International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 5 (6): 333-345, 2009
ISSN 1811-7775

Comparison of Efficacy of Turmeric and Commercial Curcumin in Immunological Functions and Gene Regulation

[1]A.K. Chakravarty, [2]S.N. Chatterjee, [1]H. Yasmin and [1]T. Mazumder
[1]Immunology and Cell Biology Laboratory, Department of Zoology,
University of North Bengal, Siliguri 734013, India
[2]Seri Biotech, Ministry of Textiles, Govt. of India, Res: 11/1,
DKM Road, Calcutta, 700035, India

Abstract: Curcumin, the active constituent of turmeric possesses anti-cancer, anti-inflammatory and other properties. Earlier we have shown ethanolic extract of turmeric rhizome (ETE) activates murine lymphocytes and induces apoptosis in tumor cells. The present investigation is intended to study comparative efficacy of ETE as used by us and others and commercially available curcumin. The efficacy of these two substances was tested for immunostimulatory, anti-inflammatory and anti-oxidant properties in murine model. The expression of a few genes such as perforin, IL-2, IL-6, TNF-αand iNOS related with these events have also been studied at transcriptional level in T-cells. The ETE promoted cell division and functions of murine lymphocytes like production of NO^{-}, up regulation of perforin, IL-2 and IL-6 genes in course of functional differentiation much better than curcumin. The ETE acts better as anti-inflammatory agent in DTH reaction than curcumin. The better efficacy of ETE over curcumin could be due to presence of other compounds in the total extract than only diferuloylmethane as curcumin. The present study intends to recommend use of turmeric over curcumin whenever possible.

Key words: Ethanolic extract of turmeric rhizome, curcumin, delayed type hypersensitive reaction, reactive oxygen species, gene expression

INTRODUCTION

In Ayurveda (since Ca 1900 BC), turmeric, occupied a very important status both for curative and prophylactic measures and it is well incorporated into the culture of Indian subcontinent (Samy *et al*., 2008; Ammon and Wahl, 1991). The long list of uses of turmeric includes antiseptic (Grant, 2000; Thiemermann, 2006), anti-inflammatory (Arora *et al*., 1971; Chandra and Gupta, 1972; Singh and Aggarwal, 1995; Siddiqui *et al*., 2006; Chan, 1995; Abe *et al*., 1999; Gulucubuk *et al*., 2006; Cheshire and Baldwin, 1997; Jobin *et al*., 1999; Satoskar *et al*., 1986; Srivastava *et al*., 1995), antioxidant (Selvam *et al*., 1995; Bosca *et al*., 1997; Mukundan *et al*., 1993), antimalarial (Rasmussen *et al*., 2000; Koide *et al*., 2002), antitumor (Rafatullah *et al*., 1990; Kuttan *et al*., 1985; Sharma *et al*., 2001), anticarcinogenic (Thangapazham, *et al*., 2006; Yamamoto *et al*., 1997) and many others. Modern research has provided considerable scientific validation of these activities of turmeric for last several decades.

The yellow component of turmeric was first isolated in 1815 and was named Curcumin after its origin from *Curcuma longa* (Daube, 1870). Its chemical structure was determined by Roughley and Whiting (1973) to be a diferuloylmethane (Fig. 1). Then onwards extensive research work has been carried out with curcumin isolated from turmeric.

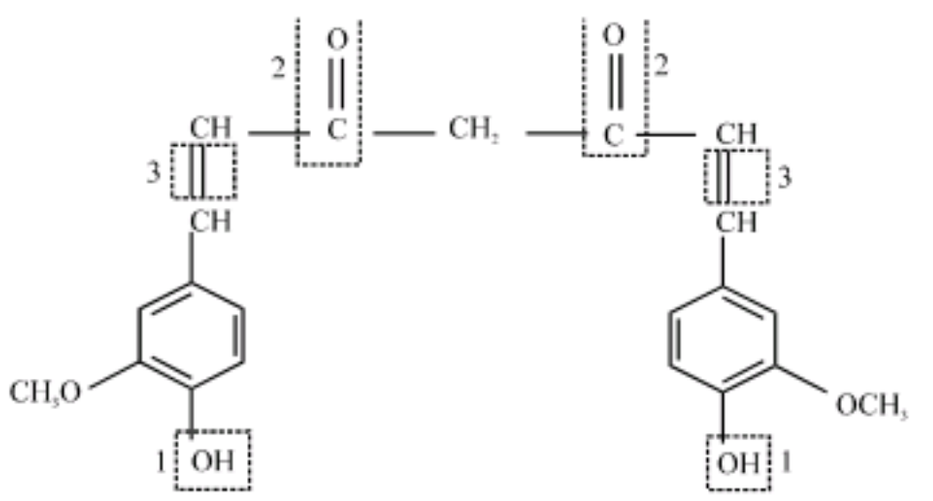

Fig. 1: Chemical structure of curcumin; 1: Parahydroxyl groups, 2: Keto groups and 3: Double bonds

Corresponding Author: Ashim K. Chakravarty, Immunology and Cell Biology Laboratory, Department of Zoology, School of Life Sciences, University of North Bengal, Siliguri-734013, West Bengal, India
Tel: +91-353-2776353

We showed Ethanolic Turmeric Extract (ETE) is inhibitory for murine fibrosarcoma and Ehlrich ascitic carcinoma c ells both *in vitro* and *in vivo* (Chakravarty *et al*., 2003; Chakravarty and Yasmin, 2005). Furthermore, we found the novelty of turmeric as pro-stimulatory for immunocompetent cells, activating T-cells for cytotoxic response against tumor cells (Chakravarty and Yasmin, 2008).

A thorough study of the comparative efficacy of total rhizome extract (ETE) as used by us and others and commercially available curcumin used by many is imperative. This was the objective of the present investigation.

The present investigation will compare the efficacy of ethanolic turmeric extract (ETE) and curcumin for immunostimulatory, anti-inflammatory and antioxidant properties in murine model. The expression of a few genes related with up and down regulation of these events has also been studied at transcriptional level in T-cells.

Perforin, a pore forming protein, plays great role in T-cell mediated cytotoxicity against targeT-cells including malignanT-cells (Henkart, 1989; Young, 1989; Tschopp and Nabholz, 1990; Kagi *et al*., 1994; Voskoboinik and Trapani, 2006). The gene encoding this pore forming molecules is not expressed in the naïve T-cells; but upon activation these molecules are produced in enhanced quantities. The level of expression of perforin gene with turmeric and curcumin treatment has been investigated. IL-2 and IL-6 belonging to type-I cytokines play major role in regulation of T-cell growth and differentiation (Stockinger and Veldhoen, 2007). That is why the expression of these two important regulatory cytokines has also been judged. TNF-α, a pleiotropic cytokine (Mace *et al*., 1988; Torisu *et al*., 2000; Rossi and Zlotnik, 2000; Balkwill and Montovani, 2001) is a powerful inducer of inflammatory response. TNF-α is one of the early genes to express with induction of various stimuli. The expression of this gene has been correlated with the degree of inflammatory reactions.

Nitric oxide is one of the important free radicals generated in activated T-cells (Niedbala *et al*., 1999, 2002). The expression of inducible nitric oxide synthase (iNOS) gene, responsible for NO generation, has also been taken into account to judge the efficacy of ETE and curcumin.

MATERIALS AND METHODS

The present investigation has been carried out at Immunology and Cell Biology Laboratory, Department of Zoology, University of North Bengal, Siliguri, India from early part of 2006 to November, 2008.

Animals: Inbred adult Swiss mice of both sexes, 8-12 weeks of age, were used for all experiments. Breeding nuclei were obtained from Indian Institute of Chemical Biology, Kolkata and are maintained with food and water *ad libitum* in our animal house.

Ethanolic turmeric extract preparation: Fresh rhizomes of turmeric (*Curcuma longa* L.) were obtained from the local market. After cleaning properly with water; 10 g of sample was crushed to a paste with mortar and pestle and 10 mL absolute alcohol was added to the paste and kept in a refrigerator at 4°C for overnight. The extract was then filtered through Whatman filter paper 1 and the filtrate was refiltered again through cellulose acetate Millipore filter paper (0.2 μm porosity, Sartorius) for sterilization and final solution obtained was aliquoted and stored at 4°C. To ascertain the dry weight of ETE, a volume of ETE in each batch was evaporated to dryness under reduced pressure (Rotary Vaccum, EYELA, Japan) at 55°C. The average dry weight of ETE was 0.435±0.032 mg mL^{-1}. As suggested by our earlier study (Chakravarty *et al*., 2003; Chakravarty and Yasmin, 2005) 25 μL dose of ETE per animal was used in the present study. The equivalent amount of ethanol (25 μL) was used for control and this protocol was maintained for all the experiments.

Preparation of curcumin solution: Curcumin (mol wt. 368.39) was purchased from Acros Organic, New Jersey, USA (MFCD00008365). Curcumin was dissolved in ethanol at 10 μM concentration, found to be most effective (Chen *et al*., 2004, 2006; Yasmin, 2007) and 25 μL per animal was used in all the experiments.

Immunological assays

Separation of T-lymphocytes: Ficoll and Hypaque gradient was employed for the separation of lymphocytes from the total cell suspension (Berger and Edelson, 1979; Tamul *et al*., 1995). Spleen and lymph node cell suspensions in 3 mL of PBS were layered on Ficoll and Hypaque solution (Type IV, Sigma Co., USA) and centrifuged at 3000 rpm for 10 min. The band of lymphocytes at the junction of Ficoll Hypaque and PBS was taken out and washed twice with PBS. The ficoll-hypaque purified lymphocytes were finally resuspended in prewarmed RPMI 1640 with 10% goat serum (Chaudhuri and Chakravarty, 1983). Then the lymphocyte preparation was poured on a nylon wool fiber

column for separation of B and T-cells, as outlined by Julius and co-worker (Julius *et al.*, 1973).

For preparing the column 0.1 g teased and sterilized nylon wool (Robins' Scientific Corporation, USA), soaked in RPMI was gently packed in a 1 mL syringe. Each of the columns was loaded with cell suspensions (6×10^6 lymphocytes in 1 mL) and incubated at 37°C for 1 h. Non adherent T-cells were eluted out with an excess amount of warm RPMI and re-suspended in fresh medium. T-cells were counted with the help of haemocytometer (Chakravarty and Yasmin, 2005).

Cell cycle analysis by FACS: For cell cycle analysis, mice in three batches were injected intravenously with 25 µL of ETE or curcumin or alcohol. After 24 h, spleen cell suspension was prepared in PBS. To 1 mL of cell suspension containing 10^6 cells, 1 mL of 80% ethanol was added and the cells were fixed overnight at 4°C. Fixed cells were centrifuged, the supernatant was decanted off and 0.5 µL of 500 µg mL^{-1} (Standard 250 µg mL^{-1}) RNAse A was added, followed by incubation for 45 min at 37°C. The 69 mM ethidium bromide was prepared in 38 mM sodium citrate. The cells were centrifuged and suspended in 0.5 mL of 69 mM ethidium bromide at room temperature for 30 min. Finally the cell cycle analysis was done in fluorescence activated cell sorter (FACS, Caliber, Becton Dickinson).

Delayed type hypersensitivity assay with 2,4-dinitrofluro benzene (DNFB): The delayed type hypersensitivity reaction depends on overall T-lymphocyte function. Primary sensitization of the mouse was carried out by applying 0.025 mL of 0.0001% DNFB made in acetone in the right foot pad subcutaneously and then resensitization was made with 0.025 mL of 0.000001% DNFB in the left foot pad on 8th day (Iizima and Katz, 1983). ETE or curcumin was administered intravenously 1 h prior to resensitization. The degree of erythema and induration was measured in terms of the diameter of the resensitized paw in cm from the ninth day onward. Size of the reaction spot was measured twice at right angles by a slide caliper and the average was taken as index for the reaction. The day of resensitization has been considered as 0 day for easy representation of the data.

Differential leukocyte count from DTH mice: Differential count of the leucocytes at the inflammation site of the DTH mice treated with ETE for 24 and 48 h after resensitization was made, to note the ratio of WBCs. For this, a thin and uniform film of the oozing fluid from the site of DTH reaction was prepared and stained with Leishman's stain for studying under microscope. The numbers of different types of white blood cells were expressed in percentage.

Separation of CD^{4+} helper T-cells from the spleen of DTH mice through Magnetic Assorted Cell Sorter (MACS) and their estimation: Cells of interest were specifically labeled with super paramagnetic MACS microbeads (Busch *et al.*, 2004; Matheu and Cahalan, 2007; Stanciu and Djukanovic, 2000). Briefly, lymphocytes isolated from the spleen of DTH mice after 24 and 48 h of resensitization were suspended in PBS for separating CD^{4+} helper T-cells. Cell suspension was then centrifuged at 1000 rpm for 10 min and the cell pellet was resuspended at a concentration of 10^7 cells in 80 µL of fresh PBS. To this cell suspension, 20 µL of $CD4^+$ (L3TH) microbeads (130-049-201, Miltenyi Biotech, Germany) were added and refrigerated for 15 min at 4 to 8°C. Cell suspension containing the microbeads were poured into the Magnetic Separator (MS) column. After collecting the total effluents of the unlabeled cell fractions the column was removed from the separator and placed upon a fresh collection tube. Finally, PBS was pipetted onto the MS column and the magnetic labeled cells were flushed out by firmly pushing a plunger into the column. The magnetic labeled live CD^{4+} T-cells were enumerated by trypan blue dye exclusion method with the help of a haemocytometer.

Estimation of TNF-" by ELISA: Solid phase sandwich ELISA KIT (PharMingen, USA) was utilized for the evaluation of serum TNF-α in DTH mice. Mouse TNF-α was assayed on microplates precoated with affinity purified polyclonal antibody specific for mouse TNF-α. The optical density of the plate was read at 450 nm. Concentration of TNF-α was extrapolated from the standard curve of TNF-α (Sirish-Kumar *et al.*, 2003).

Biochemical estimation of free radical

Superoxide scavenging assay: Scavenging of superoxide radical (O_2^-) has been measured in a reaction mixture containing 0.1 M phosphate buffer (pH-7.4), 0.1 mM EDTA, 50 µM hematoxylin, 25 µL of ethanolic turmeric extract (ETE) or curcumin or alcohol. The final volume of the reaction mixture was adjusted to 2.5 mL by adding double distilled water (Martin *et al.*, 1987). Superoxide radical (O_2^-) was generated from autoxidation of hematoxylin and was detected by an increasing absorbance at 560 nm wavelength in a UV-visible

spectrophotometer (ELICO, SL164). The inhibition of autoxidation of hematoxylin in presence of extract over the normal control was calculated.

Lipid peroxidation: Lipid peroxidation of lymphocytes in presence of turmeric extract was estimated according to Miller and Aust (1989). The reaction mixture containing 1×10^6 packed cells in 0.2 M phosphate buffer pH (7.4), with 20 mM Tris-HCl, 2 mM $CuCl_2$, 10 mM ascorbic acid and 25 µL of ETE/curcumin and were incubated for 1 h at 37°C in humidified atmosphere containing 5% CO_2 in air. In alcohol and normal controls, 25 µL ethanol or 25 µL double distilled water was added respectively instead of the extracts. Lipid peroxidation was measured as malonaldehyde (MDA) equivalent using TBA-TCA reagent (0.375% w/v TBA, 15% w/v TCA and 0.25 N HCl). OD value was determined spectrophotometrically at 535 nm. The percentage of inhibition of lipid peroxidation by the extracts and ethanol were calculated assuming the generation in normal control sets as maximum

Hydroxyl ion generation: Hydroxyl radical was generated from Fe^{2+}-ascorbate-EDTA-H_2O_2 system (Fentons' reaction) which attacks the deoxy D-ribose and a series of reaction that eventually resulted in the formation of malonaldehyde (MDA) (Halliwell *et al.,* 1987). The reaction mixture contained 2.8 mM 2-deoxy D-ribose, 20 mM of KH_2PO_4-KOH (pH-7.4), 100 mM $FeCl_3$, 104 µM EDTA, 1 mM H_2O_2, 1 mM ascorbic acid and 25 µL of ETE/curcumin. In control, 25 µL of ethanol was added instead of the extracts. The reaction mixture was incubated at 37°C in humidified atmosphere containing 5% CO_2 in air for 1 h. Then 2 mL of thiobarbituric acid-trichloroacetic acid (TBA-TCA) reagent was added in each tube and boiled for 15 min. The color of the reaction mixture changes to a pink MDA-TBA chromogen which was finally measured at 532 nm in UV- spectrophotometer (ELICO, S L164). The level of inhibition in hydroxyl radical generation caused by ETE, curcumin and ethanol was calculated in reference to the level in normal control sets.

Nitric Oxide Synthase (NOS) activity: The NOS activity was determined by measuring the conversion of oxyhemoglobin to methemoglobin according to Jia *et al.* (1996). Briefly 1×10^6 packed lymphocytes were incubated for 2 h with 50 mM Tris-HCl buffer (pH 7.4), 10 mM L-arginine, 64 mM hemoglobin, with 25 µL of ETE at 37°C in humidified atmosphere containing 5% CO_2 in air. After incubation, the mixture was centrifuged at 1000 rpm for 5 min and the optical density of supernatant was measured in UV-spectrophotometer (ELICO, S L164) at 535 nm. Results of NO production were expressed as pmol of NO produced/h.

To confirm that the production of NO was actually due to the activation of NOS, a competitive inhibitor of nitric oxide synthase (NOS), 10 µM N^G methyl-L-arginine acetate ester (NAME) was added in a separate set of experimental tubes.

Gene expression analysis: Expression of the genes of interest in this study has been carried out using single cell RNA phenotyping procedure as outlined by Rappolee *et al*. (1988).

RNA isolation: The RNA was isolated from murine splenic T-cells using RNeasy Mini kit (74104, Qiagen, Valencia, USA.), as per manufacturer's protocol. Briefly, 6×10^6 T-cells were homogenized with 300 µL RLT buffer and passing through a 2 mL syringe fitted with a 27 gauge needle. Three hundred microliter of 70% ethanol was added to the homogenate and collected in a spin column attached with a collection tube, supplied by the manufacturer. After a brief centrifugation for 15 sec at 10,000 rpm the collection tube was decanted and 500 µL of RW1 buffer was added to the spin column. Again the collection tube was decanted after a brief centrifugation and 500 µL of buffer RPE was added to the spin column. The column was centrifuged at 10000 rpm for 15 sec and was followed by a repeat wash with buffer RPE. Finally, the spin column was then fitted with a fresh collection tube and washed twice with 15 µL of DEPC treated water to come up with a total 30 µL RNA sample.

The concentration of RNA was measured spectrophotometrically at 400X dilution with Shimadzu UV-160, Japan. The extracted RNA was used for cDNA synthesis.

cDNA synthesis: The isolated RNA was used for First strand cDNA synthesis utilizing RevertAid™ First strand cDNA synthesis kit # K1621 from Fermentas following the manufacturer's protocol. For synthesis of first strand cDNA the primer used for PCR amplification was oligo(dT^{13}) synthesized by GMBH. cDNA constructed was stored at -20°C.

Primer utilized and amplification schedule: Primers were designed from various geneBank accession retrieved from PUBMED Data Bank as shown in Table 1, using primer program available on internet. The designed primers were synthesized by GMBH, Germany. Details of the primers are:

Primers	Accession No.	Sense (5′-3′)	Antisense (3′-5′)	T_m(°C)
Perforin	NM_011073	ACCCTGAATGGGCTCACA	GCAGCAGTCCTGGTTGGT	57.00
IL-2	NM_008366	GCGGAAGCACAGC	CATGCCGCAGAGG	50.20
IL-6	NM_031168	GGGAAATCGTGGA	AGGTTTGCCGAGT	43.90
TNF-α	NM_013693	TGGCACAGCCAAG	GGGACCCCTGCTC	52.36
iNOS	NM_010927	GCATCCCAAGTACGAGTGGT	AAGGCCAAACACAGCATACC	53.98

Table 1: Percentage of cells at different stages of cell cycle after 24 h *in vivo* treatment with ETE, curcumin and alcohol. Results are expressed as Mean±SD. The percentage of cells with ETE treatment at S phase is significantly different from the other three values at the level of $p<0.01$

	Cell cycle stages			
	G0	G1	S	G2-M
Turmeric	79.12±3.53	5.28±0.649	14.87±0.782*	0.46±0.043
Curcumin	89.45±5.667	2.43±0.418	7.38±0.692	0.49±0.072
Alcohol	76.62±4.451	14.70±1.793	8.04±0.491	0.480.0591
No treatment	84.27±2.127	10.37±2.015	4.88±1.121	0.44±0.327

The PCR was performed for 35 cycles in 30 µL reaction mixture using thermocycler (PeqLab, Germany) containing Taq DNA polymerase buffer, all four dNTPs, oligonucleotide primers, Taq DNA polymerase and cDNA products. After amplification PCR products were analyzed on 0.8% (w/v) agarose gel. The band density was quantified in reference to the known concentration of lambda DNA (30 ng) through ImageAide, Spectronics Corporation, NY.

Experimental sets and statistical analysis: In all the experiments, the responses of ETE and curcumin were compared with one set of control with same amount of ethanol and another set without any treatment. All the experiments were repeated at least thrice, each with triplicate samples. The data represents mean of all the repeat experiments and statistically analyzed with two way ANOVA method (Alin, 2006).

RESULTS

Cell cycle analysis: *In vivo* turmeric treatment for 24 h, induced proliferation of lymphocytes to an appreciable level compared to other treatment groups as revealed in FACS analysis. The percentage of cells entering into S phase was higher with ETE treatment (14.87%) compared to curcumin (7.38%); the difference was statistically significant. The count with curcumin was similar to the alcohol treated control (Table 1, Fig. 2).

Inhibition of DTH response after treatment: As ETE activates cell mediated immunity (Chakravarty *et al*., 2003; Chakravarty and Yasmin, 2005) so its effect on Delayed Type Hypersensitivity (DTH) response enacted by T-cells was also studied. The DTH was initiated upon 2,4-DNFB injection in the foot paw of mouse.

The untreated mice had the swelling all through the study period of 9 days (Fig. 3). The ETE and curcumin

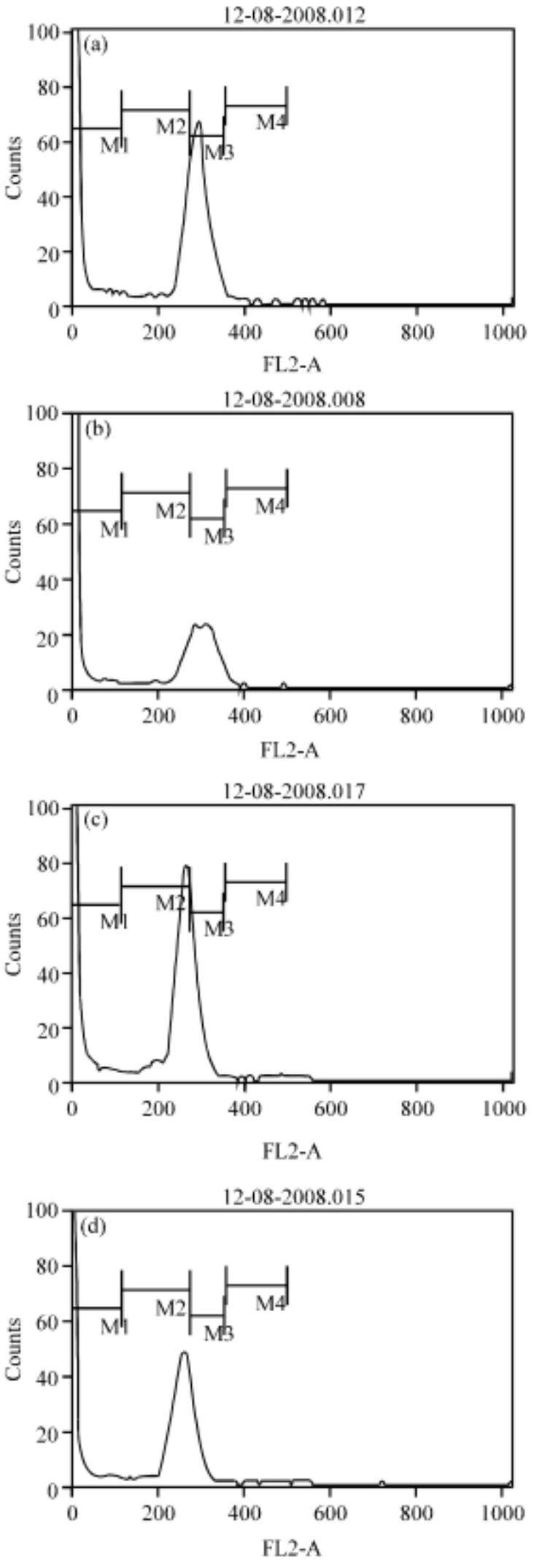

Fig. 2: DNA histograms by FACS for cell cycle analysis of splenic lymphocytes. (a) ETE treated, (b) curcumin treated, (c) alcohol treated and (d) no treatment. M1-G_0, M2-G_1, M3-S and M4-G_2

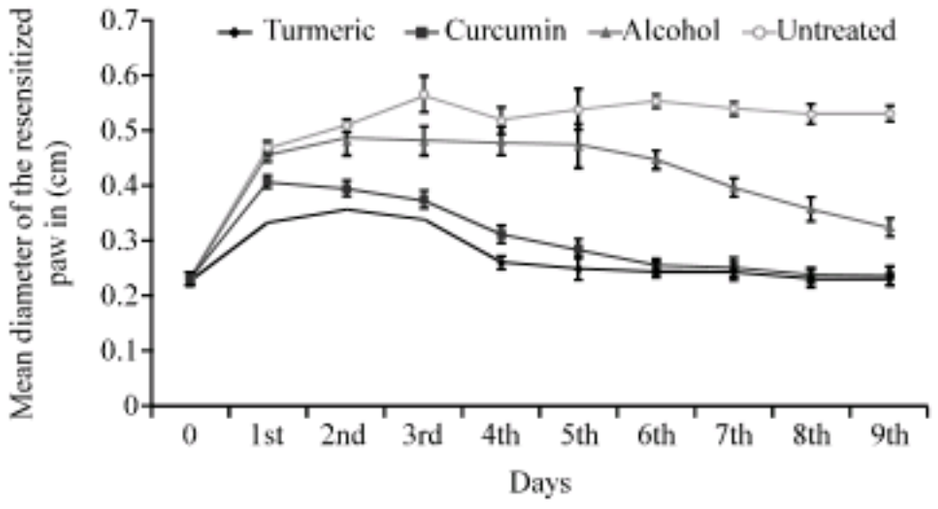

Fig. 3: Change in paw size of DTH mice with ETE and curcumin treatment given intravenously, 1 h prior to resensitization (Day of resensitization indicated by 0). There were alcohol treated and untreated controls. Results are expressed as Mean±SD

(10 μM) treatment reduced the swelling noticeably at all the points compared to the controls (Fig. 3). Severe inflammation often led to partial loss of digits and foot pad in untreated mice and foot pad in alcohol control (Fig. 4). With ETE or curcumin treatment edema subsided and the paw with digits regained normal size and appearance by the 9th day of resensitization (Fig. 4). However, in subsiding DTH response ETE seemed to do better job than curcumin (Fig. 4).

Differential count at the inflammation site of the DTH mouse after treatment: DTH reaction is mainly carried out by infiltrating lymphocytes, macrophages and neutrophils from the venous and lymphatic system at the site of inflammation. And thus to know what are the cell types that infiltrated at the site of inflammation and the effect of ETE and curcumin treatment, differential count of leucocytes was taken into account.

Increase in the percentage of lymphocytes and vis-à-vis decrease in neutrophils were noted in case of ETE and curcumin treatment than the control. Again, ETE did better in both the counts than curcumin. Although the monocyte counts remained comparable in experimental groups at 24, 48 h, an increase in the count at 48 h was observed in controls (Table 2). No appreciable change in the number of eosinophils and basophils occurred with ETE and curcumin treatment when compared to controls (Table 2).

CD^{4+} T-cell count in DTH mice after treatment: As the number of lymphocytes at the site of inflammation in mice bearing DTH response was considerably more with ETE and curcumin treatment over the alcohol and normal controls (Table 2), the contribution of CD^{4+} T-cells in this hike was studied from lymphocyte population of spleen from treated and control groups. Incidentally CD^{4+} T-cells are important participant in DTH response.

The count of CD^{4+} T-cells in spleen was indeed significantly higher in experimental groups, more so with ETE treatment (Fig. 5).

Inhibition of serum TNF-" level of DTH mice: The DTH response not only influences the recruitment of various leukocytes at the inflammatory site but also modulates secretion of various cytokines from these inflammatory cells. One such proinflammatory cytokine is TNF-α which plays significant role inflammatory reactions (Rossi and Zlotnik, 2000; Balkwill and Montovani, 2001). Quantification of it was carried out with ELISA in the present investigation.

ETE or curcumin injected intravenously 1 h prior to resensitization inhibited TNF-α production in DTH mice (Fig. 6). This is statistically significant when compared with the data of control groups.

Effect of ETE and curcumin on ROS

Inhibition of superoxide (O_2G) generation: Autoxidation of hematoxylin leads to formation of hematein and is accompanied by an increasing absorbance at 560 nm, indicating generation of O_2^- radical (Martin *et al.*, 1987). ETE could inhibit generation of O_2^- up to 58.33%. The inhibition with curcumin and alcohol were about 28 and 16% respectively (Fig. 7).

Inhibition of lipid peroxidation: Copper-ascorbate induced lipid peroxidation and malonaldehyde (MDA) production within the cell was carried out for lymphocytes. H_2O_2 generation induces lipid peroxidation in the cell membrane which in turn produces MDA. Then effect of alcohol, ETE and curcumin on lipid peroxidation and MDA production was studied. ETE inhibits generation of H_2O_2 in lymphocytes (Fig. 7) up to 57.33%. Whereas, the inhibition by curcumin was even lower than the alcohol control.

Inhibition of hydroxyl radical (OHG): The ETE also could inhibit hydroxyl radical generation upto 43% by restricting the Fenton's reaction and generation of malonaldehyde (Fig. 7). The percentage of inhibition by curcumin was comparable to that with ETE treated ones. Alcohol did not show any inhibition in OH^- radical generation.

Induction of NOG generation: The level of activity of cellular enzyme Nitric Oxide Synthase (NOS) in generation of NO from L-arginine in murine lymphocytes was maximum with ETE treatment as it could induce NO^- generation up to 17.2 pmol h^{-1}, a few fold higher than

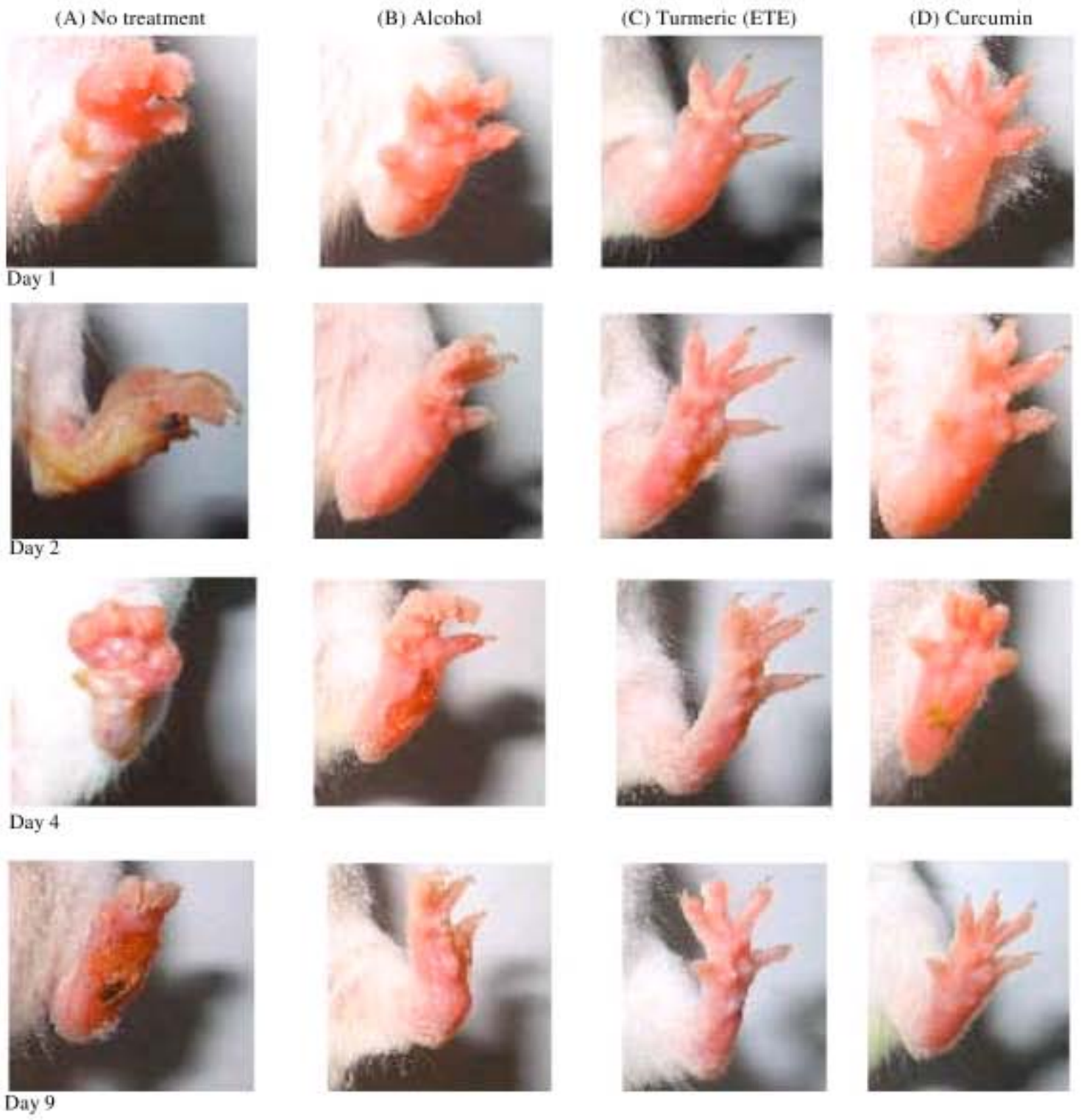

Fig. 4: Photograph showing the DTH reaction of paw in (A) untreated, (B) alcohol, (C) ETE and (D) curcumin treated mice in course of 9 days after resensitization

Table 2: Mean percentage of different leukocytes from the inflammation site of paw, after 24 and 48 h of resensitization for DTH. The ETE and alcohol were injected i.v.1 h prior to resensitization. Results are expressed as Mean±SD, * $p<0.05$ and ** $p<0.01$ compared to respective controls

	24 h			48 h		
Treatment	Neutrophil	Monocyte	Lymphocyte	Neutrophil	Monocyte	Lymphocyte
ETE	21±1.35	9.00±0.56	63±1.67**	19±1.06*	9.00±0.13*	64.0±2.45**
Curcumin	23±1.47	11.1±0.45	59±3.45**	25±1.47	13.9±0.09	53.1±1.45
Alcohol	33±1.05	16.0±0.14	42±1.03	36±2.13	24.0±0.45	33.0±2.12
Normal	39±0.97	11.0±0.23	42±1.65	42±2.24	28.0±0.42	25.0±1.98

Percentage of Eosinophil and Basophil remained in a comparable level in all the cases, in the range of 2-4%

other groups (Fig. 8). Curcumin caused no appreciable increment in NO^- generation over the control (Fig. 8).

Gene expression analysis: The level of expression of certain genes at transcription level in presence of ETE, curcumin and in control was judged by quantitating the cDNA PCR product amplified with specific primers. The quantitation was done against 30 ng of lambda DNA as standard using ImageAide, Spectronics Corporation, NY.

ETE treatment caused a heightened expression of perforin, the effector molecule to carry ouT-cell mediated immunity. It was almost double to that with curcumin which could not elicit the response beyond controls (Fig. 9, 10). Therefore, it seems that ETE mediated activation might elicit better cellular immune response. Similar results were observed in quantitation of IL-2 primer amplified PCR product, rather curcumin seems to down regulate expression of the gene (Fig. 9, 10).

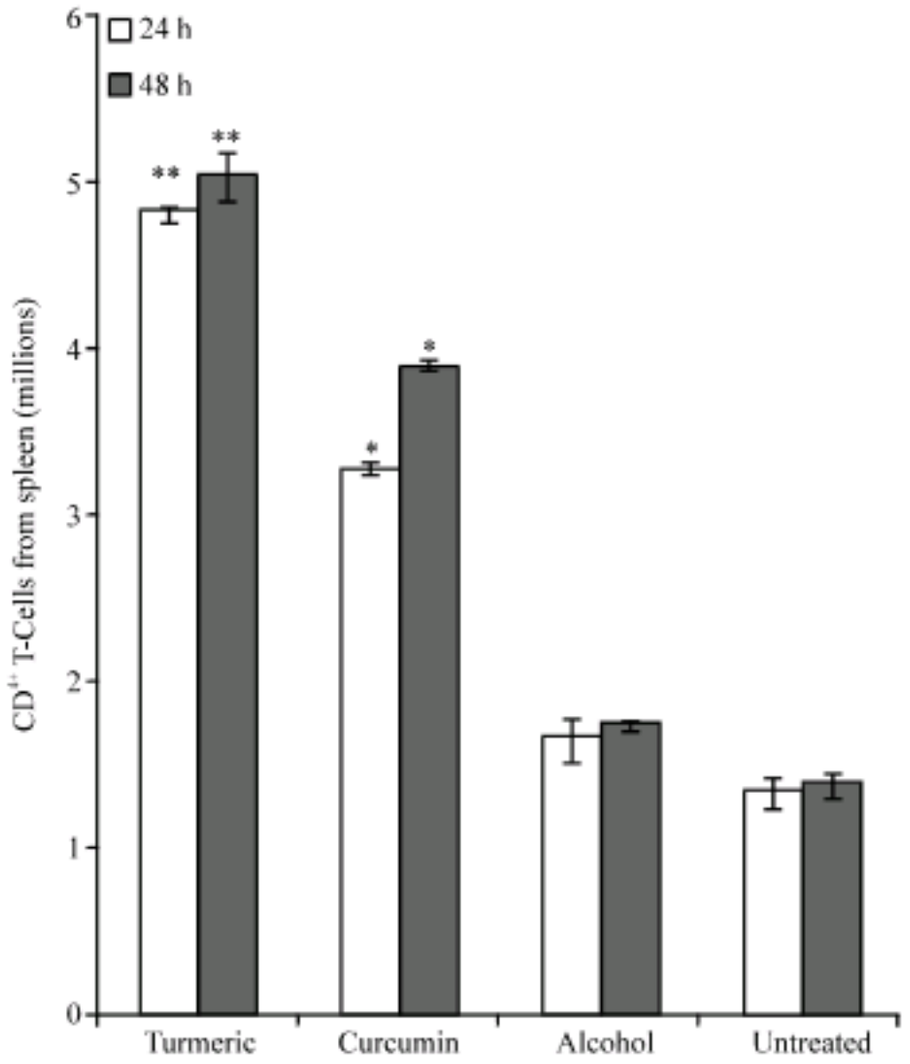

Fig. 5: Count of CD^{4+} T-cells from spleen of DTH mice after 24 and 48 h of treatment with ETE and curcumin and of control mice. Results are expressed as Mean±SD. Two way ANOVA revealed significant differences of the values with ETE ($p<0.01$) and curcumin ($p<0.05$) to the controls

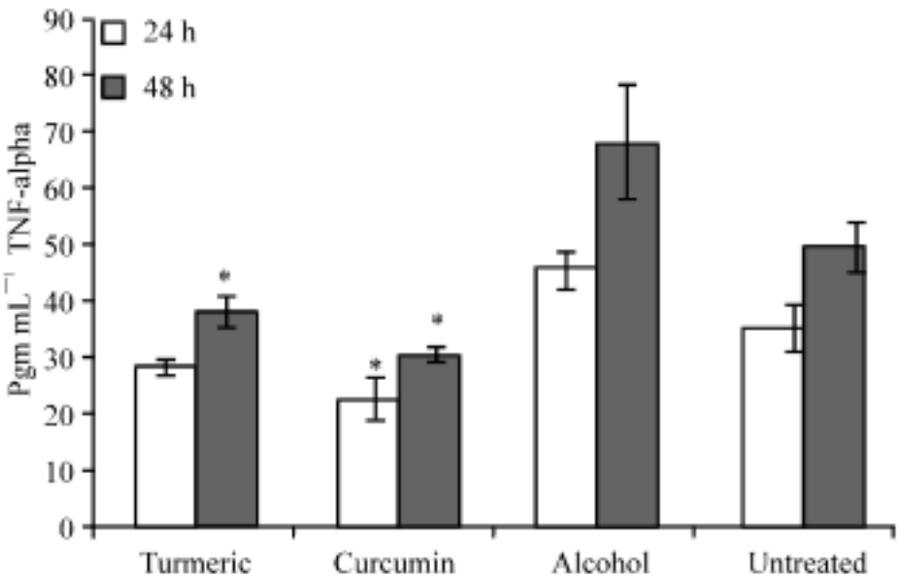

Fig. 6: Inhibition of serum TNF-α level in pg mL^{-1} by ETE and curcumin at 24 and 48 h of treatment of the mice undergoing DTH. ELISA was used for quantitation. Two way ANOVA revealed differences of the treatment values (ETE and curcumin) were significant to controls ($p<0.01$)

The higher level of expression of IL-6, another stimulatory cytokine, was observed with ETE treatment and curcumin was not effective in this count. The amount of PCR product with curcumin was comparable with the

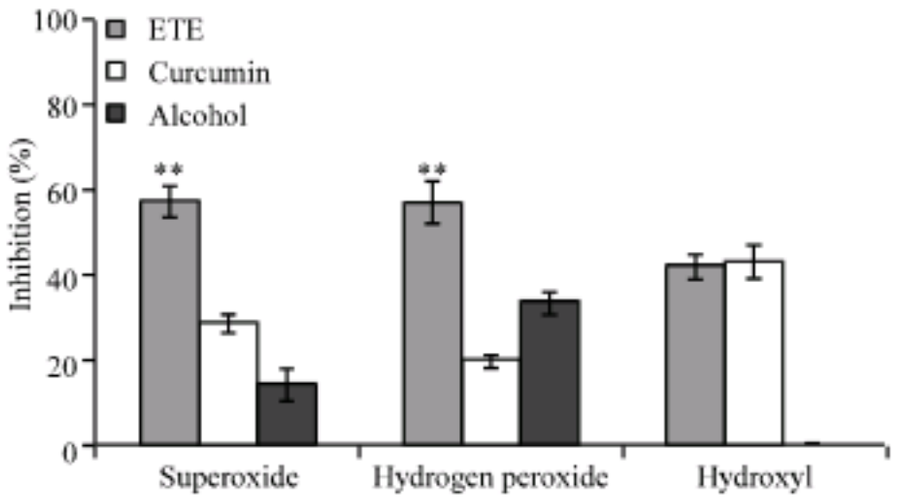

Fig. 7: Inhibition of Reactive Oxygen Species (ROS) generation by ETE, curcumin and alcohol control. In case of OH^{-} radical, alcohol could not inhibit at all, so no representation by bar. *Denotes treatment values were significant ($p<0.01$) to the controls

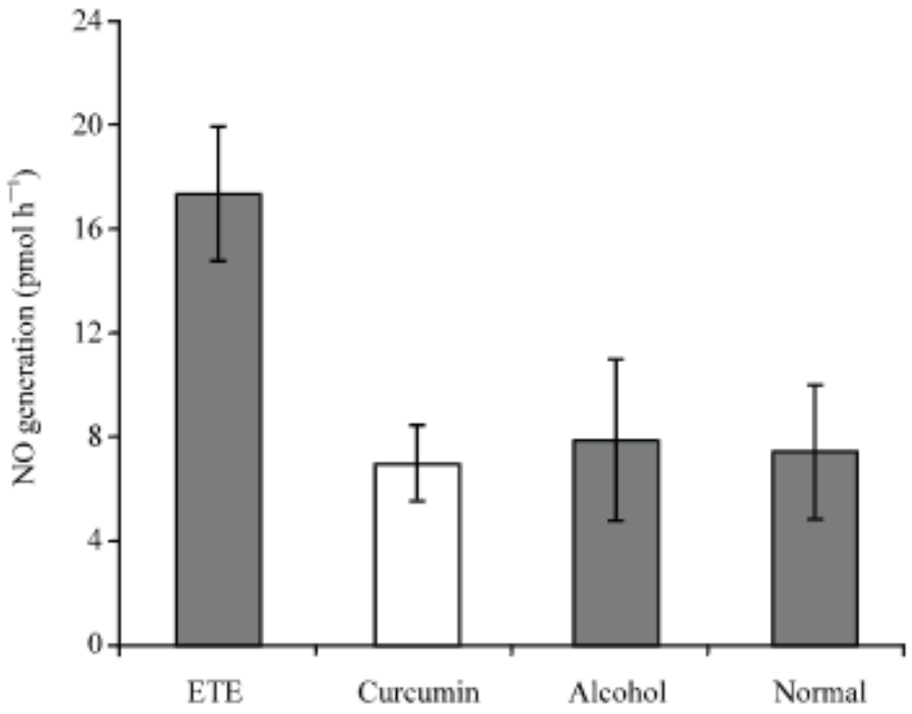

Fig. 8: Generation of NO in murine lymphocytes with ETE and curcumin treatment. Two ANOVA revealed significant difference in NO generation with ETE treatment ($p<0.01$)

control ones (Fig. 9, 10). Both ETE and curcumin inhibited the expression of TNF-α, a pro-inflammatory cytokine (Fig. 9, 10). iNOS expression with ETE and curcumin treatment were marginally better than the controls (Fig. 9, 10).

DISCUSSION

In earlier investigations we showed ethanolic extract of turmeric rhizome (ETE) activates lymphocytes and induce apoptosis in tumor cells (Chakravarty and Yasmin, 2005, 2008). Many other researchers have carried out several studies on anti-cancer property of curcumin, the

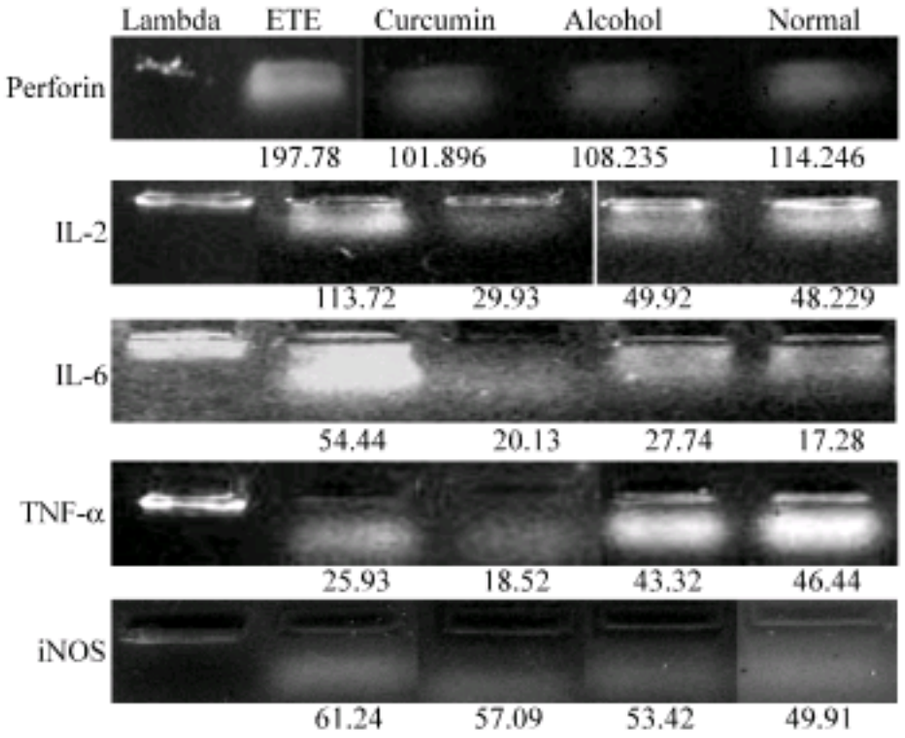

Fig. 9: Representation of mRNA expression of different genes on agarose gel

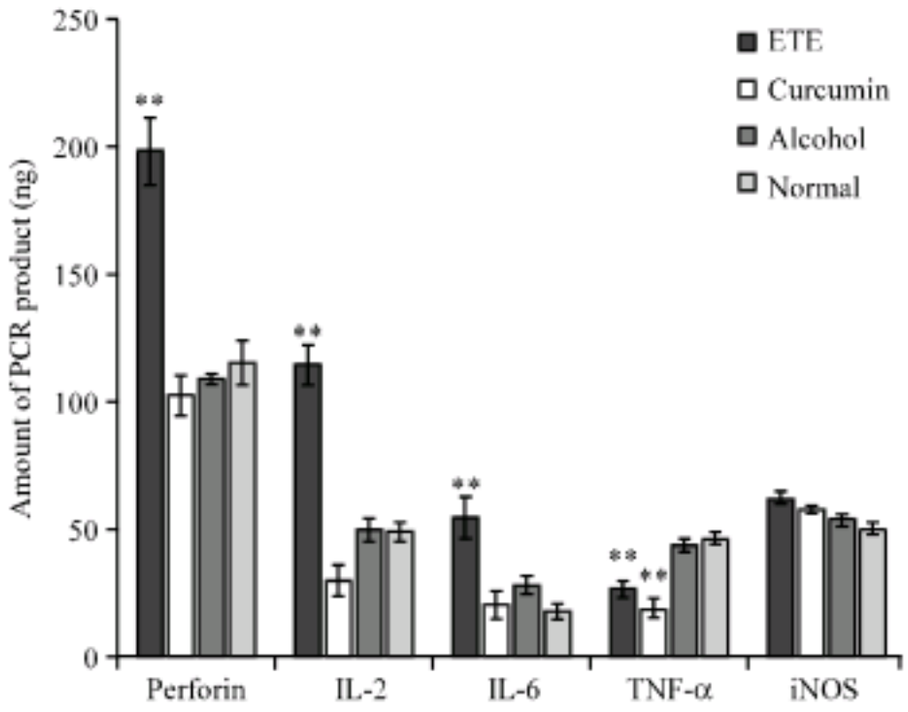

Fig. 10: Graphical representation of expression of different genes with ETE and curcumin treatment compared to necessary controls. Significant changes with ETE or curcumin treatment have marked *($p<0.01$)

active constituent present in turmeric (Aggarwal *et al.*, 2003, 2004, 2006; Anto *et al.*, 2002; Bharti *et al.*, 2003a, b; Choudhuri *et al.*, 2002, 2005).

In the present investigation, the cell cycle analysis data shows ETE can drive murine lymphocytes towards division better than curcumin (Table 1, Fig. 2). That is why the percentage of cells driven to synthetic or S phase with turmeric is almost double to that induced with curcumin. We and others showed earlier that division of lymphocytes is prerequisite for functional differentiation of the cells (Chakravarty and Clark, 1977; Das and Chakravarty, 1997). Marked differences in stimulation by ETE and curcumin is also reflected in functional acumen of the activated cells in expression of different genes, discussed later.

The ETE and curcumin were also found to inhibit delayed type hypersensitive reaction and to help in regaining normalcy of foot pad; again ETE being superior (Fig. 3, 4). The ETE inhibited inflammatory phagocytic cell recruitment at inflammation site (Table 2) slightly better than curcumin. This might help in subsiding the inflammation related injury at DTH site, foot paw in our experiments. The ETE and curcumin treatment of mice undergoing DTH caused higher CD^{4+} T-cell in splenic population of lymphocytes (Fig. 5); in this account also ETE was better performer. Normally T_{DTH} cells belong to CD^{4+} type and contribute to the production of TNF-α. TNF-α is the most important among different cytokines playing crucial ole in developing inflammation reactions. Our results suggest increase in number of CD^{4+}T-cells may not necessarily contribute towards inflammation or production of TNF-α. The level of this cytokine in serum was down along with inhibition of inflammation with both ETE and curcumin treatment as estimated by ELISA (Fig. 6). In this context, down regulation of the expression of TNF-α gene by ETE and curcumin as evidenced in our study (Fig. 9, 10) seems to be immensely meaningful.

The effect of turmeric (ETE) and curcumin on generation of Reactive Oxygen Species (ROS) namely superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl (OH^-) and nitric oxide (NO^-) radicals has been looked into. The ROS play crucial roles in developing various pathophysiological conditions including inflammation and cancer (Bandhopadhy *et al.*, 1999; Fruehauf and Meyskens Jr., 2007). The percentage of inhibition in O_2^- generation with ETE treatment was almost double to that of curcumin treated ones (Fig. 7). The ETE also could inhibit $CuCl_2$-ascorbate derived H_2O_2 generation in murine lymphocytes by higher percentage over control; whereas the percentage of inhibition with curcumin treatment was even lower than the alcohol control (Fig. 7). Inhibition of OH^- radical generation by ETE and curcumin as measured in Fentons' reaction were comparable (Fig. 7). Thus, ETE is found to be effective anti-oxidant, at times better than curcumin.

NO^- is pleiotropic molecule that acts as a messenger to mediate diverse biological functions including cell division and differentiation (Niedbala *et al.*, 1999, 2002). The ETE induced NO generation in murine lymphocytes (Fig. 8) and up regulated inducible nitric oxide synthase (iNOS) gene in splenic T-cells (Fig. 9, 10). Increase in the NO production might contribute towards overall differentiation of lymphocytes for better production of cytokines like IL-2, IL-6 and perforin molecules.

Thus ETE was found to be promoter of cell division and differentiation of murine lymphocytes, allowing them to secrete cytokines and to produce pore forming proteins

for cytolytic ability. In all these events ETE performed better than commercial curcumin. The reflection of better efficacy of ETE over curcumin was also observed when we measured the expression of concerned genes, such as IL-2, IL-6 and perforin in assay with PCR product.

For the expression of IL-2, IL-6 and perforin curcumin could not elicit response beyond controls. This seems notable to establish superiority of ETE in induction of certain immunologically important genes over curcumin. Gao *et al*. (2004) and Xiaexhung and Xiaochung (2005) also showed curcumin to suppress development of cytotoxic T-cells (CTLs) and production of IL-2 in splenic T-cells.

Both ETE and curcumin down regulated TNF-α expression in mouse splenic T-cells (Fig. 9, 10) clearly indicating their anti-inflammatory property. The results presented in Fig. 3 and 4 documented anti-inflammatory property of ETE and curcumin.

The present investigation allows recommending the use of turmeric for better result where curcumin has already been found to be effective. Therefore, a question will be why ETE performs better than curcumin in all the aspects investigated. Purification of curcumin eliminates several substances present in the whole extract of turmeric. Main component in curcumin is diferuloylmethane whereas whole extract of turmeric contains curcumin plus an antioxidant protein β-turmerin (Smitha *et al*., 2009), fats, minerals, carbohydrates, complex arabinogalactan and 2-7% essential oil. The essential oil consists of different sesquiterpenes viz., turmerone, ar-turmerone, zingiberene etc. (Leela *et al*., 2002). Some researchers observed anti-microbial (Miguel *et al*., 2002) and anti-inflammatory, wound healing, insecticidal activities (Negi *et al*., 1999) of this volatile curcuma oil which is essentially get lost in course of extraction of curcumin. Dohare *et al*. (2008) have found the curcuma oil to reduce nitrosative and oxidative stress. Raina *et al*. (2005), Srimal (1997), Nigam and Ahmad (1990) showed pinenes, eugenol and limonene in the turmeric essential oil participate specifically in the anti-inflammatory, anti-tumor activities. These components of whole rhizome extract of turmeric, some having specific anti-inflammatory and other biological activities, are left out in course of purification of commercial curcumin. These compounds in all likeliness have additive role for the immunotherapeutic value of ETE over curcumin.

The findings of this study provide a rationale for the general belief with Ayurvedic practitioners that the crude extract acts better than the purified compound from herbal sources in curing human ailments. An herbal medicine may not signify a compound, rather it includes several minor chemical components along with a main functional compound, as diferuloylmethane being main component in turmeric. Interestingly the minor components seem to act in conjunction with the major one. This may be taken into consideration to work with a bioactive fraction from herbal source.

The use of total turmeric rhizome as we find in Indian subcontinent for cooking and taking directly for medicinal purposes is lot easier and cost effective when one considers the commercial value of curcumin around $20 for 20 capsules. We may add that, it is an age old common practice in India to have oral uptake of 5-10 g of turmeric rhizome with molasses in the morning in empty stomach; the present findings suggest rationale for this practice and recommend use of turmeric over curcumin whenever possible.

ACKNOWLEDGMENTS

Authors are thankful to the University Grants Commission (UGC), India and Indian Council of Medical Research (ICMR), India for their financial assistance. Authors also express their heartfelt gratitude to Dr. Santu Bandyopahyay, IICB, Klkata, Dr. Arnab Sen, Department of Botany, NBU and Dr. Ranadhir Chakraborty, Department of Biotechnology, NBU to let us avail instrumental facilities in their laboratories. Researchers are also thankful to Dr. Bholanath Paul, IITR, Lucknow, India for providing valuable chemicals to carry out ELISA in his Laboratory.

REFERENCES

Abe, Y., Y. Hashimoto and T. Horic, 1999. Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. Pharmacol. Res., 39: 41-47.

Aggarwal, B.B., A. Kumar and A.C. Bharti, 2003. Anticancer potential of curcumin: Preclinical and clinical studies. Anticancer Res., 23: 363-398.

Aggarwal, S., Y. Takada, S. Singh, J.N. Myers and B.B. Aggarwal, 2004. Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor kappa B signaling. Int. J. Cancer, 111: 679-692.

Aggarwal, S., H. Ichikawa, Y. Takada, S.K. Sandur, S. Shishodia and B.B. Aggarwal, 2006. Curcumin (Diferuloylmethane) down-regulates expression of cell proliferation and antiapoptotic and metastatic gene products through suppression of IkBá kinase and Akt activation. Mol. Pharmacol., 69: 195-206.

Alin, A., 2006. Testing non-additivity (interaction) in two-way anova table with no replication. Stat. Methods Med. Res., 15: 63-85.

Ammon, H.P. and M.A. Wahl, 1991. Pharmacology of *Curcuma longa*. Planta Med., 57: 1-7.

Anto, R.J., A. Mukhopadhyay, K. Denning and B.B. Aggarwal, 2002. Curcumin (diferuloylmethane) induces apoptosis through activation of caspase-8, BID cleavage and cytochrome c release: Its suppression by ectopic expression of Bcl-2 and Bcl-xl. Carcinogenesis, 23: 143-150.

Arora, R.B., N. Basu, V. Kapoor and A.P. Jain, 1971. Anti-inflammatory studies on *Curcuma longa* (turmeric). Indian J. Med. Res., 59: 1289-1295.

Balkwill, F. and A. Mantovani, 2001. Inflammation and cancer: Back to virchow? Lancet, 357: 539-545.

Bandhopadhy, U., D. Das and K.R. Banerjee, 1999. Reactive oxygen species: Oxidative damage and pathogenesis. Curr. Sci., 77: 658-665.

Berger, C.L. and R.L. Edelson, 1979. Comparison of lymphocyte function after isolation by ficoll hypaque flotation or elutriation. J. Invest. Dermatol., 73: 231-235.

Bharti, A.C., N. Donato and B.B. Aggarwal, 2003a. Curcumin (diferuloylmethane) inhibits constitutive and IL-6-inducible STAT3 phosphorylation in human multiple myeloma cells. J. Immunol., 171: 3863-3871.

Bharti, A.C., N. Donato, S. Singh and B.B. Aggarwal, 2003b. Curcumin (diferuloylmethane) down-regulates the constitutive activation of nuclear factor-kappa B and IkappaBalpha kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis. Blood, 101: 1053-1062.

Bosca, A.R., M.A. Carrion-Gutierrez, A. Soler, C. Puerta and A. Diez *et al*., 1997. Effects of the antioxidant turmeric on lipoprotein peroxides: Implications for the prevention of atherosclerosis. Age, 20: 165-168.

Busch, R., D. Cesar, D. Higuera-Alhino, T. Gee, M.K. Hellerstein and J.M. McCune, 2004. Isolation of peripheral blood $CD4^+$ T cells using RosetteSep™ and MACS™ for studies of DNA turnover by deuterium labeling. J. Immunol. Methods, 286: 97-109.

Chakravarty, A.K. and W.R. Clark, 1977. Lectin driven maturation of cytotoxic effector cells: The nature of effector memory. J. Exp. Med., 146: 230-240.

Chakravarty, A.K., H. Yasmin and S.K. Das, 2003. Two way efficacy of alcoholic turmeric extract: Stimulatory for murine lymphocytes and inhibitory for ascitic fibrosarcoma. Pharm. Biol., 42: 217-224.

Chakravarty, A.K. and H. Yasmin, 2005. Alcoholic turmeric extract simultaneously activating murine lymphocytes and inducing apoptosis of Ehrlich's Ascitic Carcinoma cells. Int. Immunopharmacol., 5: 1574-1581.

Chakravarty, A.K. and H. Yasmin, 2008. Free radical scavenging and nitric oxide synthase activation in murine lymphocytes and Ehrlich ascitic carcinoma cells treated with ethanolic extract of turmeric. Proc. Natl. Acad. Sci. India, 78: 37-44.

Chan, M.M., 1995. Inhibition of tumor necrosis factor by curcumin, a phytochemical. Biochem. Pharmacol., 49: 1551-1556.

Chandra, D. and S.S. Gupta, 1972. Anti-inflammatory and anti-arthritic activity of volatile oil of *Curcuma longa* (Haldi). Indian J. Med. Res., 60: 138-142.

Chaudhuri, T.K. and A.K. Chakravarty, 1983. Goat serum as a substitute for fetal calf serum in *in vitro* culture of murine lymphocytes. Indian J. Exp. Biol., 21: 494-496.

Chen, H.W., S.L. Yu, J.J. Chen, H.N. Li and Y.C. Lin *et al*., 2004. Anti-invasive gene expression profile of curcumin in lung adenocarcinoma based on a high throughput microarray analysis. Mol. Pharmacol., 65: 99-110.

Chen, A., J. Xu and A.C. Johnson, 2006. Curcumin inhibits human colon cancer cell growth by suppressing gene expression of epidermal growth factor receptor through reducing the activity of transcription factor Egr-1. Oncogene, 25: 278-287.

Cheshire, J.L. and A.S. Baldwin Jr., 1997. Synergistic activation of NF-B by tumor necrosis factor alpha and gamma interferon via enhanced IkBα degradation and de novo IkBβ degradation. Mol. Cell Biol., 17: 6746-6754.

Choudhuri, T., S. Pal, M.L. Aggarwal, T. Das and G. Sa, 2002. Curcumin induces apoptosis in human breast cancer cells through p53-dependent Bax induction. FEBS Lett., 512: 334-340.

Choudhuri, T., S. Pal, T. Das and G. Sa, 2005. Curcumin selectively induces apoptosis in deregulated cyclin D1 expressed cells at G2 phase of cell cycle in p53-dependent manner. J. Biol. Chem., 280: 20059-20068.

Das, S.K. and A.K. Chakravarty, 1997. Characterization of primed lymphocytes on the basis of sensitivity of chromatin to Dnase I. Indian J. Exp. Biol., 54: 1156-1160.

Daube, F.V., 1870. Uber curcumin, den farbstoff der curcumawurzel. Berichte Deutschen Chemischen Gesellschaft, 3: 609-613.

Dohare, P., P. Garg, U. Sharma, N.R. Jagannathan and M. Ray, 2008. Neuroprotective efficacy and therapeutic window of curcuma oil: In rat embolic stroke model. BMC Complementary Alternative Med., 8: 55-74.

Fruehauf, J.P. and F.L. Meyskens Jr., 2007. Reactive oxygen species: A breath of life or death?. Clin Cancer Res., 13: 789-794.

Gao, X., J. Kuo, H. Jiang, D. Deeb and Y. Liu *et al*., 2004. Immunomodulatory activity of curcumin: Suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity and cytokine production *in vitro*. Biochem. Pharmacol., 68: 51-61.

Grant, K.L., 2000. Alternative therapies: Turmeric. Am. J. Health Syst. Pharm., 57: 1121-1122.

Gulucubuk, A., K. Altunatmaz, K. Sonmez, D. Haktanir-Yatkin and H. Uzun *et al*., 2006. Effects of curcumin on tumor necrosis factor-α and interleukin-6 in the late phase of experimental acute pancreatitis. J. Vet. Med. Ser. A, 53: 49-54.

Halliwell, B., J.M.C. Gutteridge and O.I. Aruoma, 1987. The deoxyribose method, a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem., 165: 215-219.

Henkart, P.A., 1989. Mechanisms of lymphocyte-mediated cytotoxicity. Annu. Rev. Immunol., 3: 31-58.

Iizima, M. and S.I. Katz, 1983. Specific immunologic tolerance to dinitrofluorobenzene following topical application of dinitrothyocyanobenzene: Mulation by suppressor T-cells. J. Invest. Dermatol., 81: 325-330.

Jia, L., C. Bonaventua, J. Bonaventura and J.S. Stamler, 1996. S-nitrosohemoglobulin: A dynamic activity of blood involved in vascular control. Nature, 380: 221-226.

Jobin, C., C.A. Bradhan, M.P. Russo, B. Juma, A.S. Narula and D.A. Brenner, 1999. Curcumin blocks cytokine mediated NF-kB activation and proinflammatory gene expression by inhibiting factor IkB activity. J. Immunol., 163: 3474-3483.

Julius, M., E. Simpson and A.L. Herzenberg, 1973. A rapid method for isolation of functional thymus derived murine lymphocytes. Eur. J. Immuno., 3: 645-649.

Kagi, D., B. Ledermann, K. Burki, P. Seiler and B. Odermatt *et al*., 1994. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. Nature, 369: 31-37.

Koide, T., M. Nose, Y. Ogihara, Y. Yabu and N. Ohta, 2002. Leishmanicidal effect of curcumin *in vitro*. Biol. Pharm. Bull., 25: 131-133.

Kuttan, R., P. Bhanumathy, K. Nirmala and M.C. George, 1985. Potential anticancer activity of turmeric (*Curcuma longa*). Cancer Lett., 29: 197-202.

Leela, N.K., A. Tava, P.M. Shafi, S.P. John and B. Chempakam, 2002. Chemical composition of essential oils of turmeric (*Curcuma longa* L.). Acta Pharm., 52: 137-141.

Mace, F.K., J. Ehrke, K. Hori and D.L. Maccubbin, 1988. Role of tumor necrosis factor in macrophage activation and tumoricidal activity. Cancer Res., 48: 5427-5432.

Martin, J.P., M. Dailey and E. Sugarman, 1987. Negative and positive assays of superoxide dismutase based on hematoxilin autoxidation. Arch. Biochem. Biophys., 255: 329-336.

Matheu, M.P. and M.D. Cahalan, 2007. Isolation of CD^{4+} T cells from mouse lymph nodes using miltenyi MACS purification. J. Vis. Exp., 2007: 409-409.

Miguel, J., A. Bernd, J.M. Sempere, J. Diaz-Alperi and A. Ramirez, 2002. The curcuma anti-oxidants: Pharmacological effects and prospects for future clinical use. A review. Arch. Gerontol. Geriatr., 34: 37-46.

Miller, D.M. and S.D. Aust, 1989. Studies of ascorbate dependent, iron-catalyzed lipid peroxidation. Arch. Biochem. Biophys., 271: 113-119.

Mukundan, M.A., M.C. Chacko, V.V. Annapurna and K. Krishnaswammy, 1993. Effect to turmeric and curcumin on BP-DNA adducts. Carcinogenesis, 14: 493-496.

Negi, P.S., G.K. Jayaprakash, L. Jagan-Mohan-Rao and K.K. Sakaria, 1999. Anti-bacterial activity of turmeric oil: A byproduct from curcumin manufacture. J. Agric. Food Chem., 47: 4297-4300.

Niedbala, W., X.Q. Wei, D. Piedrafita, D. Xu and F.Y. Liew, 1999. Effects of nitric oxide on the induction and differentiation of Th1 cells. Eur. J. Immunol., 29: 2498-2505.

Niedbala, W., X.Q. Wei, C. Campbell, D. Thomson, M. Komai-Koma and F.Y. Liew, 2002. Nitric oxide preferentially induces type 1 T cell differentiation by selectively up regulating IL-12 receptor 2 expression via cGMP. Proc. Natl. Acad. Sci. USA., 99: 16186-16191.

Nigam, M.C. and A. Ahmad, 1990. Curcuma longa terpenoid composition of its essential oil. Indian Perfumer, 35: 255-257.

Rafatullah, S., M. Tariq, M.A. Al-Yahya, J.S. Mossa and A.M. Ageel, 1990. Evaluation of turmeric (*Curcuma longa*) for gastric and duodenal anti-ulcer activity in rats. J. Ethnopharmacol., 29: 25-34.

Raina, V.K., S.K. Srivastava and K.V. Syamsundar, 2005. Rhizome and leaf oil composition of curcuma longa from the lower Himalayan Region of Northern India. J. Essential Oil Res., 17: 556-559.

Rappolee, D.A., C.A. Brenner, R. Schultz, D. Mark and Z. Werb, 1988. Developmental expression of PDGF, TGF-alpha and TGF-beta genes in preimplantation mouse embryos. Science, 241: 1823-1825.

Rasmussen, H.B., S.B. Christensen, L.P. Kvist and A. Karazmi, 2000. A simple and effective separation of the curcumins, the anti-protozoal constituents of *Curcuma longa*. Planta Med., 66: 396-398.

Rossi, D. and A. Zlotnik, 2000. The biology of chemokines and their receptors. Annu. Rev. Immunol., 18: 217-242.

Roughley, P.L. and D.A. Whiting, 1973. Experiments in the biosynthesis of curcumin. J. Chem. Soc. Perkin Trans., 20: 2379-2382.

Samy, R.P., P.N. Pushparaj and P. Gopalakrishnakone, 2008. A compilation of bioactive compounds from ayurveda. Bioinformation, 3: 100-110.

Satoskar, R.R., S.J. Shah and S.G. Shenoy, 1986. Evaluation of the anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. Int. J. Clin. Pharmacol. Ther. Toxicol., 24: 651-654.

Selvam, R., L. Subramanian, R. Gayathri and N. Angayarkanni, 1995. The anti-oxidant activity of turmeric (*Curcuma longa*). J. Ethnopharmacol., 47: 59-67.

Sharma, R.A., H.R. McLelland, K.A. Hill, C.R. Ireson and S.A. Euden *et al*., 2001. Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. Clin. Cancer Res., 7: 1894-1900.

Siddiqui, A.M., X. Cui, R. Wu, W. Dong, M. Zhou and M. Hu *et al*., 2006. The anti-inflammatory effect of curcumin in an experimental model of sepsis is mediated by up-regulation of peroxisome proliferator-activated receptor-gamma. Crit. Care Med., 34: 1874-1884.

Singh, S. and B.B. Aggarwal, 1995. Activation of transcription factor NF-B is suppressed by curcumin (diferuloylmethane). J Biol Chem., 270: 24995-25000.

Sirish-Kumar, I.V.M.L.R., B.N. Paul, R. Asthama, A.K. Saxena, S. Mehrotra and G. Rajan, 2003. Swertia chirayita mediated modulation of interleukin-1, interleukin-6, interleukin-10, interferon-and tumor necrosis factorá in arthritic mice. Immunopharmacol. Immunotoxicol., 25: 573-583.

Smitha, S., B.L. Dhananjaya, R. Dinesha and L. Sinivas, 2009. Purification and characterization of a ~34 kDa protein (α-turmerin) from turmeric (*Curcuma longa*) waste grits. Biochimie, 91: 1156-1162.

Srimal, R.C., 1997. Turmeric a brief review of medicinal properties. Fitoterapia, 68: 483-493.

Srivastava, K.C., A. Bordia and S.K. Verma, 1995. Curcumin, a major component of food spice turmeric (*Curcuma longa*) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. Prostaglandins Leukot. Essential Fatty Acids, 52: 223-227.

Stanciu, L.A. and R. Djukanovic, 2000. Isolation of T cell subsets by magnetic cell sorting. Methods Mol. Biol., 134: 133-133.

Stockinger, B. and M. Veldhoen, 2007. Differentiation and function of Th17 T-cells. Curr. Opin. Immunol., 19: 281-286.

Tamul, K.R., J.L. Schimtz, K. Kane and J.D. Folds, 1995. Comparison of the effects of ficoll-hypaque separation and whole blood lysis on results of immunophenotypic analysis of blood and bone marrow samples from patients with hematologic Malignancies. Clin. Diagnostic Lab. Immunol., 2: 337-342.

Thangapazham, R.L., A. Sharma and R.K. Maheswari, 2006. Multiple molecular targets in cancer chemoprevention by curcumin. AAPS J., 8: E443-E449.

Thiemermann, C., 2006. The spice of life: Curcumin reduces the mortality associated with *Experimental* sepsis. Crit. Care Med., 34: 2009-2011.

Torisu, H., M. Ono, H. Kiryu, M. Furue and Y. Ohmoto *et al*., 2000. Macrophage infiltration correlates with tumor stage and angiogenesis in human malignant melanoma: Possible involvement of TNF-α and IL-1β. Int. J. Cancer, 85: 182-188.

Tschopp, J. and M. Nabholz, 1990. Perforin mediated target cell lysis by cytolytic T lymphocytes. Annu. Rev. Immunol., 8: 279-302.

Voskoboinik, I. and J.A. Trapani, 2006. Addressing the mysteries of perforin function. Immunol. Cell Biol., 84: 66-71.

Xinjian, L. and L. Xiaochung, 2005. Effect of curcumin on immune function of mice. J. Huazhong Univ. Sci. Technol. Med. Sci., 25: 137-140.

Yamamoto, H., K. Hanada, K. Kawasaki and M. Nishijima, 1997. Inhibitory effect of curcumin on mammalian phospholipase D activity. FEBS Lett., 417: 196-198.

Yasmin, H., 2007. Analysis of the effects of *Curcuma longa* Linn. on lymphocytes and malignant cells in murine model for immunotherapy. Ph.D. Thesis, University of North Bengal, Sliguri, India.

Young, J.D., 1989. Killing of target cells by lymphocytes: A mechanistic view. Physiol. Rev., 69: 250-314.