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EFFECT OF DIETARY TURMERIC (*CURCUMA LONGA*) ON IRON-INDUCED LIPID PEROXIDATION IN THE RAT LIVER

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Abstract—Male Wistar rats were fed a control diet or the control diet supplemented with 1% (by weight) turmeric for 10 wk. In rats injected with 30 mg Fe^{2+}/kg body weight, lipid peroxidation was 29 and 35% lower in liver homogenates and microsomes, respectively, of turmeric-fed rats than in those of rats fed the control diet. The activities of superoxide dismutase, catalase and glutathione peroxidase were higher (by 19, 19 and 20%, respectively) in liver homogenates of rats fed the turmeric-containing diet in comparison with the controls. These studies indicate that dietary turmeric lowers lipid peroxidation by enhancing the activities of antioxidant enzymes.

INTRODUCTION

Curcumin is a component of turmeric, a yellow colouring spice which is used routinely in many of the dishes prepared in Asian countries (Govindarajan, 1980). Curcumin is a good antioxidant and inhibits lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates (Pulla Reddy and Lokesh, 1992; Salimath *et al.*, 1986; Sharma, 1976). Curcumin is also a good scavenger of reactive oxygen species and lowers the formation of inflammatory compounds such as prostaglandins and leukotrienes (Elizabeth and Rao, 1990; Huang *et al.*, 1991; Unnikrishnan and Rao, 1992). Because of these effects, curcumin has been used to lower carrageenan-induced oedema in rats and to give moderate relief of rheumatoid arthritis in human patients (Deodhar *et al.*, 1980; Srihari Rao *et al.*, 1982). These beneficial effects of curcumin may indicate that turmeric has therapeutic potential. Indeed, feeding turmeric to mice and rats inhibits the formation of tumours in response to benzopyrene, 3-methylcholanthrene and 3′-methyl-4-dimethylaminobenzene (Aruna and Sivaramakrishnan, 1992; Polasa *et al.*, 1991).

Recently, turmeric was also shown to contain a water-soluble peptide termed turmerin (Leela Srinivas *et al.*, 1992). Like curcumin, turmerin has also been reported to be a good antioxidant and protects erythrocyte membranes and phospholipid fatty acids from oxidation *in vitro* (Leela Srinivas *et al.*, 1992). Turmerin is present in turmeric at levels up to 0.1%, while curcumin constitutes up to 5% (Govindarajan, 1980). In an earlier, unpublished study we demonstrated that inclusion of lipophilic curcumin in diets containing n-6 and n-3 polyunsaturated fatty acids lowered lipid peroxidation in the serum and liver microsomes of rats (A. Ch. Pulla Reddy and B. R. Lokesh, unpublished data, 1993). However, the effect of turmerin supplementation in the diet on lipid peroxidation is yet to be ascertained. The presence of curcumin and turmerin makes turmeric an excellent dietary antioxidant. However, no studies have been carried out to test the efficacy of turmeric in lowering lipid peroxidation *in vivo*. Using a model system developed by Tappel and his colleagues for measuring *in vivo* lipid peroxidation (Hu *et al.*, 1990), we have demonstrated that dietary turmeric lowers lipid peroxidation in rat liver homogenates and microsomes. Further, we have demonstrated that feeding turmeric to rats also modulates the antioxidant enzymes in a manner that favours the lowering of lipid peroxidation.

Abbreviation: TBARS = thiobarbituric acid reactive substances.

MATERIALS AND METHODS

Thiobarbituric acid, vitamin E (DL-α-tocophorol), xanthine oxidase, glutathione, glutathione reductase, cumene hydroperoxide, hydrogen peroxide, α-cellulose, choline chloride and methionine were obtained from Sigma Chemical Co. (St Louis, MO, USA). NADPH, cytochrome c, xanthine, adenosine diphosphate, trichloroacetic acid and EDTA were purchased from Sisco Research Laboratory (Bombay,

Table 1. Diet composition

Ingredients	Amount (g/100 g diet)
Casein*	20.0
Cellulose	5.0
Sucrose	60.0
Choline chloride	0.2
Methionine	0.3
Vitamin mix†	1.0
Mineral mix†	3.5
Groundnut oil	10.0

*Kaira District Milk Producers Cooperative, Anand, India.
†AIN 76 vitamin and mineral mix.
In the experimental diet turmeric (mixed in the groundnut oil) was added at a level of 1% (1 g/100 g diet).

India). Ferrous sulphate ($FeSO_4 \cdot 7H_2O$) was obtained from Qualigen Fine Chemicals (Bombay, India). Refined groundnut oil and turmeric powder were purchased from a local supermarket.

Animals. Groups of 12 male Wistar rats weighing 70–75 g were fed the purified diets with or without turmeric for 10 wk. The rats had free access to water. The food intake and growth of the animals were monitored at regular intervals.

Diets. The composition of the basal diet is summarized in Table 1. The experimental diet (1% turmeric) was prepared by mixing the basal diet thoroughly with turmeric suspended in groundnut oil. Fresh feed was provided daily.

Liver homogenates, microsomes and lipid peroxidation. Six of the rats in each dietary group were injected ip, 1 hr before they were killed, with 30 mg iron as ferrous sulphate in 1 ml saline/kg body weight (Hu *et al.*, 1990). The remaining rats were injected with the same volume of saline alone. The rats were killed by cardiac puncture and the livers were perfused with saline and homogenized in 10 volumes of 0.15 M KCl. The liver microsomes were prepared as described by Lokesh *et al.* (1981). The washed microsomes were suspended either in 0.15 M KCl or other appropriate buffers as indicated.

The basal and incubated lipid peroxidation levels (Mathias and Dupont, 1979) in liver homogenates and microsomes was measured as thiobarbituric acid reactive substances (TBARS) following the method described by Buege and Aust (1978).

Antioxidant enzymes. Superoxide dismutase was measured by the inhibition of cytochrome c reduction mediated by superoxide anions generated by xanthine–xanthine oxidase and monitored at 550 nm (Flohe and Otting, 1984). One unit of superoxide dismutase was defined as the amount required to inhibit the reduction of cytochrome c by 50%. Catalase activity was assayed according to the method of Aebi (1984) by following the decomposition of H_2O_2 at 240 nm. Glutathione peroxidase activity was determined by NADPH oxidation in a coupled reaction system containing cumene hydroperoxide and oxidized glutathione (Tappel, 1978). Protein was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as reference standard.

Statistical analysis. Significant differences in the experimental values were determined using Student's *t*-test. Correlation coefficients were calculated to determine the relationship between TBARS and the activities of protective enzymes (Dowdy and Wearden, 1983).

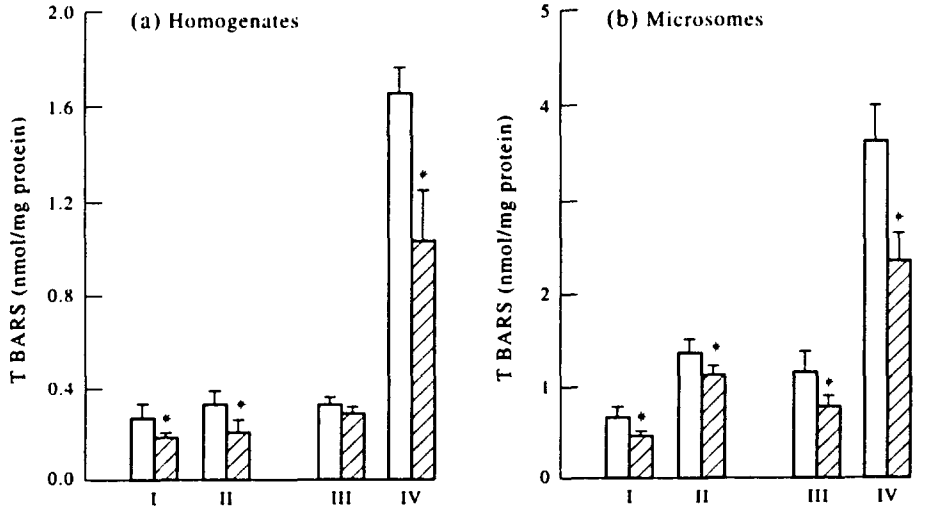

Fig. 1. Lipid peroxidation in (a) liver homogenate and (b) liver microsomes. Rats were injected with 1 ml saline (bars I and II) or Fe^{2+} (30 mg/kg body weight in 1 ml saline; bars III and IV). Lipid peroxidation was measured at zero (bars I and III) or at 60 min (bars II and IV) of incubation as described in Materials and Methods. Rats were fed the control diet (□) or turmeric-supplemented diet (▨). Results are means ± SD for six rats per group and those marked with an asterisk differ significantly (Student's *t*-test) from the corresponding value for rats fed the control diet (*$P < 0.01$).

Table 2. Effect of feeding turmeric on antioxidant enzymes in liver homogenates of rats

Enzymes	Control diet	Turmeric-supplemented diet
Superoxide dismutase (units/mg protein†)	133 ± 6.2	158 ± 3.3*
Catalase (μmol H_2O_2 decomposed/mg protein)	207 ± 3.7	247 ± 8.1*
Glutathione peroxidase (munits/mg protein‡)	54 ± 3.7	65 ± 5.0*

Results are means ± SD for groups of six rats, and those marked with an asterisk differe significantly (Student's t-test) from the corresponding control values (*$P < 0.001$).
†1 unit of superoxide dismutase was defined as the amount required to inhibit the reduction of cytochrome c by 50%.
‡1 munit = 1 nmol NADPH oxidized.

RESULTS

There were no significant differences in feed consumption (15 g/day/rat), body weight gain (180 ± 12.5 g) and liver weights (3.6 ± 0.37/100 g body weight) between rats on the control diet and those fed the turmeric-supplemented diet. (These values are combined means ± SD, for six rats/group.)

Lipid peroxidation

Injection of Fe^{2+} into the rat peritoneum significantly enhanced lipid peroxidation in liver homogenates and microsomes (Fig. 1). However, the levels of thiobarbituric acid reactive substance (TBARS) were lower in rats fed the turmeric-supplemented diet: these levels were 29% lower in the homogenates and 35% lower in the microsomes in comparison with the controls. Rats injected with saline did not show any significant enhancement in TBARS in liver homogenates and microsomes, indicating the importance of Fe^{2+} in the induction of lipid peroxidation in hepatic tissues. This is in agreement with the results published by other workers (Galleano and Puntarulo, 1992; Hu *et al.*, 1990).

Antioxidant enzymes

The effect of dietary turmeric on the activities of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase was measured. The activities of superoxide dismutase, catalase and glutathione peroxidase were 19, 19 and 20% higher, respectively, in rats fed the turmeric-containing diet than in those fed the control diet (Table 2). The correlation coefficient between lipid peroxidation and the activities of the antioxidant enzymes is shown in Table 3. These studies indicate that turmeric may lower lipid peroxidation by maintaining higher levels of activities of antioxidant enzymes.

Table 3. Linear regression correlation between lipid peroxidation and activities of antioxidant enzymes in liver homogenates from rats fed control or turmeric-containing diets and injected with saline (uninduced rats) or Fe^{2+} (induced rats)

	Correlation coefficient			
	Control diet		Turmeric-supplemented diet	
Enzymes	Unindeced rats	Induced rats	Uninduced rats	Induced rats
Superoxide dismutase	−0.815	−0.080	−0.982	−0.980
Catalase	−0.456	−0.639	−0.456	−0.769
Glutathione peroxidase	−0.788	−0.879	−0.560	−0.590

DISCUSSION

The therapeutic potential of turmeric has been known for a long time in Indian medicine (Govindarajan, 1980). Its antimicrobial and anti-inflammatory properties have been exploited over centuries. Recently, Aruna and Sivarama Krishnan (1992) demonstrated that feeding 160 mg turmeric/g diet/day can reduce tumour formation by 54% in mice injected with benzopyrene. Turmeric at levels of 10–100 mg/g diet was also shown to exert antimutagenic effects (Polasa *et al.*, 1991). Incidentally, these studies also showed that none of the rats that were fed diets containing 10% turmeric developed subcutaneous tumours (Polasa *et al.*, 1991). The therapeutic potential of components of turmeric (curcumin, 1200 mg/day; volatile oil, 0.01 ml/kg body weight) have been demonstrated in arthritic patients (Deodhar *et al.*, 1980) and in laboratory animals (Chandra and Gupta, 1972). Recently, Phase II clinical trials of curcumin in rheumatoid and osteoarthritis patients have been reported (Asthana, 1992–93). Curcumin was given to these patients at doses ranging from 1000 to 2500 mg/day. Four out of nine rheumatoid arthritis patients and three out of three osteoarthritis patients showed improvement after curcumin treatment for 2–3 months.

Turmerin, a water-soluble component in turmeric, was also shown to reduce inflammation in the mouse ear punch assay (Leela Srinivas *et al.*, 1992).

These studies clearly indicated that turmeric has anti-inflammatory potential and may also have anti-carcinogenic effects. However, not much is known regarding the mechanism by which turmeric can exert such beneficial effects.

Lipid peroxidation plays a crucial role in inflammation, cancer and heart diseases (Freeman and Crapo, 1982). Our previous studies showed that curcumin present in turmeric can lower lipid peroxidation (Pulla Reddy and Lokesh, 1992). Turmerin from turmeric is also an effective inhibitor of *in vitro* lipid peroxidation (Leela Srinivas *et al.*, 1992; Salimath *et al.*, 1986). Our current studies indicate

that whole turmeric can also have a similar inhibitory effect on lipid peroxidation *in vivo*. However we could not quantitate the individual contributions made by the curcumin and turmerin present in turmeric in lowering lipid peroxidation.

On average, curcumin is present to an extent of 2% in turmeric (Govindarajan, 1980). Based on this value, the rats in the study reported here consumed 3 mg curcumin/day. Our unpublished results (A. Ch. Pulla Reddy and B. R. Lokesh, unpublished data, 1993) indicated that rats consuming 3 mg curcumin per day showed 23% less inflammation in foot pads in response to carrageenan injection. The macrophages isolated from rats fed 3 mg curcumin/day also showed a decrease of 22% in superoxide anion formation (B. Joe and B. R. Lokesh, unpublished data, 1993).

Turmerin is reported to be present at a level of up to 0.1% in turmeric (Leela Srinivas *et al.*, 1992). From this it was calculated that the rats in our study consumed 0.067 mg turmerin/day. However, we have no reported data on turmerin to compare its efficacy with that of curcumin as an antioxidant when given orally or as a dietary supplement.

Turmeric may lower lipid peroxidation by maintaining the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) at higher levels (Table 2). These enzymes play an important role in the regulation of lipid peroxidation (Harris, 1992). We found similar effects on these antioxidant enzymes in rats fed 1% curcumin in the diet (A. Ch. Pulla Reddy and B. R. Lokesh, unpublished data, 1993). The mechanism by which turmeric or curcumin provides an environment for the enhanced activities of these enzymes is yet to be ascertained. In addition, we have also observed that curcumin can scavenge/quench oxygen free radicals such as superoxide anions and hydroxyl radicals which play an important role in the initiation of lipid peroxidation. Curcumin also prevented the oxidation of Fe^{2+} in the Fenton reaction (A. Ch. Pulla Reddy and B. R. Lokesh, unpublished data, 1993). Therefore, turmeric and one of its constituents, curcumin, can lower lipid peroxidation by influencing a number of important factors that regulate lipid oxidation.

The amount of turmeric used in the present study was 75 times higher than the normal human intake of turmeric from the diet (Thimmayamma *et al.*, 1983). However high concentrations of turmeric in the diet are not toxic. Acute toxicity studies conducted with rats, guinea pigs and monkeys revealed that turmeric is non-toxic even at the very high levels of 2.5 g/kg body weight (Bhavani Shankar *et al.*, 1980). Sambaiah *et al.* (1982) demonstrated that turmeric fed to rats at doses that are 1.25 to 125 times higher than the normal human intake did not cause any adverse effect on growth, food efficiency ratio, numbers of red and white blood cells, differential blood cell counts, levels of haemoglobin, total serum protein, albumin or globulin, or activities of serum aminotransferase and alkaline phosphatase. Aruna and Sivarama Krishnan (1992) fed mice a diet containing 160 mg turmeric/g and observed a 54% reduction in the incidence of tumours in those mice injected with benzopyrene. Similarly, Polasa *et al.* (1991) fed rats 100 mg turmeric/g diet and noticed that none of them developed subcutaneous tumours on injection with benzopyrene. These results indicate that for therapeutic purposes turmeric may be taken at doses higher than those ingested from the normal diet without any toxicity.

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