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Two-way Efficacy of Alcoholic Turmeric Extract: Stimulatory for Murine Lymphocytes and Inhibitory for Fibrosarcoma Cells

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Abstract

The ethanol turmeric extract has been found to be stimulatory for murine lymphocytes and inhibitory for ascitic fibrosarcoma cells which have been investigated on the basis of *in vitro* viability assay, cell cycle study by fluorescence activated cell sorter (FACS), and DNA synthesis by ^{3}H-thymidine incorporation. Viability of lymphocytes is better with turmeric; DNA synthesis increases and the majority of the lymphocytes are driven toward mitotic stage as observed by FACS. Whereas turmeric causes a significant level of death in ascitic fibrosarcoma cells *in vitro* and possibly causes arrest of the cell cycle at S-phase, it induces programmed cell death.

Keywords: Curcumin, DNA synthesis, FACS, fibrosarcoma inhibition, lymphocyte stimulation, turmeric, viability.

Introduction

Curcumin is a constituent of the yellow-colored Indian spice turmeric obtained from the rhizome of *Curcuma longa* Linn. (Zingiberaceae). It is used for the preparation of many Indian dishes. Its medicinal value was mentioned in the great Ayurvedic medicinal literature of India, which is several millennia old. Recently, much interest has been generated for varieties of pharmacological effects of curcumin, including anti-tumor (Huang et al., 1994; Ruby et al., 1995), anti-infectious (Mazumdar et al., 1995), and antibacterial and anti-inflammatory (Srivastava, 1989; Brouet & Ohshima, 1995) activities. Several authors (Mehta & Moon, 1991; Huang et al., 1992, 1994; Tanaka et al., 1994) have demonstrated anti-carcinogenic properties of curcumin in animal models. Our laboratory also became interested in the anticarcinogenic effects of curcumin several years ago (Choudhury, 1998; Das, 1999, 2000; Yasmin, 2000). In this paper, we report our findings that an alcohol extract of turmeric is inhibitory for murine fibrosarcoma, while it stimulates the growth of murine lymphocytes. The analysis was made on the basis of the viability of cells, cell cycle analysis, and ^{3}H-thymidine incorporation.

Materials and Methods

Tumor induction

Inbred adult Swiss mice of both sexes, 8–16 weeks of age, were used for all experiments. Ascitic fibrosarcoma cell line was obtained from Chittaranjan National Cancer Research Institute, Calcutta, and maintained in our laboratory by serial passages. To continue with the cell line, adult mice were injected intraperitoneally with 10^{6} ascitic fibrosarcoma cells per mouse in 0.1 ml of PBS for induction of ascitic tumor. A cell suspension was prepared in phosphate-buffered saline (PBS) following a standard protocol. After 10 to 15 days, tumors would develop.

Ethanol turmeric extract preparation

For the preparation of turmeric extract, fresh rhizomes of turmeric (*Curcuma longa* Linn.) were obtained from a local market, and after cleaning, 10 g of sample was crushed to a paste with a mortar and pestle. To this paste, 10 ml of absolute alcohol were added and kept in a refrigerator at

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4 °C. After 12 h, the mixture was taken out and filtered through Whatman filter paper no. 1; the filtrate was refiltered through Millipore filter paper, and the final solution obtained was stored at 4 °C for further use.

Lymphocyte preparation

Spleen was collected aseptically from mice, and the cells were dissociated in PBS, pH 7.2, with the help of stainless steel wire mesh by passing through a syringe fitted with a 27-gauge needle. The splenocytes were washed twice in cold PBS, and the suspension was then kept in cold, sterilized ammonium chloride solution (0.83%) for 10 min in order to lyse the red cells completely. Then, the cell suspension was washed twice with cold PBS. Whenever it was necessary to remove debris, the cell suspension was layered on Ficoll and Hypaque solution (Sigma Chemical Co., St. Louis, MO, USA) and spun down at 3000 rpm for 15 min. The lymphocytes were collected from the interface and then washed twice with PBS. Finally, the cells were suspended in minimum essential medium (MEM, Hi Media).

In vitro viability assay

Splenic lymphocytes and ascitic fibrosarcoma cells were transferred to culture plates at a density of 1×10^6 cells in 0.2 ml of minimum essential medium (MEM, Hi-Media) supplemented with 10% heat-inactivated sterile goat serum (Chaudhuri & Chakravarty, 1983), 10 µl or other doses of ethanol turmeric extract, and 200 U of penicillin/ml at 37 °C. As the turmeric extract was made in ethyl alcohol, an equivalent amount of ethanol for a particular dose of turmeric was used for control. Cell survival studies were performed at different times (4, 6, 8, 16, 24, 48 h) of cell culture by the Trypan blue dye exclusion test. The total number of viable cells were counted in a hemocytometer.

Cell cycle analysis by fluorescence activated cell sorter

For cell cycle analysis, mice were injected intravenously with turmeric extract, and at 16 and 24 h, spleen cells and ascitic tumor cell suspensions were prepared in PBS separately. To 1 ml of cell suspension, 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 (Standard 250 µg/ml) RNAse A were added, followed by incubation for 45 min at 37 °C. Ethidium bromide (69 mM) was prepared in 38 mM sodium citrate. The cells were centrifuged and suspended in 0.5 ml of 69 mM ethidium bromide (a fluorochrome that stains nuclear DNA) at room temperature at least for 30 min. Finally, the cell cycle analysis was done with a fluorescence activated cell sorter (FACS; Caliber, Becton Dickinson).

Measure of DNA synthesis

Proliferation of lymphocytes was determined by measuring the rate of ^{3}H-thymidine (^{3}H-TdR) incorporation into DNA. Cells were obtained from mice injected (i.v.) earlier with turmeric extract and suspended at a concentration of 2×10^6 cells/ml in culture medium (RPMI-1640), of which 200 µl of cell suspension was transferred to each well of a 96-well

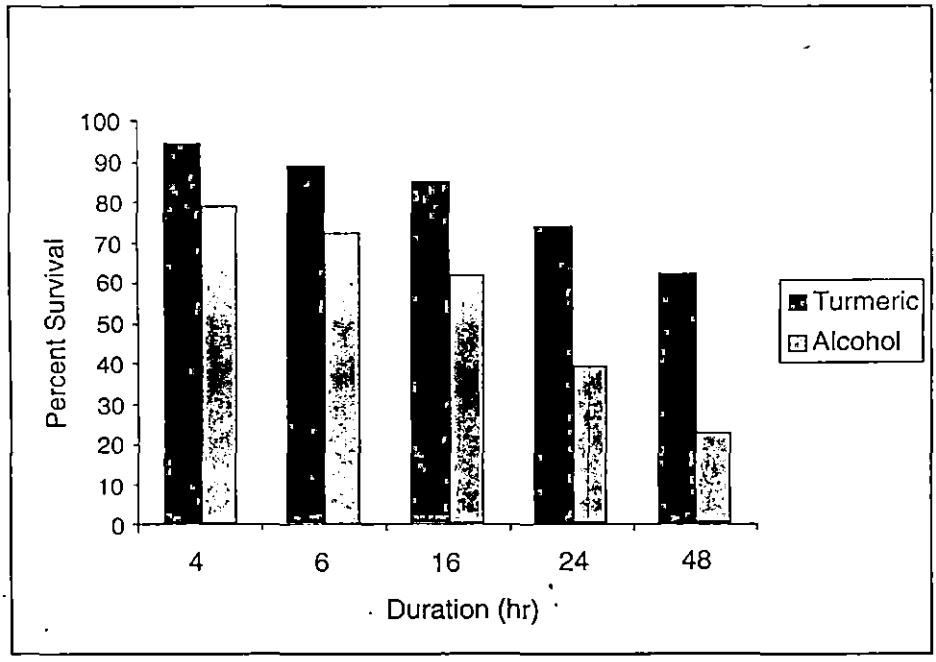


Figure 1. Diagram showing the comparative survival of splenic lymphocytes at different hours of turmeric treatment *in vitro*.

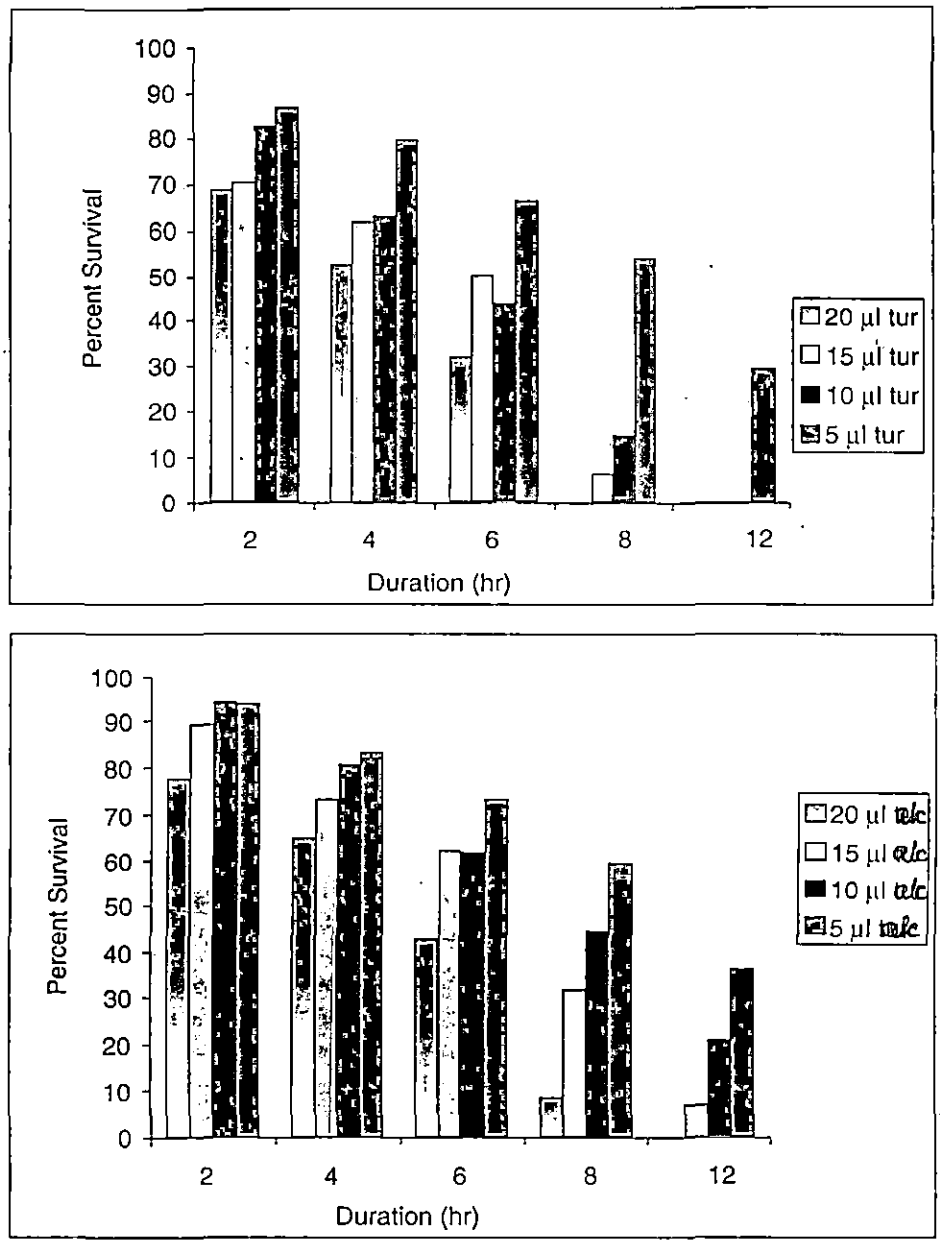


Figure 2. The comparative survival of ascitic fibrosarcoma cells at different hours of *in vitro* treatment with various doses of ethanolic turmeric extract.

microculture plate. The microculture plate was incubated for 8 h at 37 °C in a humidified atmosphere containing 5% CO_2 in air in the presence of 1 µCi of ^{3}H-thymidine (Bhaba Atomic Research Centre) per well. At the end of the culture period, cells were harvested with a PHD Cell Harvester (Cambridge, MA, USA) onto glass fiber filters, washed with methanol, dried, and kept in standard scintillation vials (Beckman). At the time of radioactivity count, 5 ml of scintillation fluid (6 gm PPO, 0.5 gm POPOP/1 of toluene) were added into each vial. All assays were done in triplicate and the level of ^{3}H-TdR incorporation was expressed as counts per minute.

Results

In vitro viability studies

For *in vitro* studies of the effect of turmeric on cell growth and viability, the splenic lymphocytes and ascitic tumor cells were cultured separately. After treatment with 10 µl of ethanol turmeric extract for 4 h, 94.37% of the lymphocytes were viable, whereas a survival of 79.21% was observed in the control (Fig. 1). Thus, the viability of lymphocytes was better with turmeric. This trend was also observed at the end of 24 h, when the percentage of live lymphocytes was 73.57% in the treatment group *versus* 38.84% in control. However,

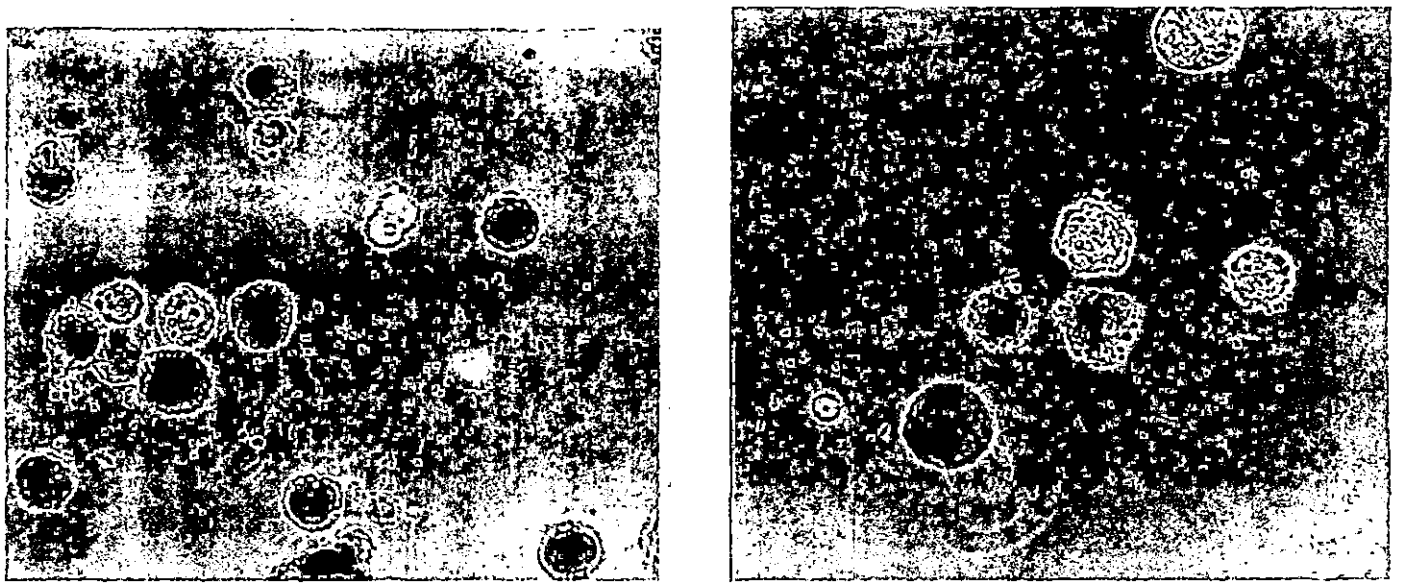


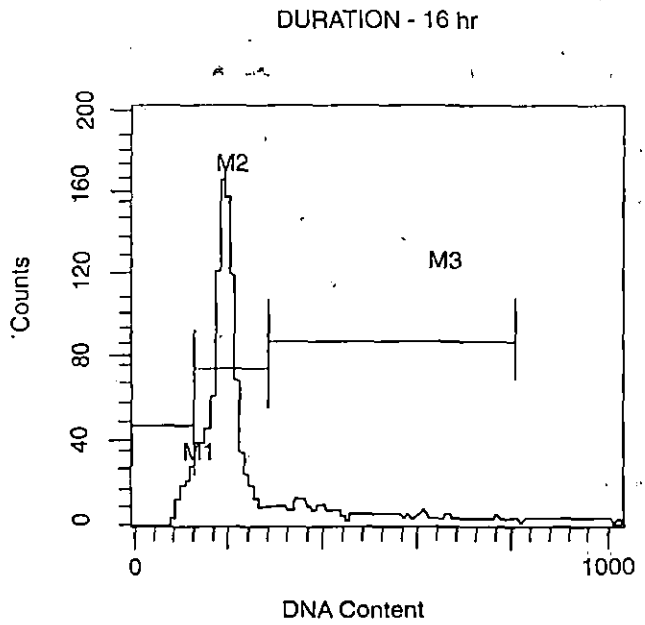
Figure 3. Trypan blue dye exclusion test for the ascitic fibrosarcoma cells at 4 h of *in vitro* treatment with (a) ethanolic extract of turmeric, (b) ethanol only (control). Turmeric treatment causes a higher rate of death to the tumor cells as evidenced from the majority of the cells turning blue in the photomicrograph (magnification ×1600).

the same dose of turmeric causes a significant level of death in ascitic fibrosarcoma cells. At 4 h, only 62.5% of viable cells could be recovered, whereas in control, 80.50% cells remained alive. Viability of tumor cells with turmeric fell dramatically after 6 h and continued to diminish with a longer incubation period in comparison with the control (Fig. 2). After 12 h of incubation, all turmeric extract – treated tumor cells were dead, whereas 21.23% of tumor cells were alive in the control. Besides 10 µl dose, a few other doses were tried (Fig. 2). The trends with other doses such as 5, 15, and 20 µl were similar to those with 10 µl, but in a graded fashion. At 12 h, total cell death was observed with 10, 15, and 20 µl doses, but not with 5 µl. A photomicrograph showing the results of the Trypan blue dye exclusion test for the ascitic fibrosarcoma cells at 4 h of *in vitro* treatment clearly indicates that in comparison to the control, the majority of tumor cells turned blue when treated with turmeric (Fig. 3). Thus, the survival indices for lymphocytes were better in alcohol turmeric extract, whereas the extract seemed to be inhibitory to murine fibrosarcoma cells.

Cell cycle analysis

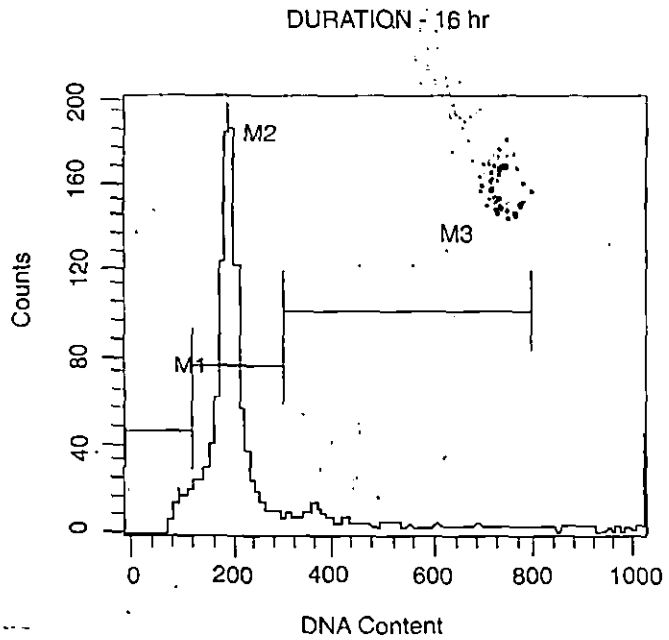
In cell cycle analysis by FACS, DNA replication was studied by tagging the fluorochrome ethidium bromide (EB). EB staining of permeabilized cells (DNA content) was plotted against cell numbers in the DNA histograms. In addition to dot-plot display and histogram display, the software of FACS also displays a table showing the percentage of cells at each cell cycle stage. In this paper, "index" refers to the percentage of cells at a particular cell cycle stage. Lymphocytes, after 16 h *in vivo* turmeric treatment, did not show any appreciable differences, relative to the control. The difference became apparent after 24 h. After 24 h of turmeric treatment, the M1 peak index was at 0.96 for lymphocytes relative to an index of 2.36 of the control (Fig. 4, Table 1). The M1 peak indicated the number of cells entered in the apoptotic state at sub G_0-G_1 phase. The observation that the index with turmeric-treated lymphocytes was lower at the M1 peak explains the higher count of viable cells with turmeric treatment as shown in Figure 1. The M2 peak showing the G_0-G_1 phase was also lower in the turmeric group in comparison to the control, suggesting that turmeric possibly has driven the cells quickly into the next phase (i.e., S-phase). At G_2-M phase (i.e. the M4 peak), the turmeric-treated lymphocytes showed an index of 12.63 in comparison with 3.34 in the control (Table 2). This indicated that turmeric has driven the majority of the lymphocytes toward mitotic stage.

However, the turmeric treatment of ascitic fibrosarcoma produced results of a reverse trend. At 16 h of treatment with turmeric, the indices for the M1 peak were 6.39, and 0.00 in control (Fig. 5, Table 1). Probably, the indices showed that the majority of the tumor cells had entered into the apoptotic state with turmeric treatment. The turmeric treatment, up to 24 h, possibly caused arrest of the cell cycle at the S-phase, as represented by the M3 peak. Thus, a fall in the M4 peak at the G_2-M phase has been observed, suggesting that the tumor cells have not entered into the mitotic stage. In summary, FACS analysis reveals that turmeric is promotional for murine lymphocytes by activating the cell cycle stages and, on the other hand, it is inhibitory for cell division and induces programmed cell death in murine ascitic fibrosarcoma cells.



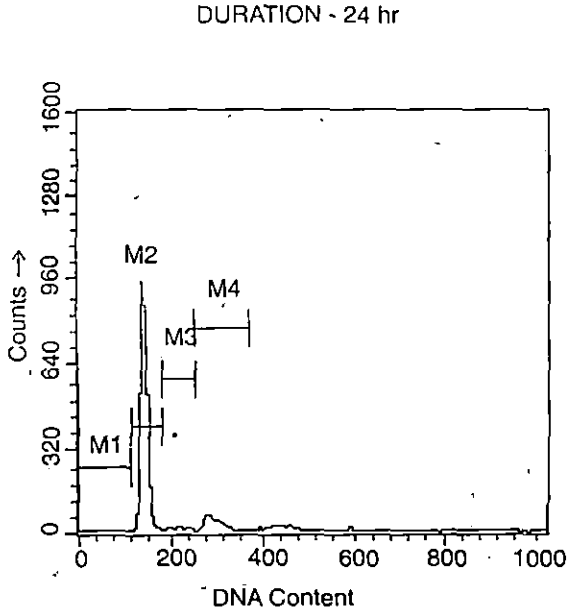
Splenic lymphocytes treated with ethanolic termeric extract

M1, sub G_0 - G_1; M2, G_0 - G_1; M3, S-G_2 - M



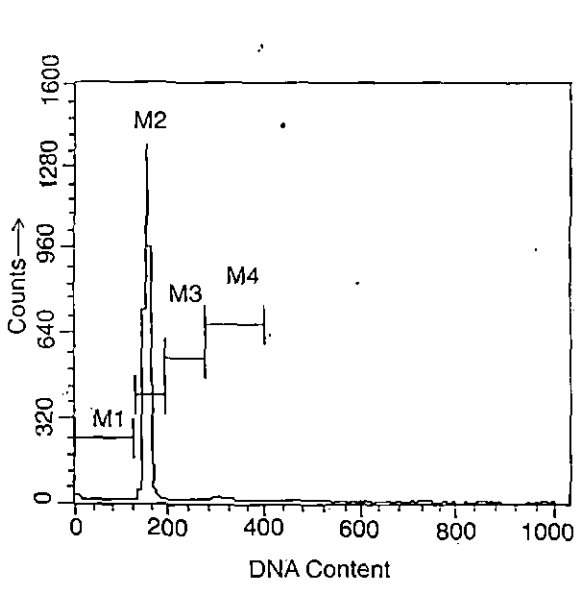
Splenic lymphocytes treated with alcohol

M1, sub G_0 - G_1; M2, G_0 - G_1; M3, S-G_2 - M



Splenic lymphocytes treated with ethanolic termeric extract

M1, sub G_0 - G_1; M2, G_0 - G_1; M3, S; M4, G_2 - M



Splenic lymphocytes treated with alcohol

M1, sub G_0 - G_1; M2, G_0 - G_1; M3, S; M4, G_2 - M

Figure 4. DNA histograms by FACS for cell cycle analysis of splenic lymphocytes.

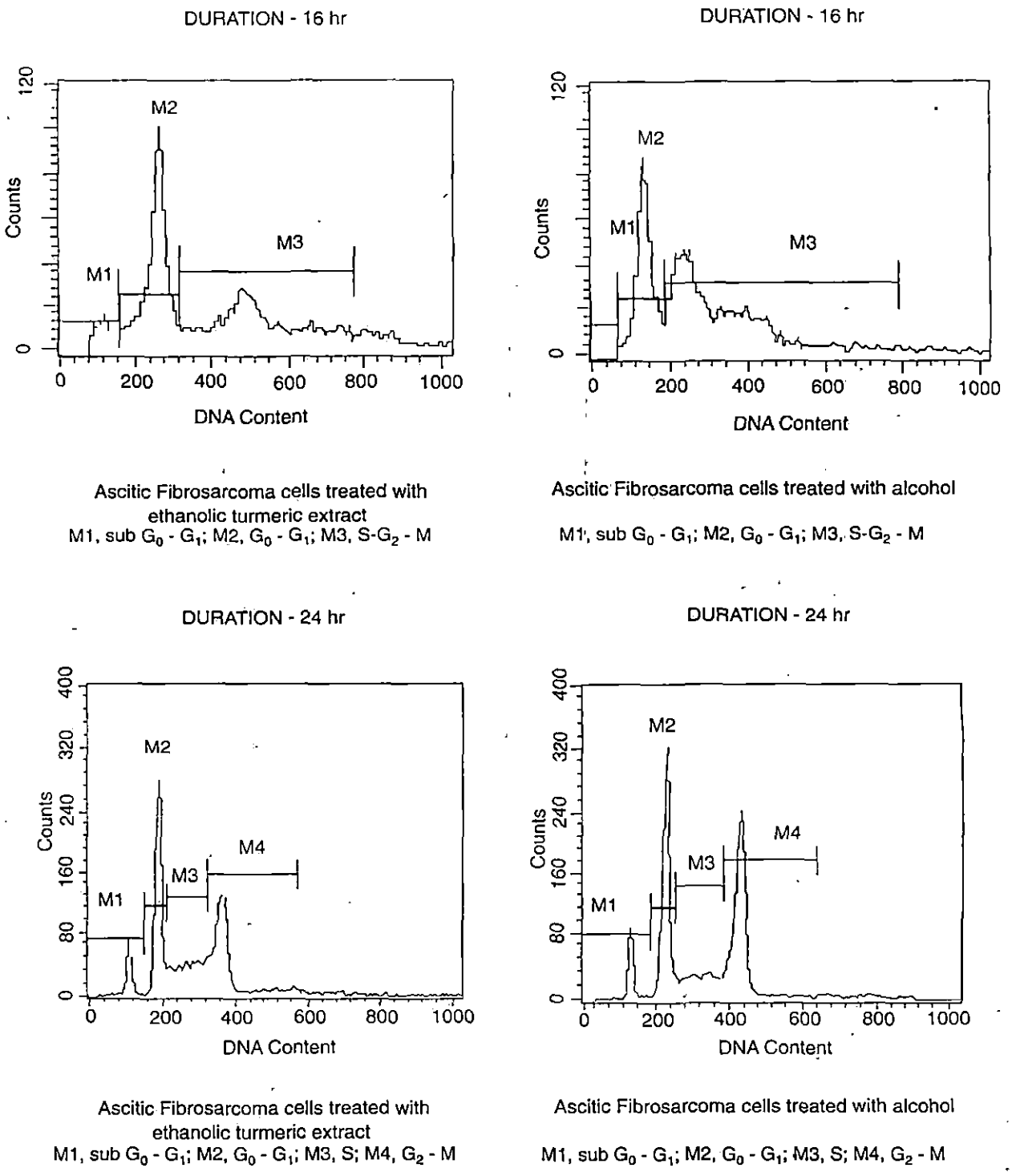


Figure 5. DNA histograms by FACS for cell cycle analysis of ascitic fibrosarcoma cells.

DNA synthesis

The inference from FACS analysis was further strengthened by the DNA synthesis data. At 24 h, ^{3}H-TdR incorporation was highest in lymphocytes with the 25 µl dose of turmeric treatment, and significantly higher than the control. The 50 µl dose of turmeric also seemed to be stimulatory, but the level of stimulation was lower than the 25 µl dose (Fig. 6). The data of ^{3}H-TdR incorporation was obtained in the course of another study using higher doses of turmeric and mentioned here for proper analysis of other observations.

Table 1. Percentage of cells at different cell cycle stages by FACS for 16 hours.

	Lymphocytes (%)		Tumor cells (%)	
Cell cycle stages	Turmeric	Control	Turmeric	Control
Sub G_0-G_1 (M1 peak)	1.89	2.72	6.39	0.00
G_0-G_1 (M2 peak)	84.10	88.92	46.41	34.27
S-G_2-M (M3 peak)	12.17	8.23	41.57	63.92

Table 2. Percentage of cells at different cell cycle stages by FACS for 24 hours.

	Lymphocytes (%)		Tumor cells (%)	
Cell cycle stages	Turmeric	Control	Turmeric	Control
Sub G_0-G_1 (M1 peak)	0.96	2.36	5.59	5.32
G_0-G_1 (M2 peak)	67.83	73.88	33.6	31.97
S (M3 peak)	2.39	1.25	22.2	15.92
G_2-M (M4 peak)	12.63	3.34	33.21	41.60

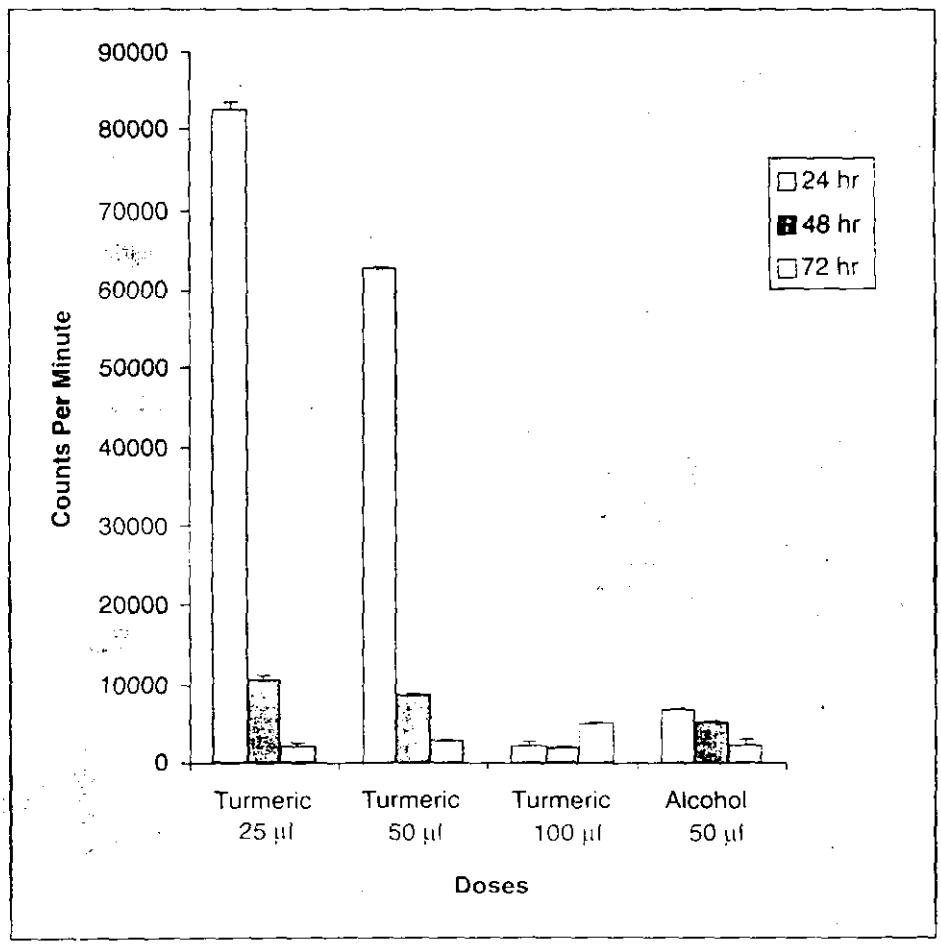


Figure 6. The level of incorporation of ^{3}H-thymidine by lymphocytes treated for different hours with different doses of ethanolic extract of turmeric, indicating the highest level of DNA synthesis is achieved with the dose of 25 µl. The control values with ethanol are shown in the extreme right.

Conclusions

The various modes of tumor-suppressive activity of turmeric have been analyzed by different authors. Curcumin, the active component of turmeric, shows antiproliferative effects with tumor cells by inhibiting protein tyrosine kinase and protein kinase C activity, as well as c-myc and bcl-2 mRNA expression (Chen & Huang, 1998). Khar et al. (1999) demonstrated that curcumin inhibited AK5 tumor growth and induced apoptosis through the activation of caspase-3 and the generation of reactive oxygen intermediates. It has also been found to inhibit the expression of several proto-oncogenes: c-Fos, c-Jun, and c-Myc in the mouse (Kakar & Roy, 1999) and c-Jun and c-Fos in JB6 cells and mouse epidermis (Lu et al., 1994).

Thus, although the inhibitory mechanisms of turmeric on tumor cells have been studied to some extent, there is not much work on the stimulatory role of curcumin for lymphocytes, as has been observed in the current investigation. This opposite effect of turmeric on ascitic fibrosarcoma cells murine lymphocytes might be additive for the effective destruction of the tumor. Thus, turmeric can be suggested for the treatment of malignancy.

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Preliminary report

Alcoholic turmeric extract simultaneously activating murine lymphocytes and inducing apoptosis of Ehlrich ascitic carcinoma cells

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Abstract

In the present investigation ethanolic turmeric extract has been found to play diabolically opposite role on murine lymphocytes and on Ehlrich ascitic carcinoma cells. Turmeric stimulates the lymphocytes into the effector pathway as studied through in vitro viability, blastogenesis and ^{3}H-TdR incorporation and also seems to be healthy under scanning electron microscopy (SEM). SEM revealed the formation of cytoplasmic blebs and plasma membrane disintegration of tumor cells with ethanolic turmeric extract treatment, suggesting turmeric to be initiating apoptosis of tumor cells. Thus, in the present work viability of the cells, blastogenesis, DNA synthesis and SEM study establish the fact that turmeric is a conducive agent for lymphocytes and inhibitory as well as apoptotic for tumor cells.

Keywords: Curcumin; Lymphocytes; Activation; Tumor cells; Apoptosis

1. Introduction

The rhizome of *Curcuma longa* Linn. (turmeric) is being widely used in different food preparations and external application in India for its medicinal value since ancient times [1]. Curcumin, 1,7-6-bis [4-hydroxy 3-methoxy phenyl]-1,6-heptadiene-3,5-dione, the major pigment and phenol compound in turmeric has been shown to possess both anti-inflammatory [2,3] and antioxidant properties [4–6] and has also been found to be inhibitory against chemical induced carcinoma in several experimental models [7–9].

In the present study we have found ethanolic turmeric extract (ETE), playing simultaneously opposite role on murine lymphocytes and Ehlrich ascitic carcinoma cells as judged through in vitro viability assay, blastogenesis, DNA synthesis and scanning electron microscopy (SEM). SEM was employed to study the changes on the cell surface topography of both murine lymphocytes and Ehlrich ascitic carcinoma cells treated with ETE.

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2. Materials and methods

2.1. Animals

Inbred adult Swiss mice of both sexes, 8–12 weeks of age, were used for all experiments.

2.2. Chemicals

Ficoll (type 400), RPMI 1640 medium, HEPES buffer, nystatin, phosphate buffer saline (PBS), toluene, POPOP and PPO for scintillation fluids were purchased from SIGMA, USA. ^{3}H-thymidine, obtained from Bhaba Atomic Research Centre (BARC), Mumbai, India and Nylon wool from Robins' Scientific Corporation, USA.

2.3. Tumor induction

Ehlrich ascitic carcinoma cell line was obtained from Chittaranjan National Cancer Research Institute, Calcutta and maintained in our laboratory by serial passages. To continue with the cell line, adult mice were injected intraperitoneally with 10^6 ascitic fibrosarcoma cells per mouse in 0.1 ml PBS for induction as ascitic tumor. After 10–15 days, a full-fledged tumor would develop.

2.4. Ethanol turmeric extract (ETE) preparation

For the preparation of turmeric extract, fresh rhizomes of turmeric (*Curcuma longa* Linn.) were obtained from local market, and after cleaning; 10 g of sample was crushed to a paste with mortar and pestle. To this paste 10 ml of absolute alcohol was added and kept in a refrigerator at 4 °C. After 12 h the mixture was taken out and filtered through Whatman filter paper 1; the filtrate was refiltered through Millipore filter paper, and the final solution obtained was stored at 4 °C for further use. One milliliter of ETE contain 0.435 mg dry weight of turmeric. For each experiment, an equivalent amount of ethanol for a particular dose of turmeric was used for control.

2.5. Cell suspension

Spleen and lymph nodes were collected aseptically and cells were dissociated in phosphate buffered saline (PBS, pH 7.2) separately with the help of stainless steel wire mesh and by repeated passage through a 27-gauge needle. Cell suspensions were separately layered on Ficoll Hypaque gradient (Sigma USA) and spun at 3000 rpm for 30 min. The band of lymphocytes at the junction of Ficoll Hypaque and PBS was taken out. Lymphocytes were finally washed twice with PBS.

2.6. Separation of B and T cells

B and T cells were separated by nylon wool fiber column technique from the lymphocyte suspension obtained from spleen or lymph nodes, as outlined by Julius et al. [10]. Briefly, a 0.1 g teased and sterilized nylon wool (Robins' Scientific Corporation, USA), soaked in RPMI was gently packed in 1 ml syringes. The columns were loaded with cell suspensions and incubated at 37 °C for 45 min. Non adherent T cells were eluted out with an excess amount of warm RPMI and re-suspended in fresh medium. The column was then filled up with chilled RPMI and further incubated in ice for 10 min. Nylon wool adherent B cells were eluted out with an excess amount of cold RPMI by vigorous agitation of the wool and then re-suspended in fresh medium. T and B cells were counted with the help of haemocytometer.

2.7. Culture medium

For in vitro culture, lymphocyte suspensions were aliquot separately in round bottom glass culture tube (vol. capacity—5 ml) at a concentration of 1×10^6 cells in 2 ml of RPMI, supplemented with glutamine, HEPES buffer, 200 mg $NaHCO_3$/100 ml, 100 U of penicillin/ml, 100 mg/ml streptomycin, 50 mg/ml nystatin and 10% heat inactivated goat serum. Finally cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2 in air.

2.8. Cell viability assay

Our earlier findings [9] showed good results with a 25 μl dose of ETE and hence this dose of ETE was used for all the experiments. Viability of splenocytes in each culture tube was counted at 8, 16, 24, 48 and 72 h with a 25 μl dose of ETE. As the turmeric extract was made in ethyl alcohol, the equivalent

amount of ethanol for a particular dose of turmeric was used for control in all the experiments. Percentages of viable cells were counted in a haemocytometer in the presence of trypan blue, considering the number of viable cells at the beginning as a hundred.

2.9. Measure of blastogenesis

Blastogenic activity of turmeric was compared with that of ConA, a polyclonal activator. ConA at a concentration of 5 μg ConA/ml was used. The percentage of blast cells was counted by a haemocytometer in the presence of trypan blue. Cells with a diameter over 6 μm and ranging more than 10 μm were considered as blast. All the experiments were repeated more than thrice and triplicate sets were kept for individual experiments.

2.10. Measure of DNA synthesis

Proliferation of lymphocytes was determined by measuring the rate of ^{3}H-thymidine (^{3}H-TdR) incorporation in vitro. Cells were suspended at a concentration of 2×10^{6} cells/ml in culture medium, from which 200 μl of cell suspension was aliquot in each well of a 96-well micro-culture plate. In each of the experimental wells 25 μl ETE and in control, an equivalent amount of alcohol was added. After 24, 48 and 72 h of incubation at 37 °C in humidified atmosphere containing 5% CO_2 in air, the cells were labeled for 8 h at the same atmosphere in the presence of 1 μCi of ^{3}H-thymidine (specific activity—15.8 Ci/mM) per well. At the end of labeling cultures were terminated by washing with cold PBS and precipitated with cold 10% trichloroacetic acid (TCA). The TCA precipitate was then filtered onto glass fiber filters; washed with methanol and dried with a PHD cell harvester (Cambridge, MA). The discs from filter paper containing individual samples were placed in standard scintillation vials (Beckman, USA). At the time of radioactivity count, 5 ml of scintillation fluid (6 g PPO, 0.5 g POPOP/1 l of toluene) was added into each vial. Radioactivity was counted in β-scintillation counter (LS 1800 BECKMAN, USA). All assays were done in triplicate, ^{3}H-TdR incorporation was expressed as counts per minute.

2.11. Scanning electron microscopy (SEM)

Murine splenocytes treated earlier with turmeric extract in vitro were fixed in Karnovsky fixative for 3–4 h at 4 °C. After fixation, cells were washed in PBS twice and then dehydrated. After dehydration cells were dried by critical point drying method and finally gold plating was done. Cells were examined and photographed under a scanning electron microscope (EDAX-9900) at AIIMS, New Delhi.

3. Results

3.1. In vitro viability of murine lymphocytes

At different hours of assay, percentage of the viable lymphocytes cultured with ETE was much higher than the alcohol treated lymphocytes (Fig. 1). At the end of 74 h, 42.89% of lymphocytes were alive with turmeric treatment, whereas in the case of alcohol treated control, the viability was zero. Hence, lymphocytes survived better with turmeric than control.

3.2. In vitro blastogenic response of lymphoid cell types in the presence of ETE

Blastogenic response has been observed in B and T cells obtained from the spleen and lymph node.

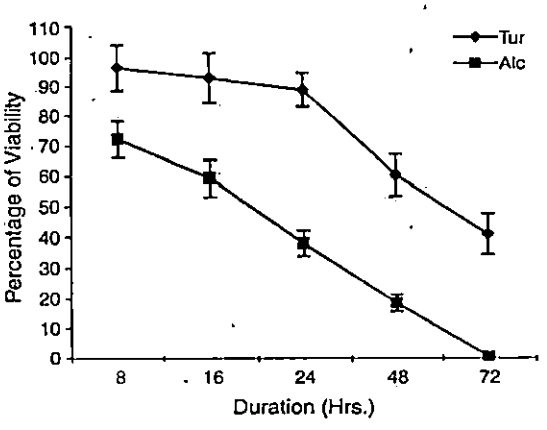

Fig. 1. Percentage of viable lymphocytes at 8, 16, 24, 48 and 72 h after treatment with ethanolic extract treatment (ETE).

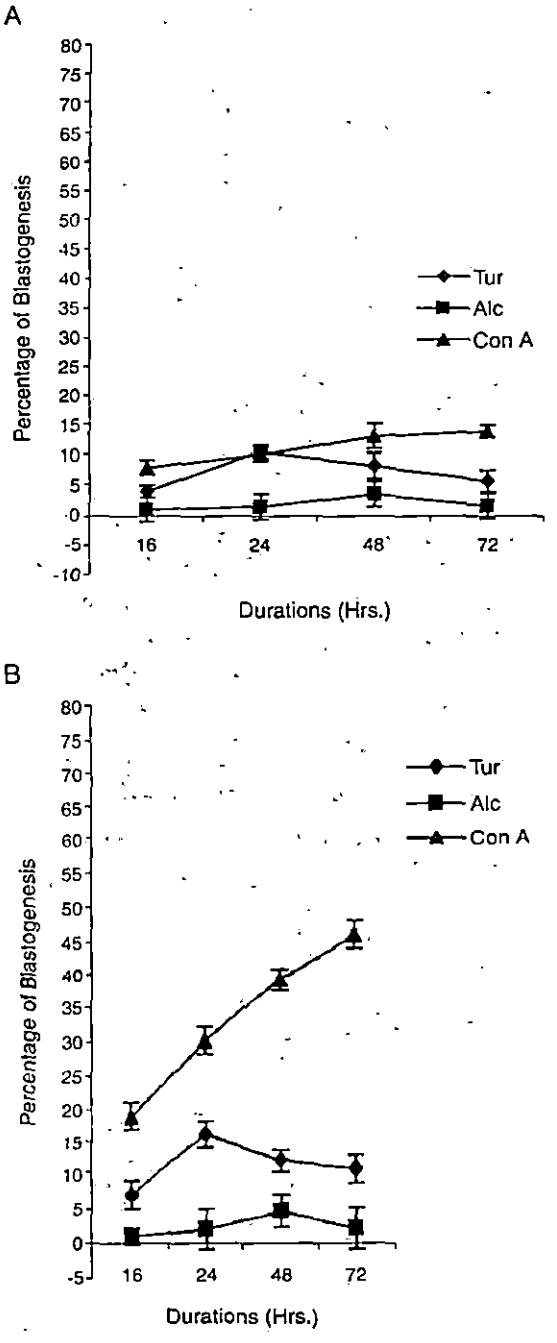

Fig. 2. Histogram showing blastogenic response of lymphocytes from spleen treated with ETE, alcohol and ConA; A) B lymphocytes, B) T lymphocytes.

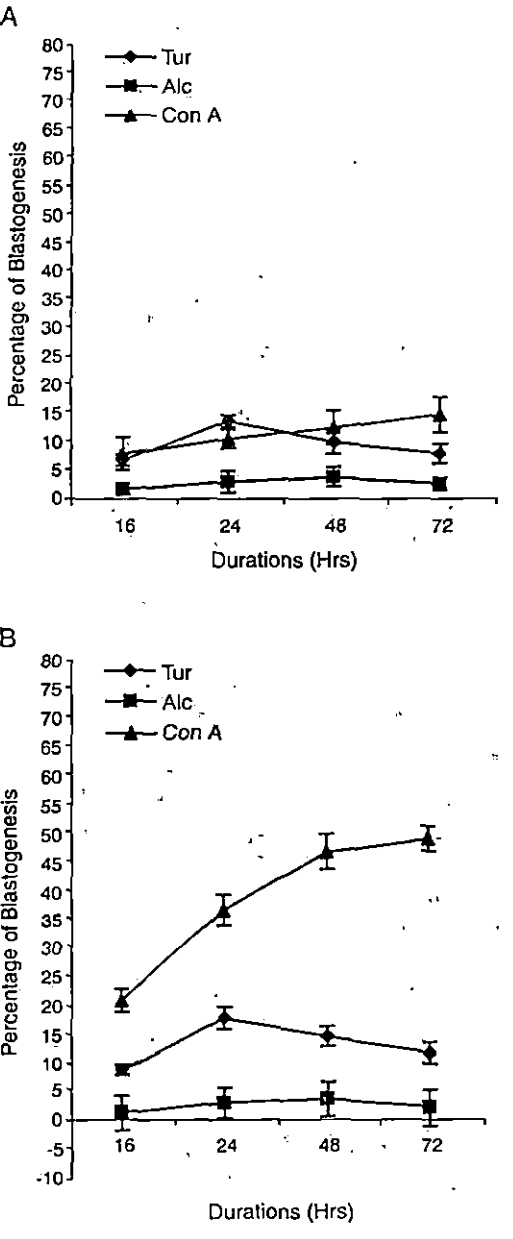

Fig. 3. Histogram showing blastogenic response of lymphocytes from lymph node treated with ETE, alcohol and ConA; A) B lymphocytes, B) T lymphocytes.

Normal lymphocytes measure about 5 μm in diameter. Blast transformation with turmeric treatment results in increment of cell size from 6 to 10 μm or more; as observed under phase contrast microscope.

The treatment of ETE caused an increase in number of blasts over the control (alcohol). Blastogenic response of T cells (Figs. 2B and 3B) was better than B cells (Figs. 2A and 3A). With turmeric treatment, the peak of blastogenesis was effectively reached by 24 h. Percentage of blasts was more in the lymph node cells (Fig. 3A and B) than splenocytes (Fig. 2A and B). When blastogenesis of T cells with ConA, an established polyclonal stimulator for murine T cells, was compared, ConA was found to be a better stimulator than ETE (Figs. 2B and 3B).

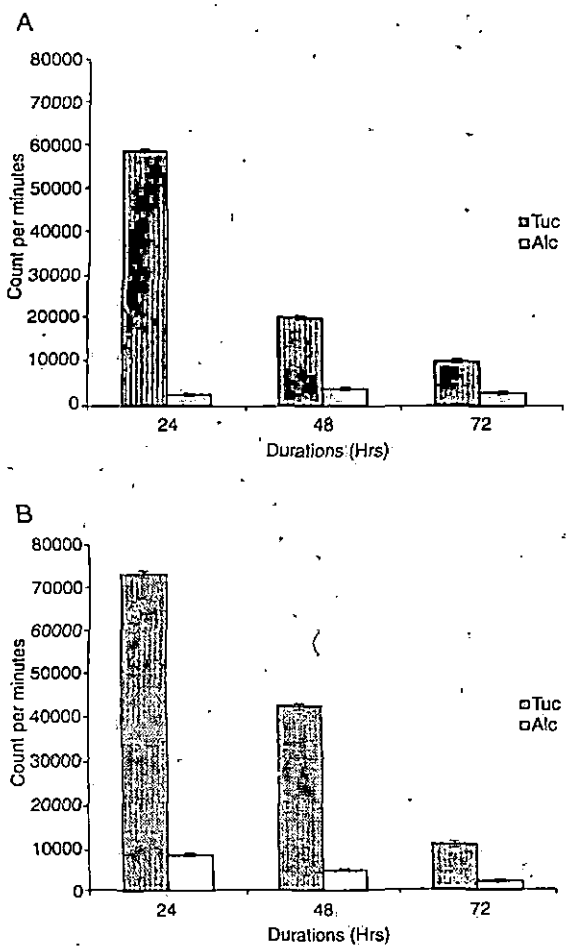

Fig. 4. Pattern of incorporation of ^{3}H-thymidine by lymphocytes treated for different hours with ETE; A) lymphocytes from spleen, B) lymphocytes from lymph node.

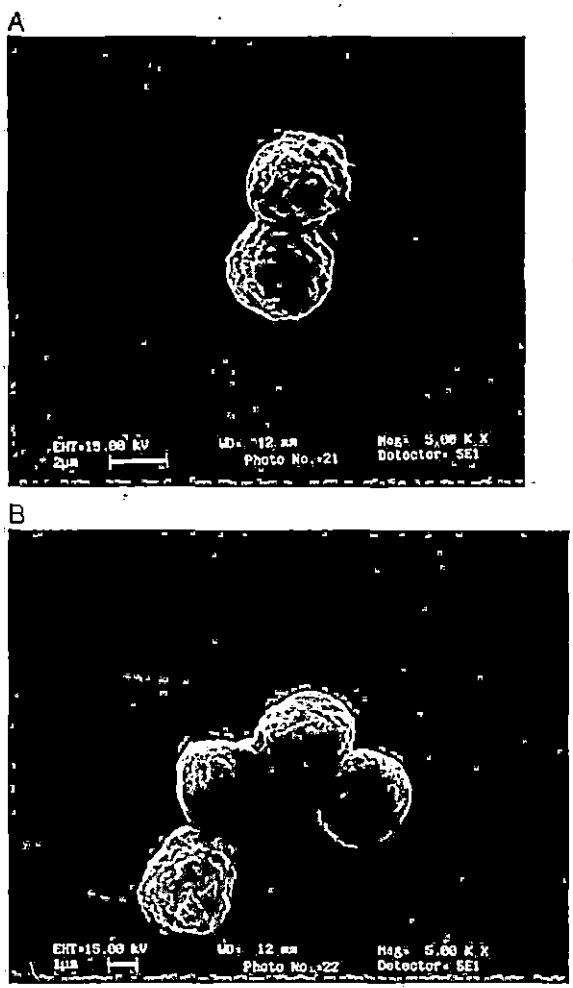

Fig. 5. Scanning electron micrographs of murine lymphocytes from spleen. A) After 16 h of turmeric treatment, lymphocytes showing no significant changes on its surface topography and seem to be healthy. B) Control, lymphocytes at 16 h.

3.3. ^{3}H-TdR incorporation in lymphocytes with ETE treatment

Blast transformation usually is a way indicative for DNA synthesis in stimulator cells. At 24 h, ^{3}H-thymidine incorporation was highest in ETE treated lymphocytes (Fig. 4A and B). Thus, the kinetics of DNA synthesis is in agreement with the blastogenesis (Figs. 2A and B and 3A and B). Lymphocytes from lymph nodes showed better response in both the counts.

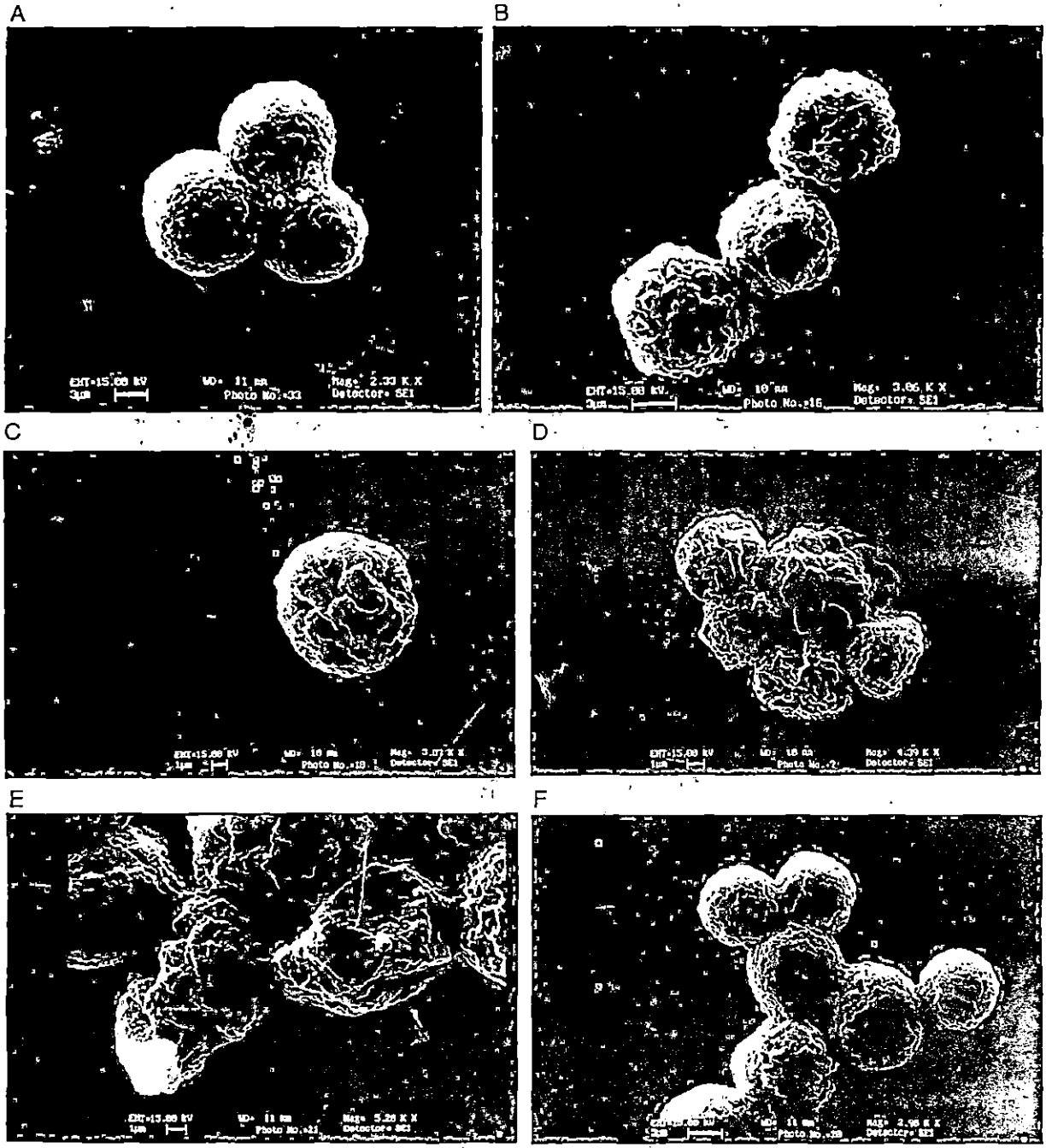

Fig. 6. Scanning electron micrographs of Ehlrich ascitic carcinoma cells. A) Tumor cells prior to turmeric treatment, showing ruffles distributed evenly all over the surface; B) after 10 min of turmeric treatment, cells showing formation of cytoplasmic blebs; C) after 4 h of turmeric treatment, cytoplasmic blebs were broader and conspicuous; D) after 8 h of treatment, cytoplasmic blebs, became numerous, cell volume tends to decrease; E) after 16 h of turmeric treatment, loss of cellular organization with disintegration of plasma membrane; F) control, after 16 h of culture tumor cells remain unchanged.

3.4. Scanning electron microscopic study

Murine lymphocytes with in vitro turmeric treatment did not show any significant changes on its surface topography from that of control after 16 h (Fig. 5A). But an increment in the size of ETE treated lymphocytes over the control was observed; this is possibly indicative for blastogenesis of treated cells. On the other hand, turmeric treatment caused progressive changes in cell surface of Ehlrich ascitic carcinoma cells (Fig. 6B–E). Ruffles on cell surface were distributed evenly over its surface prior to treatment (Fig. 6A). Within 10 min of in vitro turmeric treatment tumor cells starts showing formation of cytoplasm blebs over its surface (Fig. 6B). These changes accentuated within 4 h of treatment; particularly the cytoplasm blebs were broader and conspicuous (Fig. 6C). After 8 h of turmeric treatment, the blebs became numerous and the cell volume tends to decrease, this was indicative of initiation of disintegration of cell structure (Fig. 6D). Loss of cellular organization and disintegration of plasma membrane was more pronounced at 16 h of treatment (Fig. 6E). These changes clearly showed the onset of apoptosis in treated tumor cells, when tumor cells treated with alcohol (control) remain unchanged (Fig. 6F).

4. Discussion

The ETE has been found to stimulate the murine lymphocytes in general, causing blastoid differentiation of lymphocytes in vitro, more particularly T lymphocytes (Figs. 2B and 3B). The peak of blastogenic response was attained by 24 h, much earlier than with a polyclonal stimulator like ConA. The percentage of blasts with ETE treatment was lower than the percentage of blasts obtained with ConA. ^{3}H-TdR incorporation by the lymphocytes treated with ETE also show the peak at 24 h (Fig. 4A and B).

Comparison of the blastogenesis with ETE and ConA indicates that ETE provides blastogenic stimulus for T cells but not to the extent of a polyclonal stimulator like ConA.

Viability, blastogenic response, DNA synthesis and SEM results (Fig. 5A) in our study show the promotional effect of turmeric on murine lymphocytes. Whereas, ETE treatment of Ehlrich ascitic carcinoma cells caused pronounced cytoplasm blebing and plasma membrane disintegration leading to apoptosis. Several authors [11–14] observed apoptotic effect of turmeric on various transformed cells. Kim et al. [11] suggests curcumin-induced apoptosis was mainly due to prominent downregulation of Bcl-2 and upregulation of Bax expressions. Khar et al. [12], demonstrated that curcumin induced apoptosis through the activation of caspase-3 and by the generation of reactive oxygen intermediates. And according to some it can even inhibit the expression of several proto-oncogenes, such as c-*jun*, c-*fos* and c-*myc* in mouse skin [13] and transcription factors such as NF-κB[14].

The present investigation reports the novelty of turmeric in setting apoptosis to tumor cells and on the other hand activating lymphocytes. Our earlier studies of lymphocytes from mice treated in vivo with ETE clearly showed DNA synthesis and cells entering in mitotic cycle, whereas the treatment arrest the tumor cells at S-phase [15]. FACS performed the analysis of cell cycle.

Most of the natural antioxidants possess either a phenolic group or a β-diketone group. Curcumin and its analogues are unique, having both phenolic and β-diketone functional groups on the same molecule [16]. Like Vitamin E, curcumin is a lipid soluble antioxidant and believed to be localized within the membranous sub cellular fraction of cells [17]. The presence of curcumin in the cell membrane might have some implication for initiating the process of activation for lymphocytes and apoptosis in malignant cells.

Molecular mechanism of action of curcumin on cell physiology for two-way efficacy on two types of cells is in the agenda for our current investigation.

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