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IMMUNOMODULATORY ACTIVITY OF CURCUMIN

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ABSTRACT

Curcumin, an active ingredient present in Curcuma longa, was analysed for the immunomodulatory activity in Balb/c mice. Curcumin administration was found to increase the total WBC count (15,290) significantly on the 12th day Group of animals treated with vehicle alone showed results similar to that of normal animal (10,130 on 12th day). Curcumin increased the circulating antibody titre (512) against SRBC. Curcumin administration increased the plaque forming cells (PFC) in the spleen and the maximum number of PFC was observed on the 6th day (1,130 PFC/10^6 spleen cells) after immunization with SRBC. Bone marrow cellularity (16.9x10^6 cells/femur) and α-esterase positive cells (1,622/4000 cells) were also enhanced by Curcumin administration. A significant increase in macrophage phagocytic activity was also observed in Curcumin treated animals ($P<0.001$). These results indicate the immunostimulatory activity of Curcumin.

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INTRODUCTION

There are several herbal preparations used in the indigenous system of medicine which can enhance the body's immune status. Immunomodulators are agents that can either stimulate an individuals immune system or inhibit host defense parameters which are normal or already activated (1). A variety of materials from plant source like polysaccharides, lectins (2) peptides (3) etc. have been known to stimulate the immune system. Immunostimulators are now widely used in cancer therapy. Curcumin analysed in this study for its immunomodulatory activity is an active ingredient from Curcuma longa and is a known antioxidant (4). It has already been tested for its antimutagenic (5) and anticarcinogenic activity (6). Curcumin has been reported to have cytocidal activity towards tumour cells (7) and antitumour activity in animals (8). The chemical structure of Curcumin was elucidated by Lamp et al. (9) and it was reported to be a derivative of methane substituted by two ferulic acid residues. Phenolic structure of Curcumin together with the β-diketone structure is suggested to be responsible for the high biological activity of Curcumin (10). Curcumin has also been reported to have antimetastatic activity (11) and it inhibits TNF expression (12). The present study analyses the immunomodulatory activity of Curcumin.

MATERIALS AND METHODS

Animals: Balb/c mice (4-6 weeks old) were purchased from the National Institute of Nutrition, Hyderabad. Animals were kept in air controlled rooms and fed with normal mouse chow (Lipton, India) and water ad libitum.

Reagents: Para rosaniline hydrochloride and α-naphthyl acetate were obtained from LOBA chemie, Mumbay. All other

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chemicals were of analytical reagent grade. Sheep red blood cells (SRBC) were collected from local slaughter house in Alsever's solution.

Drug Preparation: Neutral unilamellar liposomes of Curcumin were prepared by the method of Bangham (13).

Effect of Curcumin on haematological parameters: Balb/c mice (6 numbers/group) were treated with five doses of liposomally encapsulated Curcumin (200 µM/Kg Body Weight) intraperitoneally. Blood was collected from the caudal vein and parameters such as total WBC count (haemocytometer), differential count (Leishman stain) and haemoglobin level by the cyanhaemoglobin method (14) were recorded prior to the drug administration and continued on every third day for 30 days.

Effect of Curcumin on relative organ weights: Balb/c mice were treated with liposomally encapsulated Curcumin (200 µM/Kg Body Weight/dose) on five days and vehicle liposome alone intraperitoneally. Body weights of normal animals were recorded after the drug adminisration. Animals were sacrificed the day after the last dose of drug administration and the weight of vital organs such as liver, spleen, thymus, kidney were recorded and expressed as relative organ weights.

Effect of Curcumin on antibody titre; Two groups of Balb/c mice (6/group) were immunized with sheep red blood cells (20%, .1 ml) by intraperitoneal injection. One group of animals were injected intraperitoneally with vehicle liposome alone and the second group with liposomally encapsulated Curcumin (200 µM/Kg Body Weight/dose) on five days proir in immunization. Blood was collected from the caudal vein on every third day for one month. The serum was separated and heat inactivated at 56°C.Antibody titre was determined by the haemagglutination method (15).

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Effect of Curcumin on plaque forming cells : *Two groups of Balb/c mice (7 / group) were immunized by injecting $2.5x10^6$ SRBC intraperitoneally. One group of animals was administered liposomally encapsulated Curcumin (200 µM/Kg Body Weight/dose) intraperitoneally on five consecutive days prior to immunization. Animals were sacrificed on various days, spleens were processed and used to perform the plaque assay by the method of Jerne (16, 17).*

Effect of Curcumin on bone marrow cellularity and α-esterase activity: *Bone marrow cellularity was determined by the method of Sredni et al. (18, 19). Animals were divided into four groups (six/group). To group I animals liposomally encapsulated Curcumin was administered for five consecutive days (200 µM/Kg Body Weight/dose) intraperitoneally. Group II animals were untreated control animals. A third group of animals received single exposure of whole body irradiation (400 rads/animal). This group was also injected with liposomally encapsulated Curcumin for five days after irradiation. A fourth group of animals received a single exposure of whole body irradiation (400 rads/animals). The day after the last dose of drug, animals were sacrified and bone marrow cells were collected. The number of cells was counted and expressed as the total number of live cells/femur. From the above bone marrow preparation smears were made on clean glass slides and stained with pararosaniline and haematoxyline to determine the nonspecific esterase activity (20) by simultaneous azo dye coupling method.*

Effect of Curcumin on the phagocytic activity of peritoneal macrophages: *Three groups of animals (Balb/c mice, 3/group) were used for the analysis of phagocytic activity. Liposomally encapsulated Curcumin and liposomes alone were administered respectively to drug treated group and vehicle alone treated group (5 doses, intraperitoneally). Along with the fifth dose of the drug 0.2ml of 5% sodium caseinate*

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was administered intraperitoneally. After five days macrophages wre harvested and examined for the phagocytic activity.

Effect of Curcumin on delayed type hypersensitivity (DTH) reaction: Three groups of Balb/c mice (6 mice/group) were immunized with SRBC ($1x10^6$ cells). One group of animals was administered five doses of Curcumin (200 μM/Kg Body Weight/ dose) and another group was treated with vehicle alone prior to the antigen administration. A third group was untreated control. DTH was determined by measuring the thickness of the paw 24 hours after giving a challenging dose of the antigen according to the method of Langarange et al (22).

RESULTS

Effect of Curcumin on the haematological parameters: Administration of Curcumin increased the total WBC counts in Balb/c mice. As shown in figure I the maximum count (15,290 cells/cc) was observed on the 12th day after drug administration. There was no significant difference in the haemoglobin level and body weights of animals before and after treatment. In the group of vehicle alone the treated animals maximum WBC count was observed on the 18th day (11,220).

Effect of Curcumin on organ weights: The weight of the internal organs of mice after Curcumin treatment is given in table I. The Curcumin treated group spleen and thymus weights were found to be increased significantly ($P<0.001$). Thymus weight was increased to 74.05% in the Curcumin treated group. Administration of vehicle alone to animals did not show any effect on the relative organ weights.

Effect of Curcumin of circulating antibody titre: As shown in Table II circulating antibody titres were increased in

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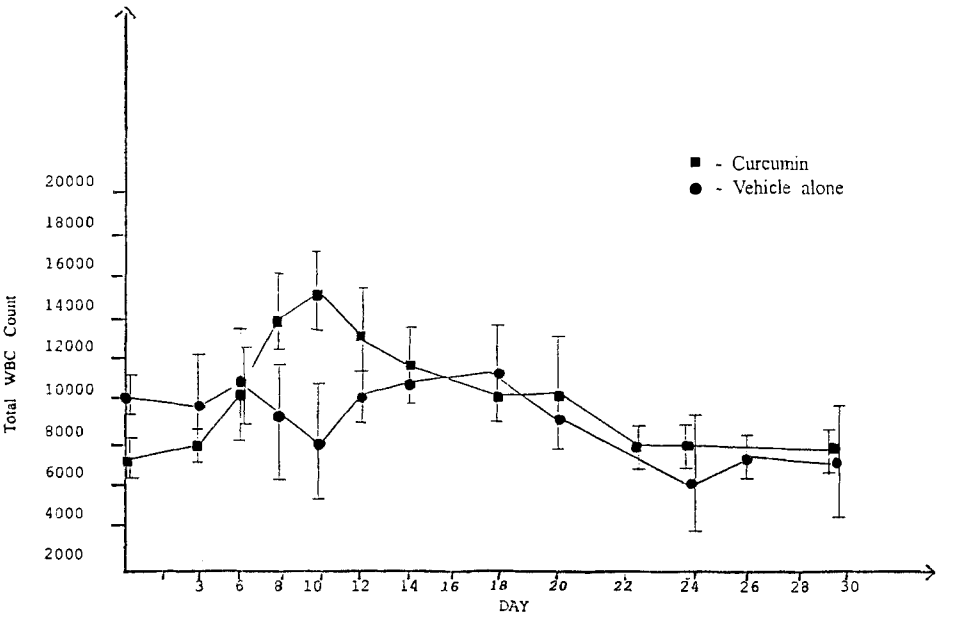

FIGURE I. Effect of Curcumin on total WBC count.
●—● verticle alone; ■—■ Curcumin

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the Curcumin treated animals compared to control animals. Maximum titre value (512) was observed on 9th day after antigen administration. In the vehicle alone treated group maximum titre (64) was similar to that of the control animals.

Effect of Curcumin on plaque-forming cells: *There was a significant increase in the number of plaque-forming cells in Curcumin treated group of animals (Fig II). The number of plaque-forming cells was increased to 1,130 PFC/10^6 spleen cells (5th day) in treated group of animals whereas the maximum number of plaque-forming cells in the untreated control animals was only 307.5 PFC/10^6 spleen cells.*

Effect of Curcumin on bone marrow cells and -esterase positive cells: *Effect of Curcumin on the bone marrow*

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TABLE I

Effect of Curcumin on relative organ weights

	Relative organ weights (gm/100gm) Treatment			% increase in organ weights after Curcumin
	Normal	Vehicle alone	Curcumin	
Spleen	0.41 ±.12	0.38 ±.02	0.50 ±.02	*22.03
Thymus	0.158 ±.03	0.23 ±.02	0.28 ±.03	* 74.05
Liver	4.67 ±.46	3.26 ±.41	4.94 ±.1	5.78
Kidney	1.42 ±.11	1.39 ±.11	1.49 ±.15	4.92

Animals were treated with five doses of Curcumin (lipsomally encapsulated, 200 μM/Kg Body Weight / dose) and vehicle alone. Next day after fifth dose animals wre sacrificed.

** P<0.001 - Significance from untreated.*

TABLE II

Effect of Curcumin on Antibody titre

Treatment modality	Antibody titre Days after treatment								
	3	6	9	12	15	18	21	24	27
Control	16	32	32	32	64	32	16	16	16
Vehicle	8	16	16	32	64	64	32	16	16
Curcumin	128	128	512	128	128	128	64	64	16

All the animals were immunized with sRBC (2%; 0.1 ml). Treated animals received Curcumin liposome (200 μM/Kg Body Weight/animal) on five consecutive days.

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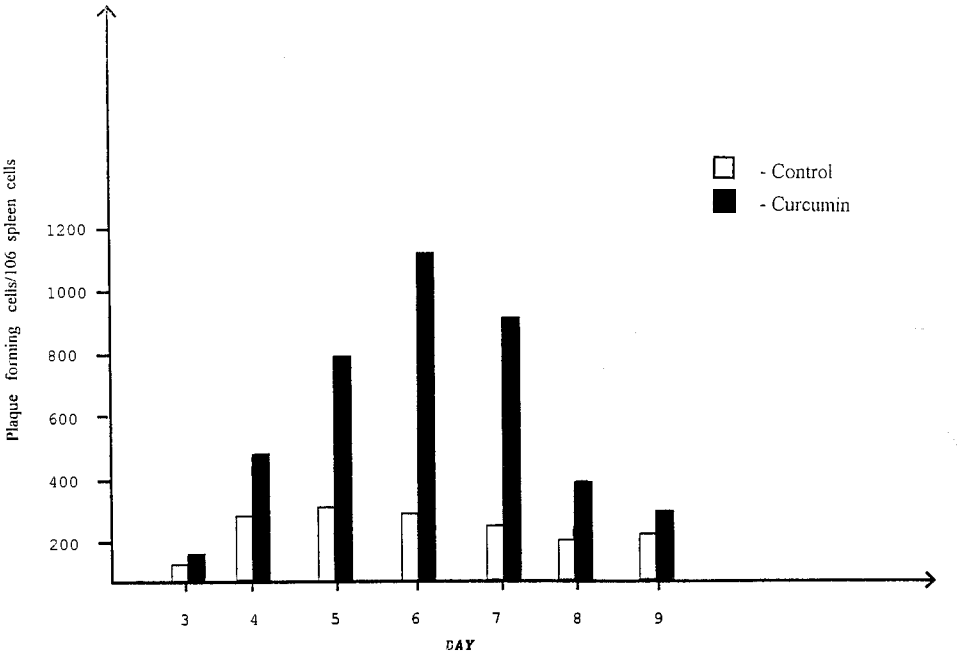

FIGURE II. Effect of Curcumin on plaque forming cells. □ *Contral;* ■ *Curcumin*

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cellularity and -esterase positive cells is given in Table III. Curcumin treated animals showed an increase in the number of bone marrow cells ($16.9x10^6$ cells/femur) compared to normal $12.9x10^6$ cells/femur). The number of α-esterase positive cells were also increased significantly ($P<0.001$) in the treated group (1,622/4,000 cells) compared to controls (1,205/4,000 cells). In the irradiated animals Curcumin treatment enhanced the cellular immune functions as shown in Table III. There was an increase in the bone marrow cell count ($10x10^6$ cells/femur) and α-esterase positive cells (434/4,000 cells) compared to control irradiated animals ($5x10^6$ cells/femur and 245/4,000 α-esterase positive cells).

Effect of Curcumin on the phagocytic activity of peritoneal macrophages: *As shown in Table IV administration of*

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TABLE III

Effect of Curcumin on bone marrow cellularity and α-esterase positive cells

Treatment modality	α-esterase positive/4,000 cells	Bone marrow cellularity
Normal	1,205 ±7.2	$12.9x10^6$
Curcumin	*1,622±30.4	$16.9x10^6$
Radiation	245±29.8	$5x10^6$
Radiation and Curcumin	*434±60.8	$10x10^6$

Treated animals received five doses of Curcumin (200 μM/Kg Body wt).

* P<0.001, Significance from untreated.

TABLE IV

Effect Curcumin on phagocytic activity of peritoneal macrophages

Treatment modality	Average number of pigmented macrophages/200 cells	% increase in phagocytic activity
Normal	42±2.8	
Curcumin	*71±2.9	*69.04

Treated animals received five doses of Curcumin (200 μM/Kg Body Weight/dose) for five days.

*P<0.001 , Significance as compared with untreated.

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TABLE V

Effect of Curcumin on delayed type hypersensitivity

Treatment modality	Differences in paw thickness (mm)	% inhibition of DTH
Control	0.166	
Vehicle	0.166	Nil
Curcumin	0.1	*39.75

All the animals were sensitized with SRBC ($1x10^6$) and treated animals received five doses of Curcumin (200 μM/Kg Body Weight).

*P<0.001 , Significance from untreated.

Curcumin enhanced the phagocytic activity of peritoneal macrophages. Numbers of macrophages with engulfed SRBC were significantly increased (P<0.001) in the treated group (71/200 cells) compared to the control group (42/200 cells).

Effect of Curcumin on delayed type hypersensitivity: In the case of Curcumin treated animals there was 39.75% inhibition of delayed type hyper sensitivity reaction (Table V). The group of animals treated with vehicle alone showed a difference of paw thickness similar to that of control animals.

DISCUSSION

Turmeric (Curcuma longa) tubers contain curcuminoids at a very high concentration (4-8%) of their dry weight. Topical application of Curcumin inhibits TPA induced epidermal DNA synthesis, tumour promotion in mouse skin and oedema of

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mouse ears(6). While the activity of Curcumin mainly resides in the conjugated diene moiety present in Curcumin, part of the activity has been ascribed to the phenolic form. Curcumin is a potent antiinflammatory agent in acute and chronic models of inflammation (23). Immunomodulators are agents that modify the relationship between antigen and host by modifying the host responses to antigen with resultant therapeutic effects. In recent years there is an increasing interest in the search for potential drugs, especially of plant origin, that are capable of modifying the immune responses with few or no side effects. Administration of liposomally encapsulated Curcumin has been shown to increase total WBC count and bonemarrow cell numbers. This may be due to the production of cytokines that regulate the proliferation and differentiation of bone marrow cells or directly acting on these cells. Curcumin treatment increased the α-esterase positive cells significantly which indicates its effects on proliferation of stem cells. Curcumin administration enhanced the humoral immunity as seen from the increase in antibody titre and antibody forming cells. Phagocytic activity of macrophages was found to be enhanced by Curcumin treatment. There was a significant increase in the relative organ weights of lymphoid organs such as spleen and thymus. Curcumin is known as a potent anticarcinogen and is being tried in phase I clinical trials as a chemopreventive (24). It has been reported that Curcumin inhibits lung metastasis induced by B16F10 melanoma cells (11). Recently Curcumin has been reported selectively to inhibit HIV-I LTR directed gene expression (25). Curcumin a component of food in several countries is nontoxic and harmless. The results presented in this study indicate the immunostimulating effects of Curcumin. The cytocidal and anticarcinogenic action of Curcumin combined with its immunopotenientiating activity makes it a potential drug for cancer therapy.

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