CHAPTER III

Role of tuftsin in apoptosis
3.1. Introduction

In biological systems, free radicals or reactive oxygen species (ROS) are continuously generated by oxygen and help in killing of pathogens (Halliwell et al. 1993, Li et al. 1994). Incidentally, ROS are generated through a variety of environmental pollutant like cigarette smoke, automobile exhaust fumes, radiation, and exposure to xenobiotic compounds. Due to their high chemical reactivity, ROS are able to induce cellular damage in a number of ways (Bendich 1990). The most deleterious effects of ROS include DNA damage (Richter et al. 1988), which can lead to a number of diseased conditions including cancer. Although, ROS act as damaging entities, at the same time they also carry out some beneficial biological events. ROS has been shown to be mediators, triggers or executioners of essential protective mechanisms such as apoptosis, phagocytosis and detoxification reactions. Increase of ROS concentration by depletion of antioxidants, enhances apoptosis and thereby inhibits neoplastic growth. Antioxidants decrease ROS level, inhibit apoptosis and suppress the elimination of cancer cells (Agostini et al. 2002 and Zhai et al. 2002).

Apoptosis is a highly organized cell death process, characterized by loss of plasma membrane phospholipid asymmetry, enzymatic cleavage of the DNA into oligonucleosomal fragments, and segmentation of the cells into membrane-bound apoptotic bodies (Kidd 1998). Genetic changes resulting in loss of apoptosis or derangement of apoptosis-signaling pathways in the transformed cells are critical components of carcinogenesis (Kastan et al. 1995 and Schulte-Hermann et al. 1997). Interestingly, induction of apoptosis of cancer cells is recognized as a valuable tool for management of cancer (kornblau 1998). In general, the cells execute apoptosis by Caspase-3, one of a family of cysteine proteases (Thornberry et al. 1998). This in turn is thought to be regulated by Bax and Bcl-2. Proapoptotic Bax forms pores in the outer mitochondrial membrane, releasing
cytochrome c while antiapoptotic Bcl-2 prevents the opening of mitochondrial transition pore by binding with Bax (Antonsson et al. 1997 and Zamzami et al. 1995). ROS have been suggested to act as an upstream signal for Caspase-3 activation (Kim et al. 2006, Jacobson 1995).

Some recent studies have revealed that the phosphatidylinositol 3-kinase (PI 3-kinase) and its downstream target, Akt, are responsible for the anti-apoptotic activity of insulin against TGF-β (Chen et al. 1998). PI 3-kinase was reported to suppress apoptotic cell death induced by a variety of stimuli (Ahmed et al. 1997, Dudek et al. 1997, Kauffmann-zeh et al. 1997, Khwaja et al. 1997, Kulik et al. 1997). Anti-apoptotic activity of PI3-kinase is mediated by through the action of the serine/threonine kinase, Akt. Recent studies have demonstrated that activated Akt can phosphorylate the proapoptotic protein BAD (Datta et al. 1997, Del-Peso et al. 1997). This phosphorylation facilitates association of BAD with 14-3-3 and dissociation from BCL-XL, which is then released to resume its function as a suppressor of apoptosis (Zha et al. 1996).

In the present study, we have tried to elucidate effect of tuftsin on dimethyl benz (a) anthracene (DMBA), induced hepatic alterations in ROS generation, lipid peroxidation, status of the antioxidant enzymes, mitochondrial membrane potential etc. The study is ought to help us understanding the mechanism for the inhibition of apoptosis by liposomised tuftsin in the liver of Swiss albino mice.

3.2. Materials and Methods

3.2.1. Chemicals

DMBA, egg phosphatidyl choline, dichlorodihydroflourescien diacetate dye (DCFH-DA), Rhodamine 123, propidium iodide (PI), β-actin (clone AC-74) and nitrocellulose membrane were purchased from Sigma (St Louis, USA). The Bcl-2 (polyclonal anti-rabbit IgG), Bax
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(polyclonal anti-rabbit IgG), and Caspase-3 (anti-mouse IgG) antibody were purchased from serotech. The anti-mouse and anti-rabbit horseradish-peroxidase conjugate secondary antibodies were obtained from Bangalore Genei (India). Cholesterol was bought from Centron Research Laboratory, Mumbai, INDIA and used after crystallizing it three times with methanol. Tuftsin was modified at C-Terminus by attaching a sufficiently long hydrocarbon fatty acyl residue to the C-terminus through an ethylenediamine spacer arm (Thr-Lys-Pro-Arg-NH-(CH$_2$)$_2$-NH-CO-C$_{15}$H$_{31}$), which permits almost quantitative incorporation into liposomes, following published procedure (Gupta et al. 1986). The rest of the chemicals were of analytical grade of purity and procured locally.

3.2.2. Preparation of tuftsin bearing liposomes

Tuftsin bearing liposomes were prepared from egg PC (49 μmol) and cholesterol (21 μmol) with tuftsin (7-8% by PC weight) following published procedure as standardized in our lab (Owais et al., 1993).

3.2.2. Estimation of liposome intercalated tuftsin

The amount of the tuftsin entrapped in the drug containing liposomes was estimated by BCA method as modified in our lab (Khan et al. 2007).

3.2.3. Animals and treatment

Female, Swiss albino mice average body weight (25 ± 2 gms body weight) were used in the study. They were randomly selected & housed in polycarbonate boxes with steel wire tops and rice husk bedding with free access to rodent diet. Experiments were conducted following mandates approved by the Animal Ethics Committee (Committee for the purpose of control and supervision of Experiments on Animals, Govt. of India). The animals were divided into five groups (Gps.), each comprising of 6 animals. The animals in Gp. I and II were given PBS and sham liposomes were administered in the animals of
Gp. III, whereas the animals of Gp. IV and V were given liposomised tuftsin (50µg/animal) intraperitonially for five consecutive days. Single dose of DMBA (50 mg/kg b. wt. dissolved in 0.2 ml corn oil) was given by gavage to the Gps. II, III and IV on day 6 after 24h from the last dose of tuftsin. Animals from all the Gps. were examined every day for gross morphological changes during the study period. All the animals were sacrificed humanly by cervical dislocation 24h after single dose of DMBA. Liver from each animal was excised and immediately washed with ice-cold saline and stored at -80 °C till further analysis.

3.2.4. Biochemical estimations

The liver tissue was homogenized individually in ice-cold phosphate buffer (pH 7.4) containing 0.15 M KCl. The homogenate centrifuged at 9,000 x g for 10 minutes and supernatant was taken as enzyme source. SOD was analyzed as described elsewhere (Kakkar et al. 1984). Briefly, the assay mixture in a final volume of 3 ml contained 0.052 M sodium pyrophosphate buffer (pH 8.3), 186 µM PMS, 300 µM NBT, 780 µM NADH, enzyme source and Milli Q water. The reaction was initiated by addition of NADH followed by incubation at 37 °C for 90 seconds. The reaction was stopped by addition of 1.0 ml of glacial acetic acid and the contents were shaken vigorously with 4.0 ml of n-butanol, allowed to stand for 10 minutes, centrifuged and butanol layer was separated. The color intensity of chromogen in butanol was measured against butanol using a spectrophotometer. A reaction mixture devoid of enzyme served as control. A single unit of enzyme activity is defined as the quantity of SOD required for 50 % inhibition of reaction. The activity of CAT was analyzed according to the method described elsewhere (Sinha 1972) using H2O2 as substrate. In brief, the reaction in a final volume of 3 ml consisted of phosphate buffer (pH 7.0), 0.2 M H2O2 and enzyme protein. The enzyme activity measured following the disappearance of H2O2 at 570 nm and was
expressed as \( \mu \) moles of \( \text{H}_2\text{O}_2 \) consumed/min/mg protein. Glutathione reductase (GR) activity was determined by the published protocol (Carlberg and Mannervic, 1985). Briefly, the assay mixtures in a final volume of 3.0 ml contained 0.067M phosphate buffer (pH 6.6), NADPH, \( 7.5 \times 10^{-3} \) GSSG (pH 6.6) enzyme and water. The reaction was initiated with enzyme preparation. The difference in optical density per 30 second was measured for 3 minutes at 340 nm against a reference cuvette devoid of GSSG and NADPH. The activity was expressed in nmoles/min/mg protein. Glutathione S-transferase (GST) was analyzed by the method as described elsewhere (Habig et al. 1974). The assay mixture in a final volume of 3.0 ml contained 0.2 M phosphate buffer (pH 6.5), GSH, CDNB, enzyme and water. The reaction was initiated by addition of CDNB. The difference in optical density per 30 seconds was measured at 340 nm for 3 minutes against a reference cuvette devoid of enzyme. The activity was expressed as nmoles CDNB-GSH conjugate/min/mg protein. Lipid peroxidation was analyzed by the method as described elsewhere (Ohkawa et al. 1974). The reaction mixture in a final volume of 3.0 ml contained enzyme, 100 \( \mu \)l of 10% sodium dodesyl sulphate (SDS), 600 \( \mu \)l of 20% glacial acetic acid, 600 \( \mu \)l of 0.8% TBA and water. The mixture was placed in boiling water bath 1 hour and immediately shifted to crushed ice bath for 10 minutes. The mixture was centrifuged at 2500 \( \times \) g for 10 minutes. The amount of thiobarbituric acid reactive substances (TBARS) formed was assayed by measuring OD of supernatant at 535 nm against a blank devoid of enzyme. The activity was expressed as n moles of TBARS/mg of tissue protein using TMP as standard. The protein content of the tissue was determined by the method of (Lowry et al.1951) using bovine serum albumin as standard.

3.2.5. Flow cytometric analysis of apoptosis
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Single cell suspension of liver tissue was prepared by using madimixer and pelleted at 2,000 rpm for 10 min at 4 °C. The cell pellet resuspended in 50 µl cold PBS and fixed in 2 ml of 70 % ice-cold ethanol. Cells were washed followed by treatment with 0.1% Triton X-100 for 5 min. After incubation, cells were again centrifuged and resuspended in 1 ml of PBS, ribonuclease (100 µg/ml) was added, and the cells were incubated at 37 °C for 30 min. After pelleting, cells were resuspended in 1 ml of PBS and 50 µg/ml PI and incubated for 18 h at 4 °C. The 10,000 cells were acquired and analyzed on a flow cytometer using ‘Cell Quest 2.0’ software (Nicoletti et al. 1991).

3.2.6. Measurement of ROS generation

ROS production was monitored by flow cytometrically (Becton-Dickinson, San Jose, CA, USA) using DCFH-DA dye as described elsewhere (Esposti and McLennan 1998). Briefly, single cell suspensions were prepared as described above in PBS supplemented with 50 mM glucose and incubated with 10 µM DCFH-DA at 37 °C for 1 hour. The fluorescence increase, due to the hydrolysis of DCFH-DA to dichlorodihydrofluroscein (DCFH) by some nonspecific cellular esterases and its subsequent oxidation by peroxides was measured. Values were given in terms of mean fluorescence intensity (MFI) using software ‘Cell Quest 2.0’.

3.2.7. Mitochondrial membrane potential (ψm) analysis

Mitochondrial membrane potential was assessed by using Rhodamine 123. Rhodamine 123 accumulates in normal mitochondria and decline of ψm will lead to leakage of Rhodamine 123 from mitochondria, while its fluorescent intensity is reduced. The single cell suspensions were incubated with rhodamine 123 (5µg/ml) for 60 min in dark at 37 °C, harvested and suspended in PBS. Prior to Rhodamine 123, a mitochondrial uncoupler FCCP added for 10 minutes in one sample of control. The mitochondrial membrane
potential was measured using flow cytometry (Becton- Dickinson, San Jose, CA, USA) by the fluorescence intensity (FL-1) of 10,000 cells (Bai et al. 1999).

3.2.8. Western Blotting

Western blotting was carried out as described elsewhere (Towbin et al. 1979) in the liver tissue homogenate. Briefly, protein content of the homogenate was estimated by the routine method using bovine serum albumin as a standard (Lowry et al. 1951). Proteins (30 μg) were resolved on 10% gel followed by electroblotted onto nitrocellulose membranes. The blots were probed with appropriate antibodies (i.e., Bcl-2, Bax, Caspase-3, PI 3-K and Akt at dilutions recommended by the suppliers. To quantify equal loading, membranes were re-probed with β-actin antibody. Data were presented as the relative pixel density of each bands normalized to band of β-actin. The intensity of the bands was quantitated using Alpha Image Analysis software on Alpha Image Gel Documentation System.

3.3. Results

3.3.1. Activities of antioxidant enzymes

Administration of liposomised tuftsin in the model animals resulted in a significant protection against DMBA-induced alteration in antioxidant enzymes activities. Table 3.1 depicts the level of antioxidants SOD, CAT, GR and GST in the liver of control and experimental mice. The extent of the oxidative stress in the liver of animals upon exposure to DMBA (Gp. II) was evident from the significant reduction in the activities of antioxidant enzymes. The activities of SOD, CAT, GR and GST were decreased upto an extent of 39.4 %, 44.5 %, 44.1 % and 48.4 % respectively in comparison to control animals not exposed to DMBA. The pretreatment with tuftsin protect against DMBA induced reduction in antioxidant enzymes. There was 55.8 %, 66.5 %, 61.8 %, 74.8 % residual activity of SOD,
CAT, GR and GST respectively in the animals pretreated with liposomised tuftsin (p<0.001) when compared with animals treated with DMBA only (Gp. II). In the group of animals pretreated with sham liposomes (Gp. III), the activities of SOD, CAT, GR and GST increased but it was not significantly different from the group of animals exposed to DMBA only with no pretreatment with sham liposomes. These results clearly demonstrated that tuftsin could effectively counteract oxidative stress induced by DMBA in liver, the major site of metabolism.

3.3.2. Lipid peroxidation level

Estimation of lipid peroxidation is a reliable marker for xenobiotic induced oxidative stress. As depicted in Table 3.1, DMBA administration resulted in a significant (p<0.001) increase in the TBARS upto an extent of 87% over untreated control group animals (Gp. I). The animals pretreated with sham liposomes (Gp. III) did reduce the level of lipid peroxidation but not to any significant degree when compared with the group of animals exposed to DMBA and not treated with liposomised tuftsin (Gp II). Pretreatment with liposomised tuftsin resulted in significant reduction in level of TBARS, upto an extent of 52 % (p<0.001) when compared with animals treated with DMBA only (Gp. II).

3.3.3. Cell death inhibition

In order to evaluate ability of tuftsin to protect against DMBA induced cell death, we observed appearance of sub G1 peak in liver cells. In general, ROS produced in DMBA metabolism led to the apoptotic cell death (Gp. II), which was observed as appearance of sub G1 peak, whereas no such peak was observed in control healthy animals that were not exposed to DMBA (Gp. I) and also in the group of animals that were treated with tuftsin alone (Gp. V). As depicted in Fig. 3.1, the considerable reduction in cell death events was recorded in the animals pretreated with liposomised tuftsin (Gp. IV).
upon administration of DMBA (Ft/Fu 2.05) (p< 0.05) in comparison to untreated control healthy animals (Ft/Fu 4.2). The loss of mitochondrial membrane potential was restored in the group of animals (Gp. IV) that were pretreated with liposomised tuftsin (Ft/Fu 3.22) (p< 0.05). However, no significant changes were observed in the animals pretreated with sham liposomes (Ft/Fu 2.08) in comparison to DMBA treated animals (Gp. II).

3.3.5. Tetrapeptide tuftsin modifies the expression of PI-3K, Akt, Bcl-2, Bax and Caspase-3.

The exposure of animals to DMBA induced reduction in the expression level of PI-3K, Akt and Bcl-2 (Fig. 3.4B, 3.4D, 3.4E). On the other hand, DMBA ensued in significant upregulation of Bax and Caspase-3 in exposed animals comparison to untreated control animal (Fig. 3.4A, 3.4C). The treatment with liposomised tuftsin resulted in upregulation of PI-3 K, Akt and Bcl-2 levels. While same treatment ensued in decreased expression of Bax and Caspase-3. Treatment of healthy animals with liposomised tuftsin did not induce any significant change in expression level of any protein (Gp. V). The pretreatment with sham liposomes, did not induce any significant change in expression of any proteins, and were almost same to that of DMBA treated animals (Gp. II). These results, supporting our contention that increased expression of PI-3K, Akt, Bcl-2 and decreased expression of Bax and Caspase-3 deactivated the DMBA induced apoptotic pathway in Swiss mice.
Table 3.1. Activities of antioxidant enzymes and lipid peroxidation induced by tuftsin against DMBA in liver of Swiss albino mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Superoxide dismutase (U/mg protein)</th>
<th>Catalase (μmoles/min/mg protein)</th>
<th>Glutathione reductase (μmoles/min/mg protein)</th>
<th>Glutathione S-transferase (μmoles/min/mg protein)</th>
<th>Lipid peroxidation (μmoles TBARS/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>7.1 ± 0.43</td>
<td>290.4 ± 14.7</td>
<td>74.9 ± 5.6</td>
<td>25.4 ± 1.4</td>
<td>1.40 ± 0.1</td>
</tr>
<tr>
<td>DMBA</td>
<td>4.3 ± 0.29 (39.4%)*↓</td>
<td>161.2 ± 12.2 (44.5%)*↓</td>
<td>41.9 ± 2.8 (44.1%)*↓</td>
<td>13.1 ± .87 (48.4%)*↓</td>
<td>2.62 ± 0.18</td>
</tr>
<tr>
<td>DMBA + Sham liposomes</td>
<td>4.6 ± .24 (7 %)</td>
<td>173.2 ± 8.3 (7.5)</td>
<td>45.7 ± 2.6</td>
<td>14.2 ± .78 (8.4 %)</td>
<td>2.43 ± .13</td>
</tr>
<tr>
<td>DMBA + Tuftsin bearing liposomes</td>
<td>6.7 ± 0.34 (55.8 %)↑</td>
<td>268.5 ± 15.5 (66.5 %)↑</td>
<td>67.8 ± 4.1 (61.8 %)↑</td>
<td>22.9 ± 1.4 (74.8 %)↑</td>
<td>1.52 ± 0.8</td>
</tr>
<tr>
<td>Tuftsin bearing liposomes (50μg)</td>
<td>7.3 ± 0.42</td>
<td>302.4 ± 16.2</td>
<td>77.6± 4.13</td>
<td>26.2 ± 1.3</td>
<td>1.38± 0.72</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals. *represent significant decrease over untreated control group (Gp. I), while "represent significant increase over DMBA treated group (Gp. II). Similarly, †represent significant increase over untreated control group (Gp. I), while ‡represent significant decrease over DMBA treated group (Gp. II).
Figure 3.1: *Effect of liposomised tuftsin on cell cycle as revealed by flow cytometric analysis in* DMBA *exposed animals*

Single cell suspension of liver tissues from all treated animals were prepared as described in Materials and Methods. PI fluorescence was measured using a flow cytometer with FL-2 filter and were expressed as histogram. The effect of liposomised tuftsin was expressed by the reduction in sub G1 peak that was induced by the treatment of DMBA indicating increased cell death. (A) Untreated, (B) DMBA, (C) sham liposomes + DMBA, (D) Lip-Tufts + DMBA and (E) Lip-Tufts.
Figure 3.2: Effect of liposomised tuftsins pretreatment by flow cytometric analysis on ROS level in DMBA treated animals.

ROS production was monitored flow cytometrically using DCFH-DA as described in Materials and Methods. The fluorescence increase, due to the hydrolysis of DCFH-DA to dichlorodihydrofluroscein (DCFH) by some nonspecific cellular esterases and its subsequent oxidation by peroxides was measured. Values were given in terms of mean fluorescence intensity (MFI) using software 'Cell Quest 2.0'.

(A) Untreated, (B) DMBA, (C) sham liposomes + DMBA, (D) Lip-Tufts + DMBA and (E) Lip-Tuft.
Figure 3.3: Effect of liposomal tuftsin on mitochondrial membrane potential in the animals exposed to DMBA.

Rhodamine 123 fluorescence was measured using a flow cytometer with FL-1 H filter. (A) Untreated, (B) DMBA, (C) sham liposomes + DMBA, (D) Lip-Tufts + DMBA and (E) Lip-Tufts. Values are expressed as mean ± S.E of six animals.
Figure 3.4: Effect of liposomised tufts in on the expression level of (A) Bcl-2, (B) Bax, (C) Caspase-3, (D) PI3K and (E) Akt in DMBA treated animals. Lysates were prepared as described in Materials and Methods and proteins (30-50 μg) were employed for Western blots. Lane 1, Untreated control; Lane 2, DMBA; Lane 3, sham liposomes; Lane 4, Lip-Tufts + DMBA; Lane 5, Lip-Tufts. Equal loading was confirmed by reprobing the membrane with β-actin. The bands shown here are from a representative experiment repeated three times with similar results. Data were expressed as mean values ± SD of four separate sets of experiment.
3.4. Discussion

The carcinogen DMBA has been shown to form free radicals, which play a critical role in induction of various forms of cancers (Cavalieri et al. 1978). DMBA is metabolized by cytochrome P4501A1 and cytochrome P4501B1 in liver microsomes to form diol epoxides and other toxic ROS such as peroxides, hydroxyl and superoxide anion radicals. In the present study we have shown that DMBA exposure increases the ROS level during its in vivo metabolism. These results are in concurrence with the earlier studies performed on liver and other tissues in other laboratories (Kalra et al. 2005). The metabolites of DMBA including diol epoxides are capable of binding to adenine residues of DNA causing chromosomal damage (Guerin 1978).

ROS play pivotal role in triggering apoptosis, the antioxidant deplete them to halt their activity (Schnabel et al. 2006 and Labriola 1999). Our data demonstrate that apoptosis induced by DMBA, is accompanied by an increase in ROS generation. The observed inhibition of apoptosis in DMBA induced mouse hepatocytes is consistent with the view that tetrapeptide tuftsin deactivates apoptosis machinery. It seems, liposomised tuftsin induces Bcl-2 on one hand and suppression of Bax and Caspase-3 on other. The tuftsin treatment was found to be associated with the increase in the expression level PI3K and Akt, which inhibit the DMBA induced apoptosis.

Mitochondrial transmembrane potential is often employed as an indicator of cellular viability, and its disruption has been implicated in a variety of apoptosis phenomena (Marchetti et al. 1996). Mitochondria outsource ROS during apoptosis and reduction of their membrane potential leads to increased generation of ROS and apoptosis (Zamzami et al. 1995). In the present study tuftsin restored the DMBA induced reduced mitochondria membrane potential and found to prevent normal cell death.
It has been shown that the Bcl-2 family plays an important regulatory role in apoptosis, either as activator (Bax) or as inhibitor (Bcl-2) (Liu et al. 2006, Green et al. 1998, Adams et al 1998). Since Bcl-XL can bind to Bax and prevent Bax insertion into the outer membrane of mitochondria, the decrease in total and mitochondrial Bcl-XL promotes the changes of Δψm. It has also been demonstrated that the gene products of Bcl-2 and Bax play important roles in apoptotic cell death (Jacobson et al. 1995, Jacobson et al. 1994, Oltvai 1993). In the present study, inhibition of DMBA induced cell death by tuftsin in mouse liver can be associated with increased level of PI3K, AKT, Bcl-2, and decreased level of Bax and Caspase-3. Analysis of data indicates that tuftsin may increase the PI-3K and Akt level presumably by the activation of IL-6 inhibiting TGF-β that ultimately disturb the Bcl-2 and Bax level and, therefore, lead to decrease in apoptosis (Chen et al. 1999, Grannoth et al. 1997).

Oxidative stress due to increased ROS levels coupled with deficiency of host antioxidant defense mechanism, might be a determining factor contributing in the development of cancer. To protect cells from oxidative stress, radical and non-radical reactive species including peroxides and superoxides need to be inactivated enzymatically by CAT, SOD, and GPx (Vang et al. 1993). Antioxidant enzymes provide the cellular defense against the intermediates of dioxygen reduction (superoxide radical, hydrogen peroxide and hydroxyl radicals). For example, SOD converts superoxide radicals into hydrogen peroxide, which in turn has to be removed by CAT and GPx. Thus, CAT and GPx protect SOD against inactivation by hydrogen peroxides. Reciprocally, SOD protects CAT and GPx from inhibition by superoxide radicals (Pigeolet et al. 1990). There was a significant decline in the activities of SOD and CAT after DMBA administration, indicative of oxidative stress. The reduced antioxidant enzyme activities in liver were prevented by the pretreatment of tuftsin liposomes (Table. 1). The protection provided by tuftsin liposomes is
possible through radicals scavenging of superoxide radicals and peroxyl radicals. GST perform functions ranging from catalyzing the detoxification of electrophilic species including metabolites of DMBA via spontaneous enzyme catalyzed conjugation reaction to protect the cells against peroxidative damage (Ishikawa et al. 1986). The reduced activity of GST observed upon DMBA administration may be partly due to the lack of its substrate (GSH) (Bhuvaneswari et al. 2005), which usually occurs due to reduced activity of GR.

Lipid peroxidation is one of the main manifestations of oxidative damage initiated by ROS and it has been linked to the altered membrane structure and enzyme activation. It is initiated by the elimination of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane (Bergendi et al. 1999). The present data reveal that DMBA exposure causes a marked oxidative impact as evident from the significant increase in lipid peroxidation. The increase in lipid peroxides might result from increased production of free radicals on one hand and a fall in antioxidant status on the other. Tuftsin liposomes significantly lowered lipid peroxidation and the values were comparable with that of the control animals.

Finally, we can conclude that the present study may open novel prospective in cancer chemoprevention, mostly because of the fact that the central mechanism of apoptosis is evolutionarily conserved, and that ROS and Caspase activation is an essential step in this complex apoptotic pathway. The data also imply that mitochondrial cell death pathway can be used as target for studies on prevention of different type of cancer and that tuftsin merits further investigations for developing strategies for chemoprevention and chemotherapeutics.