

INHIBITION OF TUMOUR PROMOTION IN MICE BY EUGENOL

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Abstract: Number of tumours (papillomas) produced by the application of 7, 12-dimethyl benz (a) anthracene as initiator and croton oil promotor in mice were considerably inhibited (84%) by the prior application of eugenol. Moreover, there was considerable decrease in the number of tumour bearing animals and their onset. Eugenol inhibited superoxide formation and lipid peroxidation and the radical scavenging activity may be responsible for its chemopreventive action.

Key words: eugenol tumour promotion superoxides lipid peroxidation

INTRODUCTION

The concept of two-stage carcinogenesis consisting of initiation and promotion was first proposed by Berenblum (1). The former stage is an irreversible process while the latter is associated with reversible and irreversible changes, 12-O-Tetradecanoyl phorbol 13-acetate (TPA), present in croton oil is a typical tumour promotor having various biological and biochemical effects on susceptible tissues (2).

Recent studies have shown that several naturally occurring compounds exhibit anti-tumour promoting activity. These include quercetin, oleanolic acid, ursolic acid and kaempferol (3). Various spices like garlic, asafoetida and mace (4, 5) and curcumin the active ingredient present in turmeric (6,7), are also reported to possess this activity. Eugenol (4-allyl-2-methoxyphenol) is a naturally occurring compound which is used as a food flavour and fragrance agent (8). Eugenol is the main component of the oil of clove and is also present in the essential oils and in the extracts of many other plants including cinnamon basil and nutmeg (9).

In the present study, we have evaluated the effect of eugenol on croton oil-induced tumour promotion on mouse skin. Inhibitory effects of eugenol on superoxide production as well as lipid peroxidation were also studied.

METHODS

Female Swiss albino mice (shaved on dorsal skin two days earlier), were divided into two groups of 10 animals each. They were initiated with single topical application of a solution of 470 nmol, of 7,12-dimethyl benz (a) anthracene (DMBA) in 200 μ l acetone (7). After one week, control mice (Group I) received topical applications of croton oil (50 m1) as a promotor twice weekly for 6 weeks. Animals in Group II were also treated with Eugenol (2 mg in 0.1 ml acetone) 30-40 min prior to the application of promotor croton oil. Number of skin tumour (papilloma) formation on the mouse skin were recorded weekly and tumours greater than 1 mm in diameter were included in the cumulative total if they persisted two weeks or more. Values are mean of two independent experiments.

The lipid peroxidation was determined by the

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thiobarbituric acid (TBA) method (10). Mouse liver homogenate (25%, 100 μ l) in cold tris-HCl buffer (0.2M, pH 7.0) was incubated with and without eugenol for 1 hr at 37°C in the presence of 150 mM KCl (100 μ l), 0.3 mM ascorbic acid (100 μ l) 0.8 mM ferrous ammonium sulphate (100 μ l) in a total volume of 500 μ l. After incubation 20% trichloroacetic acid (1 ml) followed by 0.67% TBA (2 ml) were added to each tube and boiled for 15 min. After cooling and centrifugation at 200 g, the optical density of the supernatant was measured at 540 nm. The amount of lipid peroxidation was expressed as nmoles of malonaldehyde formed in each tube.

The scavenging effect of eugenol on photochemically-induced superoxide production was determined by the nitroblue tetrazolium (NBT) method (11). The assay mixture (3 ml) contained 0.1 M EDTA containing 0.0015% sodium cyanide (200 μ l), 1.5 mM NBT (100 μ l), 0.12 mM riboflavin (50 μ l) eugenol (different concentration) and phosphate buffer (pH 7.8). Optical densities were recorded before and after illuminations (15 min) at 560 nm. The difference in optical density in the control tubes and those containing eugenol was taken as a measure of superoxide production.

Drugs : Eugenol was purchased from Romali, India and 7.12-dimethyl benz (a) anthracene (DMBA) from Sigma Chemicals, St-Louis, USA. Croton oil was prepared from the seeds of *Croton tiglium* by petroleum ether extraction (4).

RESULTS

In the control group, the first tumour appeared at week 5, whereas in the group treated with eugenol the first tumour appeared at week 12. Significant ($P < 0.001$) inhibition of tumour formation was observed on the 16th week of initiation. Eugenol also lowered the percentage of tumour bearing mice (Fig.1). All the control animals developed tumours on 16th week of initiation while only 40% of the eugenol-treated group developed tumours at week 16. The average number of tumours per mouse at week 16 were 2.56 ± 0.78 in the control group, and in the eugenol treated group 0.4 ± 0.50 ($P < 0.001$). Thus, treatment with eugenol caused 84% reduction in the average number of tumours per mouse at week 16.

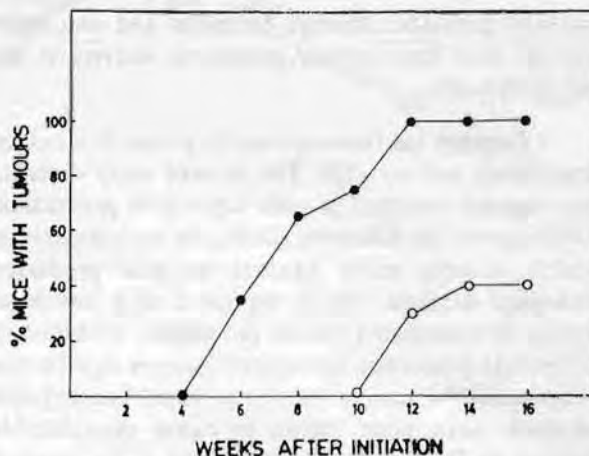


Fig. 1 : Effect of eugenol on papilloma formation. The figure indicates the percentage of tumour bearing mice in ●—● control animals treated with DMBA and croton oil ○—○ animals treated with eugenol prior to croton oil application. Values are average of 2 independent experiments of 10 animals in each set.

Eugenol was found to inhibit superoxide production and lipid peroxidation in a significant manner ($P < 0.001$) (Table I). The concentration required to produce 50% inhibition in the former was found to be 125 μ M, while the corresponding figure in the latter was 25 μ M.

TABLE I : Effect of eugenol on superoxide production and lipid peroxide formation *in vitro*.

Concentration of eugenol (μ M)	Percentage inhibition	
	Superoxide production	Lipid peroxidation
25	0	28.7 ± 2.6
25	0	51.8 ± 3.4
50	12.5 ± 0.23	ND
100	39.6 ± 1.91	68.9 ± 5.2
500	79.2 ± 11.08	ND
1000	92.5 ± 10.2	89.4 ± 6.2

ND = not determined.

DISCUSSION

It has been suggested that reactive oxygen species play an important role in tumour promotion (12). Free radical generating compounds such as

benzoyl peroxide, lauroyl peroxide and chloroper benzoic acid have tumour promoting activity in the mouse skin (6).

Eugenol has been reported to possess free radical scavenging activity (13). The present study revealed that eugenol inhibited *in vitro* superoxide production. Since superoxide dismutase (SOD), the metalloenzyme, which protect cells against oxygen mediated biological damage (14) is expressed at a low level during TPA-mediated tumour promotion, inhibition of superoxide production by eugenol assumes significance in reducing the tumour incidence. Lipid peroxidation products have been shown to cause considerable damage to DNA (15). In the present study, eugenol was found to inhibit *in vitro* lipid peroxidation. Moreover, we have shown that Eugenol could also

inhibit the chemical carcinogenesis induced by topical application of dimethyl benzanthracene followed by croton oil promotion. Because of the known free radical scavenging activity of eugenol it could be inferred that eugenol's action is mainly in the inhibition of promotion.

The effect of curcumin on TPA-induced tumour promotion has been attributed to the inhibition of arachidonic acid metabolism via the lipoxygenase and cyclo-oxygenase pathways, which result in the formation of reactive oxygen species and other free radicals (16). Eugenol has been reported to inhibit prostaglandin synthesis via promotion by inhibiting arachidonic acid metabolism and/or by functioning as a scavenger of reactive species that are produced during the metabolism of arachidonic acid.

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