α-Tocopherol attenuates NF-κB activation and pro-inflammatory cytokine IL-6 secretion in cancer-bearing mice

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Synopsis

Cancer development and progression are closely associated with inflammation. NF-κB (nuclear factor κB) provides a mechanistic link between inflammation and cancer, and is a major factor controlling the ability of malignant cells to resist tumour surveillance mechanisms. NF-κB might also regulate tumour angiogenesis and invasiveness and the signalling pathways that mediate its activation provide attractive targets for new chemopreventive and chemotherapeutic approaches. ROS (reactive oxygen species) initiate inflammation by up-regulation of pro-inflammatory cytokines and therefore antioxidants provide a major defence against inflammation. α-Tocopherol is a lipid-soluble antioxidant. In addition to decreasing lipid peroxidation, α-tocopherol may exert intracellular effects. Hence, the aim of this study was to test the effect of α-tocopherol supplementation in cancer prevention via suppression of NF-κB-mediated pro-inflammatory cytokines. α-Tocopherol treatment significantly down-regulates expression, synthesis as well as secretion of pro-inflammatory cytokine IL-6 (interleukin-6) in cancerous mice. It also suppresses NFκB-B-dependent pathway.

Key words: α-tocopherol, cancer, interleukin-6 (IL-6), nuclear factor κB (NFκB), pro-inflammatory cytokine

INTRODUCTION

Inflammation is a critical component of tumour progression. Many cancers arise from sites of infection, chronic irritation and inflammation [1]. It is now becoming clear that the tumour microenvironment, which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration [2]. All inflammatory cells (neutrophils, monocytes, macrophages, eosinophils, dendritic cells, mast cells and lymphocytes) are recruited to the site of damage or infection which secrete a large number of cytokines and chemokines that promote the outgrowth of neoplastic cells. ROS (reactive oxygen species) and RNS (reactive nitrogen species) are produced under the stimulus of pro-inflammatory cytokines in phagocytic and non-phagocytic cells. There is a significant interaction and synergy among these mediators of inflammation, and hence a sustained microenvironment provides a constant supply of a variety of RNS and ROS, reactive aldehydes, cytokines, chemokines and growth factors, leading to genomic instability and cancer development [3]. Pro-inflammatory cytokines such as IL-1 (interleukin-1), IL-6 and TNFα (tumour necrosis factor α) play a major role in the maintenance of a sustained inflammatory microenvironment around tumour cells and thus these cytokines favour tumour growth.

IL-6 acts as pro-inflammatory as well as anti-inflammatory cytokine. It is secreted by T-cells and macrophages to stimulate immune response to trauma, especially burns or other tissue damage leading to inflammation. A pivotal role for this cytokine is to direct leucocyte trafficking and to facilitate transition between innate and acquired immune responses [4]. However, IL-6 promotes inflammatory events through the activation and proliferation of lymphocytes, differentiation of B cells, and leucocyte recruitment. ROS are generated during inflammation and cancer that influence cytokine production by several related mechanisms. ROS can initiate and/or amplify inflammation via the α-κB (nuclear factor-κB), pro-inflammatory cytokine IL-6, interleukin-6; MDA, malondialdehyde; EMSA, electrophoretic mobility-shift assay; HRP, horseradish peroxidase; IKK, inhibitory κB kinase; IL-6, interleukin-6; MDA, malondialdehyde; NFκB, nuclear factor κB; PKC-α, protein kinase Ca; PUFA, polyunsaturated fatty acid; RNS, reactive nitrogen species; ROS, reactive oxygen species; RT-PCR, reverse transcription-PCR; SNK, Student–Newman–Keuls; TNFα, tumour necrosis factor α.

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up-regulation of several different genes involved in the inflammatory response, such as those that code for pro-inflammatory cytokines and adhesion molecules via activation of certain transcription factors, such as nuclear transcription factor κB [5]. The ability of NF-κB (nuclear factor κB) to suppress apoptosis and to induce expression of proto-oncogenes such as c-myc and cyclin D1 that directly stimulate proliferation, suggest that NF-κB participates in many aspects of oncogenesis. NF-κB also regulates the expression of various molecules such as cell adhesion proteins, MMPs (matrix metalloproteinases), cyclo-oxygenase 2, inducible nitric oxide synthase, chemokines and inflammatory cytokines, all of which promote tumour cell invasion and angiogenesis [6].

α-Tocopherol protects against oxidant-mediated inflammation and tissue damage by virtue of its ability to scavenge free radicals. Therefore, one representative pro-inflammatory cytokine IL-6 is selected for the study. Regulation of IL-6 expression via NF-κB is designed to highlight the signalling mechanism operative in modulation of the cytokine by the antioxidant α-tocopherol. Targeting IL-6 expression, production and secretion can be useful in attenuating the cellular injury and dysfunction observed in some cancers.

**MATERIALS AND METHODS**

**Chemicals**

Analytical grade chemicals were used for all the experiments. Molecular biology grade chemicals were used wherever necessary. Chemicals and modifying enzymes were stored, diluted and used as per the manufacturer’s instructions.

**Animals**

Male albino mice of AKR strain (*Mus musculus*) were used for the experiments because of their short lifespan. Mice were maintained at 25 ± 2°C under a 12 h light/12 h dark schedule with *ad libitum* supply of standard mice feed and drinking water as per norms of the Institutional Animal Ethical Committee.

**Induction of lymphoma in mouse**

DL (Dalton’s lymphoma) is an inducible non-Hodgkin’s T-cell lymphoma. It was introduced into adult (10–15-week-old) male mice by serial transplantation of live DL ascites cells (approx. 1 × 10⁶ ascites cells) in normal saline (pH 7.4) as described previously [7]. DL ascites cells were gifted by Professor Ajit Sodhi (School of Biotechnology, Banaras Hindu University, Varanasi, India).

**Treatment of cancerous mice with α-tocopherol**

DL transplanted cancerous mice were treated with α-tocopherol (Sigma) from the day after transplantation. α-Tocopherol was dissolved in 25% ethanol and administered by gavage. DL cancerous mice were divided into four groups (*n* = 10). Three groups were treated with different doses of α-tocopherol: 1.5 mg [50 μg/kg of bw (body weight)], 3 mg (100 μg/kg of bw) and 4.5 mg (150 μg/kg of bw) to each mouse per day orally for 15 days consecutively, and the control group received 25% ethanol in a similar manner. In addition, one group of normal mice (15–20 weeks old) was used without any treatment. The doses of α-tocopherol were standardized on the basis of a previous study by Soo et al. [8].

All the groups of mice were killed on day 19 of DL transplantation by cervical dislocation. Liver was excised on ice immediately, washed in 0.9% NaCl and used for further studies.

**Experimental procedure**

**Lipid peroxidation**

Lipid peroxidation, an indicator of oxidative stress, was determined in liver using the TBARS (thiobarbituric acid-reacting substance) assay method of Ohkawa et al. [9] with small modifications. In brief, liver was homogenized in potassium phosphate buffer (pH 7.4), supernatant was collected after centrifugation at 14000 g and was incubated with 8.1% SDS, 20% acetic acid (pH 3.5), 0.8% TBA (thiobarbituric acid) for 1 h at 95°C. After cooling, samples were mixed with 15:1 butanol/pyridine and the aqueous phase was recovered. Absorbance was determined at 532 nm, and results were expressed as nmol of MDA (malondialdehyde)/mg protein.

**Isolation of total RNA and RT–PCR (reverse transcription–PCR)**

Expression of the IL-6 gene in liver was studied by semi-quantitative RT–PCR. For this, RNA was isolated using TRI Reagent as per the manufacturer’s instructions. The tissue was homogenized in TRI Reagent and phase separation was done using chloroform. Colourless aqueous phase was precipitated with isopropanol. Precipitated RNA was washed with 70% ethanol and dissolved in DEPEC (diethylpyrocarbonate)-treated water after air drying. For cDNA synthesis, reaction mixture (20 μl) containing 2.0 μg of total RNA, 200 ng of random hexamer primers, 1 mM dNTPs, 20 units of RNase inhibitor and 200 units of MMuLV (Moloney murine leukemia virus) reverse transcriptase was incubated for 1 h at 42°C. cDNA thus obtained, was amplified by RT–PCR using specific primers.

Specific oligonucleotides selected are as follows: IL-6, forward primer 5’-ATGAAGTTCCCTCCTCTGCAAGAGCT-3’ and reverse primer 5’-CCTAGTTTTCGGAGAATGTCTC-3’; β-actin, forward primer 5’-ATCGTGCCCCTAGCTAGGG-3’ and reverse primer 5’-CTCTTTGATGTCAGCAGACACCA-3’.

These primers were synthesized by Operon. Parameters for IL-6 amplification were as follows: denaturation at 94°C for 30 s, primer annealing at 62°C for 45 s, elongation at 72°C for 1 min and number of cycles 38: for β-actin: denaturation at 94°C for 30 s, primer annealing at 57°C for 30 s, elongation at 72°C for 30 s and number of cycles 26. The number of cycles used for PCR
was chosen to be within the exponential phase of amplification. The PCR product was visualized in an imager EC (α Innotech) on 2% agarose gel. The relative amount of IL-6 was expressed as absorbance relative to that of β-actin.

Nuclear protein extraction

Nuclear extract was prepared from liver of mice according to the method of Dignam et al. [10] with a few modifications. In short, 10% of tissue homogenate was prepared in buffer A [10 mM Hepes/KOH, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 0.1 mM EGTA, 0.5 mM DTT (dithiothreitol), 0.5 mM PMSF and 0.3 M sucrose]. The homogenate was centrifuged at 1000 g for 15 min at 4°C and pellet was suspended in buffer A containing 0.3 M sucrose and then 0.2% Triton X-100 was added. An equal volume of buffer B (buffer A containing 1.8 M sucrose) was added and mixed thoroughly. It was then centrifuged at 25 000 g for 30 min at 4°C. The crude nuclear pellet was washed at 1000 g for 10 min at 4°C in buffer A containing 0.3 M sucrose and observed under microscope till clear nuclei were seen without tissue debris. The pellet was resuspended in buffer A and washed in buffer A. The nuclear pellet was suspended in buffer C containing 20 mM Hepes/KOH, pH 7.9, 25% glycerol, 0.42 M NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF and 2 μg/ml protease inhibitor cocktail (Sigma). The suspension was kept on ice for 30 min and stirred intermittently to lyse the nuclei to extract nuclear proteins. The chromatin was pelleted by centrifugation at 25 000 g for 40 min at 4°C. The supernatant was divided into aliquots and stored at −80°C. The nuclear proteins were quantified according to the method of Bradford [11].

EMSA (electrophoretic mobility-shift assay)

EMSA was carried out as described by Liu et al. [12]. In brief, purified synthetic oligonucleotide probes corresponded to NF-κB binding sequences of the IL-6 promoter (Bioserve). The sequence for NF-κB was sense 5′-AGTTAGGGGACTTCCCCAGG-3′ and antisense 5′-GCTTGGAAGTCCCTCAA-3′. NF-κB oligonucleotides were annealed and end labelled with [α-32P]-CTP using Klenow enzyme (Fermentas). Labelled oligonucleotides were separated from free nucleotides by Sephadex G50 column (spun column) chromatography. 0.1–0.2 ng (approx. 5500 cpm) labelled ds-oligonucleotides was taken per reaction. In 40 μl reaction mixture, 10 μg of nuclear protein was taken in the presence of binding buffer (5 mM Hepes, pH 7.9, 10% glycerol, 25 mM KCl, 0.05 mM EDTA and 0.125 mM PMSF), 1 μg of poly(dI-dC)·poly(dI-dC) and 0.1 ng of radiolabelled probe. The mixtures were incubated at 25°C for 30 min. The reaction was terminated by adding 5 μl of 5× loading dye (6% sucrose, 2 mM Tris/HCl, pH 8.0, 0.05% Bromophenol Blue and 0.05% Xylene Cyanol FF). The samples were loaded on to 5% non-denaturing polyacrylamide gel in a 0.5× TBE running buffer (1× TBE = 45 mM Tris/borate and 1 mM EDTA). After resolving, the gel was fixed in 10% acetic acid and 10% methanol for 15 min. It was then dried and exposed to X-ray film for autoradiography. The autoradiograph was quantified by densitometry.

Western blotting

Liver of N (normal), DL mice and DLT mice (DL-treated mice with different doses of α-tocopherol) were used. Tissue was homogenized in 50 mM Tris/HCl (pH 7.4), containing 0.2% Triton X-100, 5 mM EDTA, 5 mM EGTA, 2 mM PMSF, 5 mM benzamidine, 2 mM 2-mercaptoethanol and protease inhibitor cocktail (Sigma–Aldrich). Protein was estimated by the method of Bradford [11]. Then 100 μg of protein were resolved by SDS/PAGE (12% gel) and transferred on to PVDF membrane, blocked with 3% BSA in PBS for 4 h, then incubated overnight at 4°C with rabbit anti-IL-6 primary antibody (1:200; Abcam) in 1% BSA. After washing with PBS (pH 7.4), immunoreactive proteins were revealed with an enhanced chemiluminescence Super Signal West Pico kit (Pierce Biotechnology), and their expression level was compared by densitometry where band density values were normalized to that of β-actin.

Immunohistochemistry

Immunohistochemical staining of liver was performed to demonstrate the cellular source of IL-6 secretion. Mice were anaesthetized with chloroform and perfused transcardially with ice-cold PBS followed by 4% (w/v) paraformaldehyde. Liver was removed and post-fixed with 4% formaldehyde overnight and then embedded in paraffin wax. Then 5-μm-thick sections were de-paraffinized followed by re-hydration through alcohol series. After antigen retrieval with 0.1% trypsin for 10 min, the sections were blocked with 1% BSA for 30 min. The blocking solution was drained out and the tissue sections were treated with anti-IL-6 antibody (1:50; Abcam) overnight at 4°C. After incubation, the gel was washed with 1× PBS and probed with HRP-conjugated goat anti-rabbit IgG secondary antibody. For detection of substrate, DAB (diaminobenzidine) was used. Sections were counter-stained with haematoxylin and differentiated with water.

ELISA

IL-6 level in mice serum was measured by using highly specific quantitative Sandwich ELISA Kits (Pierce Biotechnology). The sensitivity of IL-6 was 7 pg/ml. ELISA was performed according to the manufacturer’s instructions. Briefly, ELISA plate was coated overnight with diluted anti-IL-6 antibody (1:100) at 4°C. After incubation, 200 μl of blocking solution was added to each well of the ELISA plate and the plate was incubated for 1–2 h at room temperature. Then 100 μl serum was added to each well and incubated overnight at 4°C, followed by washing with 1× PBST. Biotinylated diluted secondary antibody was added to the plate and kept for 1 h at room temperature. After decantation and washing, 100 μl of streptavidin HRP solution was added to each well and incubated for 30 min. Then 100 μl of TMB (3,3′,5,5′-tetramethyl benzidine) substrate solution was added, mixed well and kept for colour development for 20 min.
Reaction was terminated by addition of stopping solution and absorbance was measured at 450 nm.

Data and statistical analysis
The gels and X-ray film exposures were photographed by gel doc. system (α Innotech). The bands were analysed and quantified using computer-assisted densitometry (αEase FCTM software; α Innotech). For RT–PCR and Western blots, the signal intensity of the band of interest was measured after normalization with β-actin and expressed as RDV (relative densitometric value). Results represent the means ± S.E.M. of data obtained from three different sets of experiments. The means ± S.E.M. were analysed by Sigma Stat 3.5 software. All the data were examined by one-way ANOVA followed by SNK (Student–Newman–Keuls) test. *Indicates that groups differ significantly from DL mice at the level of significance P < 0.05 using one-way ANOVA followed by the SNK test.

RESULTS

Effect of α-tocopherol on oxidative stress
Lipid peroxidation is a marker of oxidative stress; hence, it was used as a parameter to measure oxidative stress in cancer-bearing mice. Lipid peroxidation was found to be elevated 2-fold in DL mice liver as compared with normal ones, which confirms oxidative stress. α-Tocopherol is a well-known chain-breaking antioxidant as it can donate two electrons and then it is oxidized and according to Buettner [13] it is thermodynamically suitable to serve as an antioxidant because it reacts poorly with oxygen. Consistent with its role as an ROS-scavenging agent, treatment of α-tocopherol significantly reduced (P < 0.05) lipid peroxidation in DL mice as measured in the form of MDA concentration (Figure 1). α-Tocopherol reduced MDA concentration by 26% and 54% with 50 mg and 100 mg doses, given to DL mice. A higher dose of 150 mg of α-tocopherol is less effective than other two doses (50 mg and 100 mg/kg of bw) in lowering MDA level. It may be because α-tocopherol is a lipid soluble vitamin. All lipid soluble vitamins are known to be harmful at higher doses. Hyper vitaminosis E has been reported earlier. During standardization of the doses of α-tocopherol, 200 mg/kg of bw was found to be toxic, as mice did not survive up to 20 ± 2 days and therefore this dose was not selected (results not shown).

Regulation of transcription of IL-6 by α-tocopherol
ROS can activate intracellular signalling pathways that are known to regulate cytokine genes. Therefore, the effect of the antioxidant α-tocopherol on activation of IL-6 expression was assessed, as α-tocopherol has been found to possess functions that are independent of its antioxidant/radical scavenging ability [14]. IL-6 expression was found to be up-regulated 2.4-fold in liver of DL mice which was down-regulated in α-tocopherol-treated cancerous mouse liver by 34% and 50% (P < 0.05) after treatment with 50 mg and 100 mg/kg of bw doses of α-tocopherol respectively (Figures 2a and 2c). However, the effect of a 150 mg/kg of bw dose of α-tocopherol was not significant. This dose is less effective than the other two doses as found in the case of lipid peroxidation.

Effect of tocopherol on NF-κB binding to IL-6 promoter
It is reported that increased IL-6 release from monocytes under pathological conditions is mediated via up-regulation of both PKC-α (protein kinase C-α) and PKC-β, through p38 MAPK (mitogen-activated protein kinase) and NF-κB. IL-6 promoter has an NF-κB-binding site at −75 and −63, and hence NF-κB activation can activate IL-6. Our result indicates increased NF-κB-binding activity (3-fold) in liver of DL mice, which is...
**α-Tocopherol attenuates NF-κB activation**

**Figure 3** Effect of tocopherol on NF-κB-binding activity to IL-6 promoter in liver of N, DL and DLT mice (n = 10)

N, normal; DL, Dalton’s lymphoma-bearing mice; DLT, Dalton’s lymphoma-bearing mice treated with 50, 100 and 150 mg/kg of bw doses of α-tocopherol. (a) Autoradiogram showing binding activity of NF-κB. (b) Densitometric scanning of autoradiogram. Values are expressed as means ± S.E.M. *Indicates that groups differ significantly from DL mice at the level of significance P < 0.05 using one-way ANOVA followed by the SNK test.

coherent with increased expression of IL-6. Therefore, we tested if suppression of IL-6 expression in α-tocopherol-treated cancerous mice is associated with NF-κB activity in liver. Densitometric analysis of autoradiogram shows that the increased NF-κB-binding activity in liver of cancerous mice was down-regulated up to 50% in cancerous mice receiving 100 mg of α-tocopherol/kg bw (Figure 3). Taken together, all the results emphasize that α-tocopherol can control oxidative stress as well as inflammation by down-regulating pro-inflammatory cytokines via an NF-κB-mediated pathway in cancer. The difference in the variation pattern of IL-6 expression and NF-κB-binding activity with an 150 mg dose of α-tocopherol supports that NF-κB is not the only transcription factor for IL-6 expression. The expression is regulated by other transcription factors such as AP-1 (activator protein 1), GRE (glucocorticoid-response element), CRE (cAMP-response element), etc. [15,16].

**Effect of α-tocopherol on production and secretion of IL-6**

Oxidative stress pathways are activated and ROS are generated in cancerous conditions. Because α-tocopherol is an ROS scavenging agent, it was used to determine if decreasing oxidative stress would inhibit IL-6 synthesis and secretion. IL-6 protein level was found to be elevated 2.5-fold in liver of cancer-bearing mice as compared with normal mice (Figures 4a and 4c). Different doses of α-tocopherol significantly reduced (P < 0.05) IL-6 level. The 50, 100 and 150 mg/kg of bw doses of α-tocopherol reduced IL-6 levels by 63.6, 60.6 and 58.5% respectively. The difference in expression and protein level of IL-6 with a 150 mg/kg of bw dose of α-tocopherol treatment to DL mice shows that the antioxidant α-tocopherol regulates IL-6 at the transcriptional as well as translational level. It may also be operative at the post-translational level.

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**Figure 4 Level of IL-6 in the liver of N, DL and DLT mice (n = 10)**

N, normal; DL, Dalton’s lymphoma-bearing mice; DLT, Dalton’s lymphoma-bearing mice treated with 50, 100 and 150 mg/kg bw dose of α-tocopherol. (a) Western blot of IL-6. (b) Western blot of β-actin. (c) Densitometric scanning of IL-6 level after normalization with β-actin. Values are expressed as means ± S.E.M. *Indicates that groups differ significantly from DL mice at the level of significance P < 0.05 using one-way ANOVA followed by the SNK test.
level making it more sensitive for degradation and thus decreasing protein level to a higher extent as compared with its mRNA level.

The cellular source of IL-6 was investigated by immunohistochemical staining of liver, where IL-6 is observed in the cytoplasm of randomly scattered hepatocytes. The result indicates a significant increase in IL-6 production by hepatocytes of DL mice as compared with normal; and α-tocopherol leads to a significant decrease in IL-6 production (Figure 5). IL-6 secretion in the serum was measured by ELISA which was approx. 10-fold higher in the case of cancerous mice. Treatment with 50, 100 and 150 mg doses of α-tocopherol significantly suppresses IL-6 secretion up to 40, 49 and 35% respectively (Figure 6).

DISCUSSION

Elevated oxidative status of lymphoma-bearing mice is confirmed by our finding of elevated lipid peroxidation in DL mice that may be due to the organism’s poor antioxidant defence. Inactivation of antioxidant systems in cancerous conditions has been reported earlier in our laboratory [17–20]. In the present study, we find that the increased level of lipid peroxide observed in cancerous conditions has been reduced back to near-normal level in α-tocopherol-administered cancerous mice. Vitamin E is a well accepted antioxidant to provide a first line of defence mechanism against lipid peroxidation. It protects PUFAs (polyunsaturated fatty acids) in cell membranes through its free radical quenching activity at an early stage of radical attack [21,22]. α-Tocopherol is a lipid-soluble vitamin, present in the outer membrane of cells and cell organelles. Lipid peroxy radicals initiate the chain reaction of oxidizing neighbouring PUFAs in a chain-like reaction and by quenching the lipid peroxy radical formed by the oxidized PUFAs, α-tocopherol breaks the chain and protects membranes from further oxidation [23]. ROS are generated during inflammation and cancer [24,25] which influence cytokine production by several related mechanisms. Down-regulation of ROS availability by α-tocopherol should in turn decrease ROS-dependent inflammation. This hypothesis has been confirmed by down-regulation of expression, production and secretion of pro-inflammatory cytokine IL-6 in α-tocopherol-treated cancerous mice.

The IL-6 gene is induced in response to bacterial endotoxin-like LPS (lipopolysaccharide), viral infection,
phytohaemagglutinin or by a variety of other cytokines such as TNFα, IL-1 and PDGF (platelet-derived growth factor) or in other words it is regulated by all substances that trigger inflammation. IL-6 is a cytokine released by activated monocytes that plays a crucial role in the immune response. Even though expression of IL-6 gene is tightly controlled, unregulated expression of IL-6 appears to be involved in certain pathological conditions, especially rheumatoid arthritis, Castleman’s disease, certain types of tumours and human T-cell lymphotrophic virus type I-infected T-cells. The apparent NF-κB-binding site found upstream (between −75 and −63) of the IL-6 gene seems to be an indispensable component of the IL-6 control region for induction of IL-6 gene expression by a variety of stimuli [26]. Our finding that α-tocopherol down-regulates IL-6 transcription by inhibiting NF-κB-binding activity to IL-6 promoter is in accordance with the hypothesis. α-Tocopherol inhibits NF-κB-mediated cytokine production probably by reducing the activation of stress kinases that are upstream of NF-κB. Stress kinases are known to phosphorylate IKK (inhibitory κB kinase) and remove IκB (inhibitory κB) inhibition from NF-κB [27].

By increasing oxidative defences, α-tocopherol may reduces the availability of ROS to activate stress kinases upstream of IKK and thus attenuate NF-κB stimulated cytokine production pathways. Such regulation by α-tocopherol has been reported earlier [28]. Our results showed higher IL-6 levels in cancerous mice serum than those in normal mice serum, indicating that production and secretion of the cytokine are increased by the tumour cells under oxidative stress and antioxidant α-tocopherol reduces NF-κB-binding activity and pro-inflammatory cytokine production by hepatocytes of cancer-bearing mice. In conclusion, our findings suggest that besides the powerful free radical scavenging effects, α-tocopherol down-regulates pro-inflammatory cytokine IL-6 expression by inhibiting NF-κB-mediated gene transcription in cancer-bearing mice that in turn leads to decreased synthesis and secretion of IL-6.

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