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INHIBITION OF LIPID PEROXIDATION AND CHOLESTEROL LEVELS IN MICE BY CURCUMIN

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Abstract: Effect of oral administration of curcumin (diferuloyl methane) on lipid peroxidation in various organs of mice like liver, lung, kidney and brain was studied in control animals as well as those given carbon tetrachloride, paraquat and cyclophosphamide. Oral administration of curcumin significantly lowered the increased peroxidation of lipids in these tissues produced by these chemicals. Administration of curcumin was also found to lower significantly the serum and tissue cholesterol levels in these animals, indicating that the use of curcumin helps in conditions associated with peroxide induced injury such as liver damage and arterial diseases.

Key words: curcumin lipid peroxidation cholesterol antioxidants

INTRODUCTION

Endothelial injury in the vascular wall has been shown to be the initial event in the atherosclerosis and related problems of coronary heart diseases. Implication of lipids, especially low density liporoteins in the causation of atherosclerosis has been documented (1). Lipid droplets get deposited in the aortic wall and the lipids undergo peroxidative changes in presence of reactive species of oxygen which eventually produce endothelial injury (2). Subsequent platelet aggregation and release of platelet factor, infiltration of smooth muscle cells and collagen deposition are some of the events leading to plaque formation (3). Compounds that can scavenge the reactive species of oxygen and inhibit peroxidation of lipid could be useful as preventive agents against atherosclerosis.

Tumeric (Curcuma longa), as well as its active ingredient curcumin, inhibit lipid peroxidation *in vitro* (4). Moreover, curcumin also decreases serum cholesterol levels in hyperlipidaemic rats and rabbits (5, 6). In the present investigation the efficiency of curcumin in inhibiting peroxidation of lipids under the influence of known free radical inducing drugs such as carbon tetrachloride, paraquat and cyclophosphamide, has been studied in mice. Studies on the level of cholesterol in serum and tissues after giving curcumin have also been carried out.

METHODS

Male Swiss albino mice, 8 weeks old, weighing 20-25 g were housed in ventilated cages and fed with mouse chow (Lipton, India) and water *ad libitum*. Groups of six animals were used for each set of experiment. Curcumin (5 mg/ml) was suspended in gum acacia (1%) and 1 ml (ie 250 mg/kg) was given to each animal orally, using gastric tube, for 14 days. Animals were sacrificed by cervical dislocation, blood was collected by heart puncture. Serum and tissues were kept at –100°C till the assays were done.

In animals given CCl_4, paraquat and cyclophosphamide, curcumin treatment was started two days prior to the drug treatment and continued till the animals were sacrificed. Control animals received the same volume of vehicle containing 1% gum acacia.

In one set of animals, carbon tetrachloride (0.5 ml/kg) diluted in liquid paraffin was given as one acute dose (i.p) and animals were sacrificed after 24 h. At this dose it produces necrotic lesion in liver within 24 h (7). Tissues obtained from sacrificed animals were frozen immediately. Blood was collected by heart

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puncture, serum separated and kept at –100°C till the assays were done.

In the second set of animals single dose of paraquat (125 mg/kg) was given orally and the animals sacrificed after 24 h. Tissues and serum removed from them were frozen immediately. Pulmonary emphysema has been reported to result from this dose (8).

Administration of cyclophosphamide has to be continued for 10-14 days in order to increase lipid peroxidation (9). Hence in the third set of animals cyclophosphamide (50 mg/kg) was given i.p. for 10 days and during this period animals received curcumin (250 mg/kg) orally everyday. These animals were sacrificed on 11th day and tissues and serum withdrawn were immediately frozen .

Assay of lipid peroxidation : Tissues were weighed and homogenized in 4 volumes of tris-HCl buffer (0.2M, PH 7.0) at 4°C and clarified by cheese-cloth. 0.1 ml of the homogenate (protein, 27 mg/ml) was used for measuring lipid peroxides by thiobarbituric acid method (10). This method involved initial precipitation with 20% trichloroacetic acid and further reaction with 0.67% thiobarbituric acid (2 ml) at 100°C for 15 minutes.

In order to determine the peroxidizable bonds (11) homogenates (0.1 ml) were incubated with KCl (150 mM, 0.1 ml), tris-HCl buffer (0.2M, PH 7.0, 0.2 ml) and ascorbic acid (0.3mM, 0.1 ml) in a total volume of 0.5 ml at 37°C for 1 h. After the incubation increased peroxides formed were estimated by thiobarbituric acid reaction.

Total cholesterol was estimated in the tissues and serum by Liebermann-Burchard reaction (12). Unless otherwise indicated, all values are expressed as mean of six animals with standard deviation. Statistical significance was determined by Student 't' - test.

Drugs used in the study were carbon tetrachloride (E, Merck, India Ltd.,) Paraquat dichloride (Gramoxone, Imperial Chemical Industries, London), Cyclophosphamide (Endoxan, Khandelwal Laboratories Pvt. Ltd., Bombay), Thiobarbituric acid (BDH Chemicals, Poole, (England), Curcumin 98% pure (Gift from Bombay Oil Industries Ltd., Angamali).

RESULTS

Effect of Curcumin treatment on lipid peroxidation in normal animals : Animals which were given curcumin for 14 days showed a significant decrease in the lipid peroxide values in tissue homogenates. When these homogenates were activated with ascorbate, the lipid peroxides was found to be significantly increased in lung (139.5%), kidney (88.7%) and brain (23%). This activation was not inhibited by curcumin treatment (Table I).

Effect of curcumin on lipid peroxidation in vitro: Addition of curcumin to normal liver homogenate reduced the lipid peroxides substantially (Fig. 1). This inhibition was found to be concentration dependent requiring a concentration of 10^{-5}M for 50% inhibition. Curcumin did not have any effect on ascorbate induced peroxidation *in vitro;* as the difference in the values

TABLE I : Effect of curcumin administration on lipid peroxidation in some mice tissues.

Tissue	*Lipid peroxidation (n moles of malonaldehyde /g tissues) M ±SD*			
	Without ascorbate activation		*With ascorbate activation*	
	Control	*Treated with Curcumin*	*Control*	*Treated with Curcumin*
Liver	112.0 ± 27.8	75.2 ± 7.2**	115.3 ± 27.0	120.0 ± 12.3
Lung	58.7 ± 14.9	47.0 ± 6.0*	140.6 ± 35.3	138.0 ± 49.9*
Kidney	147.3 ± 19.5	111.2 ± 25.0**	278.0 ± 18.5	267.2 ± 55.9
Brain	247.3 ± 9.6	199.2 ± 48.8	304.0 ± 27.0	280.8 ± 51.7*

*P < 0.05, **P < 0.01

of peroxides formed with and without curcumin remained almost the same (Fig. 1).

Effect of curcumin treatment on lipid peroxidation in paraquat treatment animals : There was a substantial increase in the lipid peroxides in liver (141%) and kidney (48%) after paraquat treatment, while it was lesser in brain (8%) and lung (18%). The greater increase in liver and kidney may be due to they are being the target tissues during the detoxification. Curcumin treatment decreased the increased lipid peroxide values in liver by 28% (P<.01) and in kidney by 25% (P<.05) (Table II).

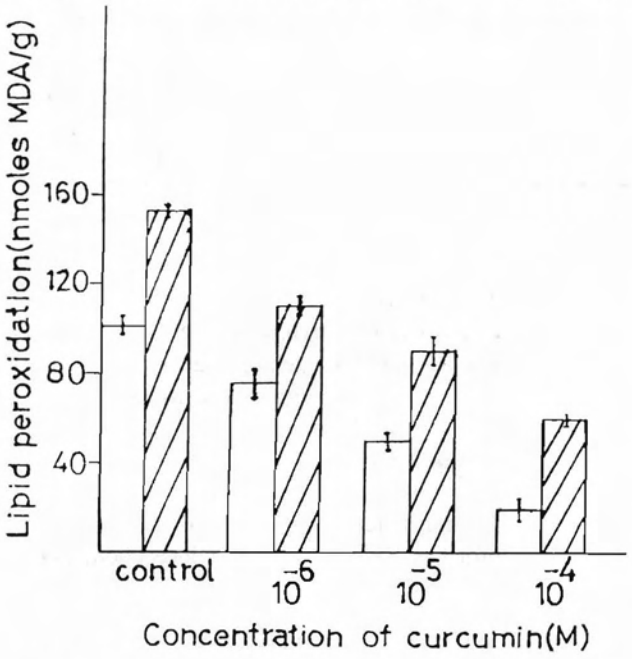

Fig. 1 : The effect of curcumin on lipid peroxides in normal liver homogenate (20%) incubated with (Cross - batched bars) and without (Open bars) ascorbate.

Effect of curcumin administration on lipid peroxides induced by Carbon tetrachloride : Administration of CCl_4 significantly increased the lipid peroxide in liver (82.6%) lungs (72.5%), kidney (23%) and brain (1%). When curcumin was administered during carbon tetrachloride treatment, these values were lowerd (P<.001) in liver by 25%, in lungs 34%, in kidney 18%, and in brain 1.8% (Table II).

Effect of curcumin on lipid peroxidation induced by cyclophosphamide: Cyclophosphamide increased the lipid peroxides in liver (57.8%) and lung (165.7%). Oral administration of curcumin along with cyclophosphamide significantly reduced (P<0.001) the lipid peroxides in these tissues in liver by 46% and in lungs by 29.7% (Table II).

Effect of curcumin on cholesterol levels in animal tissues and serum: Oral administration of curcumin for 14 days significantly reduced (P<0.001) the tissue cholesterol in liver, lungs, kidney and brain (Table III). Similar decrease in cholesterol was also found in the

TABLE II : Effect of curcumin administration on lipid peroxidation induced by Paraquat, CCl_4 and Cyclophosphamide.

	Treatment	*Lipid peroxidation (n moles/g tissue) M ± SD*			
		Liver	*Lungs*	*Kindney*	*Brain*
1.	Paraquat alone	270.0 ± 57.3	69.3±11.2	218.0 ± 63.8	268.6±50.9
	Praquat + Curcumin	194.0 ± 8.0**	64.0±08.0*	164.0±22.3*	208.6±7.3**
2.	CCl4 alone	204.6 ± 6.9	101.3±7.9	181.3±15.5	250.6±6.0
	CCl4 + Curcumin	154.0 ± 20.8***	66.6±5.5***	148.6±10.3***	246.0±7.8*
3.	Cyclopospamide alone	176.8 ± 39.6	156.0±18.1	183.2±38.7	270.4±33.8
	Cyclophosphamide + Curcumin	95.2 ± 15.8***	109.6±18.9***	170.4±27.4*	220.0±13.9**

(n = 6); *P<.05, **P<.01, ***P < .001

TABLE III: Effect of Curcumin on cholesterol levels in tissues and serum.

Tissue	*Cholesterol (mg/g tissue) M ± SD*	
	Non-treated	*Treated with curcumin*
Liver	3.82 ± 0.9	1.91 ± 0.55***
Lung	3.12 ± 0.85	0.98 ±0.13***
Kidney	5.02 ± 1.34	2.00 ± 0.22***
Brain	17.96 ± 4.1	7.76 ± 0.26***
Serum	217.5	182.5

(n = 5); Cholesterol levels is expressed in mg/100 ml serum. *** P < 0.001

serum of curcumin treated animals indicating its cholesterol lowering effect.

DISCUSSION

The results presented confirm the lipid peroxide scavenging activity of curcumin in animals. Earlier results had shown its effectiveness *in vitro* to reduce lipid peroxidation using erythrocyte ghost preparation (13). In the present study carbon tetrachloride, cyclophosphamide and paraquat were used for production of free radicals. The former, which is frequently used in showing free radical induced liver damage, has CCl_3 ion as the active species (14). In the case of cyclophosphamide, the alkylating agent gets converted into a phosphoramide mustard which is the active species and during its conversion produces free radicals (15). Similarly the lung toxicity of the herbicide paraquat is due to the superoxide radical formed and mimics the oxygen induced toxicity (16).

Increased peroxides formed by the administration of these chemicals were significantly reduced by the administration of curcumin which may be either due to the scavenging of peroxides and other activated oxygen species formed, or due to the neutralization of the free radicals. Although addition of curcumin reduced the peroxides in the homogenate, it did not inhibit the nonenzymatic peroxidation reaction by addition of ascorbate. Similar observation has also been made in the case of 2-mercaptopropionyl glycine, a radioprotector which is in clinical trials.

Curcumin has been shown to inhibit cyclooxygenase activity (17) and thereby lower prostaglandin synthesis. The metabolism of arachidonic acid via the lipoxygenase and cyclooxygenase pathways results in the formation of reactive oxygen species and other free radicals which are implicated in chemical carcinogenesis. Recently it has been shown that curcumin is a potent inhibitor of TPA - and arachidonic acid induced inflammation and of cyclooxygenase and lipoxygenase activities (18) and hence could inhibit tumour promotion. Curcumin has been shown to reduce chemically induced papillomas in animals (19).

Curcumin administration also reduces the cholesterol content in tissues such as liver, lung, kidney and brain. Such an effect has already been reported in rats and rabbits fed with hyperlipidaemic diet (5), (6). The mechanisim for such a decrease in cholesterol of the tissues and in serum is not known, although preliminary studies indicate that this could be due to decreae in absorption (5). Curcumin administration has also been shown to reduce serum cholesterol in human volunteers, with increase in the HDL cholesterol (under publication), indicating that curcumin may be mobilizing cholesterol from extrahepatic tissues to liver where it is catabolised.

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