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Antioxidant activity of curcumin and related compounds*

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The inflammatory response induced experimentally in animals appears to be correlated with disturbances in the regulation of cellular oxidative processes as evident from the anti-inflammatory action of well known antioxidants [1]. An earlier report from this laboratory gave evidence of a parallel between oedema formation in mice induced by carrageenin and the *in vitro* production of lipid peroxides by liver [2].

Curcumin 1,6-heptadiene-3,5-dione-1,7-bis (4-hydroxy-3-methoxyphenyl) the anti-inflammatory principle of *Curcuma longa* [3] inhibits *in vitro* lipid peroxide formation by liver homogenates of oedemic mice [2]. To investigate the correlation of anti-oxidant action with the anti-inflammatory action of curcumin, the effect of a few compounds chemically related to curcumin on *in vitro* lipid peroxide formation was studied.

Male albino rats (30–50 g body wt) from the CDRI stock colony were fasted overnight but given access to drinking water. After decapitation, the whole brain was taken out and washed with 150 mM KCl. All further operations were carried out at 4°C. Homogenates (10% w/v) were prepared in 150 mM KCl and centrifuged at 800 *g* for 10 min. The supernatant was used for the study of *in vitro* lipid peroxide formation. Aliquots of the supernatant (2 ml) with

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Table 1. Effect of curcumin and ferulic acid on *in vitro* lipid peroxidation in rat brain

Additions (M)	μmole MDA/ 100 g/3 hr	Inhibition (%)
—	17.760	—
Curcumin 5.15×10^{-3}	0.880	95.0
Curcumin 1.03×10^{-3}	10.160	42.8
Curcumin 1.03×10^{-4}	15.360	13.5
Curcumin 1.03×10^{-5}	16.640	5.4
Curcumin 1.03×10^{-6}	17.280	2.7
Ferulic acid 5.15×10^{-3}	5.168	70.9
Ferulic acid 1.03×10^{-3}	15.360	13.5
Ferulic acid 1.03×10^{-4}	17.760	0.0
Ferulic acid 1.03×10^{-5}	18.240	+8.1
Ferulic acid 1.03×10^{-6}	17.600	0.9

* MDA = malonyldialdehyde.

Table 2. Effect of bis-3,4-dihydroxycinnamoyl methane (I) and caffeic acid (II) on *in vitro* lipid peroxidation in rat brain

Additions (M)	μmole MDA/ 100 g/3 hr	Inhibition (%)
—	14.000	—
I 1.03×10^{-3}	0.000	100.0
I 1.03×10^{-4}	0.000	100.0
I 1.03×10^{-5}	0.000	100.0
I 1.03×10^{-6}	5.115	63.5
II 1.03×10^{-3}	0.269	98·6
II 1.03×10^{-4}	0.077	99.5
II 1.03×10^{-5}	0.769	94.5
II 1.03×10^{-6}	7.615	45.6

Table 3. Effect of cinnamic acid, *o*-hydroxycinnamic acid, *p*-hydroxycinnamic acid and 3,4,5-trimethoxycinnamic acid on *in vitro* lipid peroxidation in rat brain

Additions (M)	μmole MDA/ 100 g/3 hr	Inhibition (%)
—	16.480	—
Cinnamic acid 2.90×10^{-2}	21.472	+30.3
Cinnamic acid 1.45×10^{-2}	20.944	+27.1
Cinnamic acid 1.45×10^{-3}	16.120	2.2
—	11.936	—
o-Hydroxycinnamic acid 1.79×10^{-2}	9.152	23.0
o-Hydroxycinnamic acid 1.79×10^{-3}	11.552	3.2
o-Hydroxycinnamic acid 1.79×10^{-4}	12.432	+ 4.0
—	17.248	—
p-Hydroxycinnamic acid 1.79×10^{-2}	0.208	98.8
p-Hydroxycinnamic acid 8.95×10^{-3}	6.128	64.5
p-Hydroxycinnamic acid 1.79×10^{-3}	15.792	8.4
p-Hydroxycinnamic acid 1.79×10^{-4}	16.304	6.4
—	15.456	—
Trimethoxycinnamic acid 1.34×10^{-2}	11.376	26.4
Trimethoxycinnamic acid 2.68×10^{-3}	18.688	+20.8
Trimethoxycinnamic acid 3.35×10^{-4}	14.848	4.0

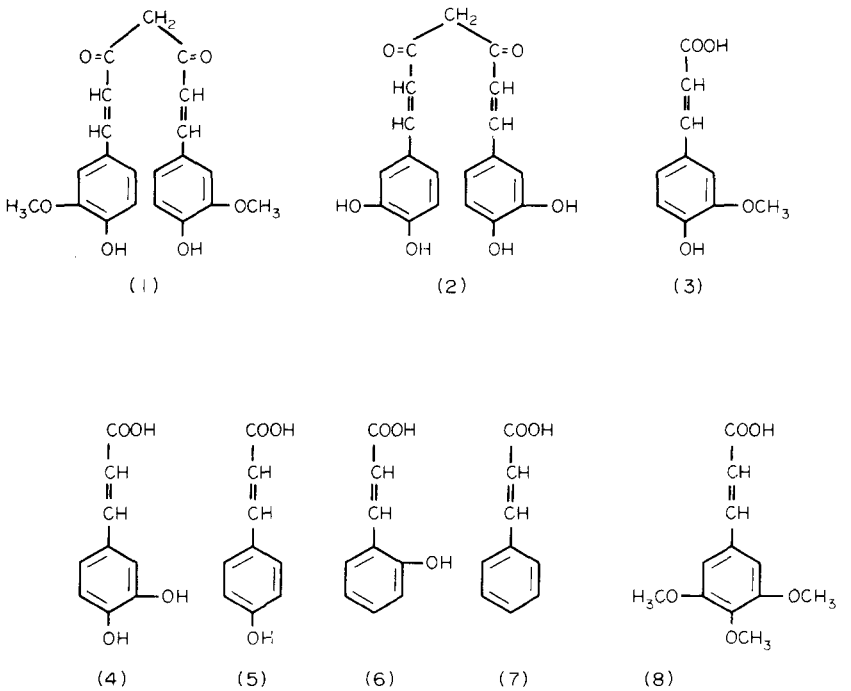

Fig. 1. Structures of curcumin and related compounds: 1. Curcumin (*trans, trans*); 2. bis-3,4-Dihydroxycinnamoylmethane (*trans, trans*); 3. Ferulic acid (*trans*); 4. Caffeic acid (*trans*); 5. *p*-Hydroxycinnamic acid (*trans*); 6. *o*-Hydroxycinnamic acid (*trans*); 7. Cinnamic acid (*trans*); 8. 3,4,5-Trimethoxycinnamic acid (*trans*).

or without additions were shaken at 37 ± 1° in 25 ml Erlenmeyer flasks in a metabolic shaker at a rate of 120 strokes/min. Samples were withdrawn after 3 hr and lipid peroxides formed were estimated by reacting with thiobarbituric acid [4]. The results were expressed as μmole malonyldialdehyde, using an extinction coefficient of 1.56×10^5 at 535 nm [4].

The effect of curcumin and some related compounds on *in vitro* output of lipid peroxides is summarised in Tables 1–3. The structural relation of the different compounds is shown in Fig. 1. Demethylated derivatives of curcumin and ferulic acid, viz. bis-3,4-dihydroxycinnamoylmethane and caffeic acid, are the most potent inhibitors of lipid peroxidation. Complete methylation as in 3,4,5-trimethoxycinnamic acid (Table 3), also leads to abolition of antioxidant capacity. The hydroxyl group in the benzene ring must be at the *p*-position. Cinnamic acid has no inhibitory effect, on the other hand it potentiates lipid peroxidation.

The salient features which emerge from this investigation are: (a) The caffeic acid moiety of curcumin is very potent as an *in vitro* inhibitor of lipid peroxidation. Two caffeic acid molecules when joined together through a methylene bridge lead to bis-demethoxy-curcumin which is the most potent antioxidant studied in this series. (b) Methylation depresses the antioxidant character, as is evident from lipid peroxidation in the presence of ferulic acid and caffeic acid, curcumin and bis-3,4-dihydroxycinnamoyl methane, and trimethoxycinnamic acid. (c) The brain homogenates used for studying the antioxidant action of the above compounds do not appear to be capable of bringing about demethylation.

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Division of Biochemistry,
Central Drug Research Institute,
Lucknow,
India.

O. P. SHARMA

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