eugenol (μM)	P-450 (nmol/mg protein)	P-450 degraded %
Control	-	0.450 ± 0.015	0
Fe^{+3}-ADP-NADPH	-	0.127 ± 0.007	72
Fe^{+3}-ADP-NADPH	-	0.310 ± 0.020	31
Fe^{+3}-ADP-NADPH	-	0.380 ● 0.012	15
CumOOH	-	0.143 ± 0.010	68
CumOOH	20	0.264 ● 0.011	41
CumOOH	100	0.320 ± 0.013	29
CCl_4-NADPH	-	0.285 ± 0.012	37
CCl_4-NADPH	10	0.318 ± 0.021	29
CCl_4-NADPH	50	0.345 ± 0.018	23

The contents of incubation mixtures of Fe^{+3}-ADP-NADPH, CumOOH and CCl_4-NADPH were as mentioned in Fig. 1, Fig. 4 and Fig. 3 respectively. The assay mixtures were incubated at room temperature for 20 min. The CO binding spectrum of P-450 was determined as mentioned in materials and methods. Values are mean ± SEM for 5 determinations.

postulated initiating species of Fe^{2+}-ADP such as perferryl, ferryl, ferrous-ferric dioxygen complexes and several lipid derived radicals to inhibit peroxidation.[38] According to Sevanian *et al.*[31] ADP-FE^{+3} is reduced by P-450 and subsequent addition of oxygen results in the formation of perferryl radical (ADP-Fe^{+3}-O_2^-) which then abstracts H atoms from polyunsaturated fatty acids to form carbon centered lipid radicals. Eugenol itself could be a potential donor of H atoms at this stage to compete with polyunsaturated fatty acids. The time course of Fe^{+3}-ADP-NADPH mediated lipid peroxidation showed a lag period in presence of eugenol. Redox recycling of phenoxyl radicals arising from phenolic antioxidants can occur by intracellular reductants like ascorbate, superoxide and microsomal electron transport.[39] The recycling of chromanoxyl radicals of α-tocopherol by non-enzymatic and enzymatic intracellular reductants has been considered and the higher recycling efficiency of short side chain homologs leading to longer lag periods in the onset of peroxidation has been reported.[40] Such recycling of phenoxyl radicals arising from eugenol could result in the lag period observed in this study. We have shown in our previous paper[19] the incorporation of eugenol into the mitochondrial membrane in a concentration dependent manner and the resultant inhibition of iron mediated peroxidation. In microsomal lipid peroxidation also eugenol incorporated into microsomal membrane may inhibit "site specific generation of radical species", a localised process proposed by Halliwell *et al.*[41] The inhibition exerted by eugenol 5 min after progress of peroxidation indicates its effect on the propagation sequence.

The hepatotoxicity of CCl_4 is due to reductive dehalogenation by cytochrome P-450 to form trichloromethyl free radical. Alcohol inducible cytochrome P-450 II E1 is reported to be highly effective in catalyzing the demethylation of NDMA[42] and dehalogenation of CCl_4.[43] Eugenol could inhibit NDMA-demethylase suggesting the possibility of its inhibitory effect on CCl_4 bioactivation. However the concentration required to inhibit NDMA-demethylase was approximately 5 times more than that required to inhibit lipid peroxidation. Hence quenching of $\dot{C}Cl_3/CCl_3O\dot{O}$ or other propagating radicals appears to be mainly responsible for the inhibition. Similarly eugenol inhibited CumOOH mediated peroxidation as well as CumOOH supported monooxygenase activities. In the absence of a monooxygenase substrate, $Cum\dot{O}$ generated could attack lipids to initiate peroxidation.[44] The amount of eugenol necessary to inhibit these reactions was considerably less (IC_{50}-15 μM). However, in the presence of monooxygenase substrates, the amount of eugenol needed for inhibition of monooxygenase activities was quite high (IC_{50}-375 μM). Hence quenching of $Cum\dot{O}$ or other propagating radicals by eugenol could result in the inhibition of peroxidation. The antiperoxidative potency of eugenol, when compared to that of BHT or α-tocopherol, differed with different peroxidation inducing systems. In some recent reports[45,46] considerably lower concentrations of α-tocopherol have been shown to inhibit ADP-Fe^{+3}-NADPH mediated lipid peroxidation. The higher concentrations which we used (Table 1) are comparable to those reported by Kagan *et al.*[40] The difference could be due to the procedure adopted for the addition of α-tocopherol to microsomes, temperature, time given for incorporation into the membranes and the solvent used to dissolve the antioxidant. With α-tocopherol, the IC_{50} values show big variations (Table 1). In NADPH containing system the recycling of chromanoxyl radicals of α-tocopherol is possible. The extent of peroxidation itself varied considerably with different peroxidation inducing systems (~35 nmoles TBARS with Fe^{+3}-ADP-NADPH, ~16 nmoles with CumOOH system and ~4 nmoles with CCl_4-NADPH system). With CumOOH recycling of chromanoxyl radicals is unlikely and hence the higher requirement for α-tocopherol. With

CCl_4-NADPH, the extent of peroxidation itself is considerably lower. Moreover α-tocopherol may be more efficient quencher of $CCl_3O\dot{O}$ than eugenol or BHT. The effects of eugenol are similar to other phenolic antioxidants such as BHT, BHA and propyl gallate with respect to inhibition of monooxygenase activities and interaction with cytochrome P-450.[47,48]

There is an ever increasing demand on cytochrome P-450 to cope with a variety of new xenobiotics entering into the environment of industrialised countries. Lipid peroxidation during the metabolism of several xenobiotics leads to the destruction of P-450. Eugenol is very effective in protecting the P-450 degradation during lipid peroxidation *in vitro* and it may offer some protection *in vivo* also. In recent times there is a growing concern regarding the safety of synthetic antioxidants and their use is being restricted considerably.[49] The use of naturally occurring antioxidants like eugenol may be more desirable than synthetic ones from health point of view. Considering the natural occurrence and wide spread usage of eugenol further studies on its protective action against free radical damage may throw light on as yet unknown health benefits.

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