Protective effect of liposomal formulation of tuftsin (a naturally occurring tetrapeptide) against cyclophosphamide-induced genotoxicity and oxidative stress in mice

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Received 02 August 2008; revised 16 January 2009

Tuftsin, a naturally occurring tetrapeptide with a sequence Thr-Lys-Pro-Arg was evaluated for its in vivo protective effect against cyclophosphamide-induced genotoxicity and oxidative stress in Swiss albino mice. The anticancer drug cyclophosphamide (CP) was administered intra-peritonially to induce mutagenic effect. The drug treatment caused significant increase in chromosomal aberrations, formation of micronucleated polychromatic erythrocytes (MNPCE’s), as well as oxidative stress and decrease in lipid peroxidation in liver of the animals. The pretreatment with tuftsin abolished such effects in dose-dependent manner and also increased mitotic index in the experimental animals. Results of the present study validated chemo-preventive properties of tuftsin against CP-induced chromosomal mutations and cellular injury of liver by oxidative stress.

Keywords: Genotoxicity, Cyclophosphamide, Tuftsin, Liposome, Oxidative stress

The present day polluted environment harbours many chemicals that are potential cause for cancer and mutagenic risks. These include a broad spectrum of both naturally occurring, as well as synthetic chemicals present in the air, soil, water, food, and the region in which we work and live1. Thus, it is important that chemicals to which people are exposed intentionally (e.g., therapeutics) in the course of their daily chores (e.g., in domestic products, cosmetics etc.) or inadvertently (e.g., pesticides) should be tested for their potential to produce cancer and genetic damage.

Management and therapy for cancer cases involve invariable usage of antineoplastic agents. These agents are toxic to rapidly proliferating cells and, therefore, kill neoplastic tissues of the cancer patient. However, because of their low therapeutic index, they damage proliferating normal tissue as well. Thus, long-term usage of antineoplastic agents is a compromise with many destructive untoward effects and is the subject of increasing concern2. Monitoring mutagenic potential of anticancer agents will help in minimizing immediate harmful effects on the genetic material and also on induction of another kind of cancer in the patients undergoing chemotherapy.

Cyclophosphamide (CP), a commonly used chemotherapeutic drug, is also a well known mutagen and clastogen3. It is an alkylating agent, producing the highly active carbonium ion, which reacts with the extremely electron-rich centers of nucleic acids and proteins. It has been extensively tested to induce dominant lethal mutation, mononuclei, DNA damage and generation of free radicals or reactive oxygen species (ROS) in vivo as well. Free radicals due to their high chemical reactivity induce cellular damage in a number of ways4. The most deleterious effects of free radicals include DNA damage, which can lead to a number of pathological conditions, including cancer5. However, while acting as damaging entities, ROS can at the same time also carry out some important beneficial biological effects. ROS have been shown to be mediators or executioners of essential protective mechanisms, such as apoptosis, phagocytosis and detoxification reactions etc. Increase of ROS concentration by depletion of antioxidants enhances apoptosis and thereby inhibits

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Abbreviations: BCA, bicinconinic acid; CP, cyclophosphamide; GST, glutathione-s-transferase; LPO, lipid peroxidation; MNPCEs, micronucleated polychromatic erythrocytes; PC, phosphatidylcholine; ROS, reactive oxygen species.
neoplastic growth. Excessive antioxidants decrease ROS level, inhibit apoptosis and suppress the elimination of cancer cells.6,7

Tuftsin, a naturally occurring tetrapeptide with the sequence Thr-Lys-Pro-Arg is an integral component of immunoglobulin G and is released from its parent molecule by the enzymatic cleavage.8 It activates various components of the host upon its administration and can be used as a potent activator of macrophages.9,11 Besides its immunopotentiating effect, reports have indicated its role against carcinogenesis as well.12,13 It has also been observed that tuftsin-treated mouse peritoneal macrophages exert cytostatic activity against tumor cell proliferation.12 Although tuftsin displays cytotoxic effect on tumor cells, it does not affect natural killing activity of lymphocytes against K562 tumor cell line at doses of 5 × 10−2 to 5 × 10−5 µg/ml.14 Tuftsin is also shown to significantly increase survival rates among Rauscher leukemia virus-infected mice and demonstrates antitumor activity against murine melanoma in vivo.15,16

Phospholipids, the structural units of plasma membrane, as well as artificial vesicles (liposomes) are also fascinating in terms of important bio-functional compounds. Phospholipids, such as phosphatidylcholine (PC) with 18:0 in the sn-1 position and one of the following fatty acids in the sn-2 position: 18:0, 18:1 omega 9 (oleic), 18:3 omega 3 (α-linolenic), 20:4 omega 6 (arachidonic), 22:6 omega 3 (docosahexaenoic) have been shown to possess antitumor effects in vivo, leading to enhanced longevity of the tumor-bearing animals.17 The effect of polyunsaturated PC and phosphatidylserine (PS) on butyrate-induced growth inhibition, differentiation and apoptosis using Caco-2 cells has also been reported.18

In the present set of experiments, we have developed liposomal formulation of tuftsin and evaluated its ability to reduce CP-induced genotoxicity in bone marrow lymphocytes and hepatic alterations, in terms of lipid peroxidation status of the antioxidant enzymes in mice. The extent of CP-induced mutagenesis has been determined on the basis of incidences of chromosomal aberrations, micronucleated polychromatic erythrocytes (MNPCEs) and biochemical estimation of antioxidant enzymes of liver tissues in the animals.

Materials and Methods

Materials

Cholesterol was obtained from Centron Research Laboratory, Mumbai, India and used after crystallizing three times with methanol. Giemsa stain, foetal bovine serum, May-Grunwald stain and colchicine were purchased from Sigma Chemical Co., USA. Egg phosphatidyl choline (egg PC), cyclophosphamide (CP) and other reagents used in the study were of the highest purity available. Tuftsin was modified by attaching a sufficiently long hydrocarbon fatty acyl residue to the C-terminus through an ethylene-diamine spacer arm (Thr-Lys-Pro-Arg-NH-(CH2)2-NH-CO-C15H31), which permitted almost quantitative incorporation into liposomes, following the procedure described previously.19

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) as described previously.20 Briefly, the mixture of lipids and tuftsin was dissolved in methanol and chloroform and then reduced to a thin dry film in a round bottom flask. Traces of organic solvents were removed by subjecting the flask to vacuum overnight at 4°C. The dried lipid film was hydrated with 150 mM sterile saline, followed by sonication for 1 h at 4°C under N2 atmosphere in bath-type sonicator. The sonicated preparation was centrifuged at 10,000 g for 1 h at 4°C to remove undispersed lipid and extensively dialyzed against saline for 24 h at 4°C in the dark to separate free tuftsin from the liposomal preparations. The tuftsin-bearing liposomal preparation was found out to be of unilamellar type with size range of 80 ± 10 nm, as revealed by electron microscopy (data not shown).

Estimation of liposome intercalated tuftsin

The amount of tuftsin incorporated in liposome was estimated by bicineconic acid (BCA) method following protocol as modified in our lab.21 Solutions A and B of BCA reagent (1:49 ratio v/v) was added to an aliquot of Triton X-100 digested tuftsin-bearing liposomes. The reaction mixture was incubated at 37°C for 45 min. Absorbance was measured at 562 nm and tuftsin content in the preparation was determined using a standard curve of tuftsin plotted in the presence of Triton X-100. Intercalation efficiency of tuftsin was found out to be about 98% in tuftsin-containing liposomes.

Animals and treatment

Female Swiss albino mice, 8-10 weeks old and with average body weight (b.w.) of 20 ± 2 g were used in the study. They were randomly selected and housed in polycarbonate boxes with steel wire tops
and rice husk bedding and 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).

**Treatment schedule**

Pretreatment of the animals with tuftsin was performed using published protocol as described elsewhere. The animals were divided into six groups of 12 animals each. The animals of group I were used as control group and received no drug treatment. Group II animals served as positive control and treated with CP at the single dose of 40 mg/kg b.w. intraperitoneally (i.p.). In group III, the animals were pretreated with sham liposomes for 5 consecutive days, followed by administration of CP. In groups IV, V and VI, liposomised tuftsin was administered to the animals through i.p route, respectively for 5 consecutive days. In groups IV and V, single dose of CP (40 mg/kg b.w.) was administered to the animals 1 h after the last dose of tuftsin pre-treatment. In group VI, animals were treated with tuftsin, followed by no post-CP treatment.

**Chromosomal analysis**

After completion of the treatment schedule, five animals from each group were sacrificed by cervical dislocation. Colchicine was administered at the dose of 4 mg/kg b.w. by i. p. route at 22 h, prior to sacrifice of animals. The bone marrow smears of animals in each group were prepared, following protocol as described elsewhere. Briefly, the bone marrow cells from both femurs of each animal were flushed in the form of fine suspension into a centrifuge tube containing fetal bovine serum (FBS). The cell suspension was centrifuged at 2000 g for 10 min and the supernatant was removed. The pellet was resuspended in a drop of FBS, before being used for slide preparation. The air-dried slides were stained with May Grunwald and Giemsa stains. Two thousand polychromatic erythrocytes (PCEs) were scored per animal to determine the frequency of micronucleated polychromatic erythrocytes (MNPCEs). To avoid personal error, the same person scored all the slides.

The parameters analyzed included the percentage of CP-induced micronuclei as well as its percent suppression caused by tuftsin pre-treatment and parameters were calculated according to the following formula:

\[
\% \text{ MNPCEs in tuftsin and } \times 100 = \frac{100 \times (\text{CP post - treated group}) - (\text{CP alone - treated group})}{\text{% MNPCEs CP post - treated group}}
\]

**Biochemical estimations**

The liver tissue isolated from individual animal was placed in ice-cold sodium phosphate buffer (5 mM, pH 7.4) containing 0.15 M KCl. The homogenate was centrifuged at 9,000 g for 10 min and supernatant was analyzed for various enzymes. Superoxide dismutase (SOD) level was estimated as per the protocol describe elsewhere. Briefly, the 3 ml assay mixture contained sodium pyrophosphate buffer (52 mM, pH 8.3), 186 µM phenazine methosulphonate (PMS), 300 µM nitroblue tetrazolium salt (NBT), 780 µM reduced nicotinamide adenine dinucleotide (NADH), enzyme source and MilliQ water. The reaction was initiated by addition of NADH, followed by incubation at 37 °C for 90 s. It 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 min, centrifuged and butanol layer was separated. The color intensity of chromogen in butanol was measured against neat butanol as a blank. A reaction mixture devoid of enzyme served as control. A single unit of enzyme activity was defined as the quantity of SOD required for 50% inhibition of reaction.
The activity of catalase (CAT) was estimated using H$_2$O$_2$ as substrate$^{25}$. In brief, the reaction in a final volume of 3 ml consisted of phosphate buffer (5 mM, pH 7.0), 0.2 M H$_2$O$_2$ and the enzyme. The enzyme activity was measured, following the disappearance of H$_2$O$_2$ at 570 nm and expressed as μmoles of H$_2$O$_2$ consumed/min/mg protein.

Glutathione reductase (GR) activity was determined by the previously described procedure$^{26}$. Briefly, the assay mixtures in a final volume of 3.0 ml contained sodium phosphate buffer (67 mM, pH 6.6), nicotinamide adenine dinucleotide phosphate (NADPH), 7.5 x 10$^{-3}$ M glutathione disulfide (GSSG) enzyme and water. The reaction was initiated with the enzyme preparation. The difference in optical density (O.D.) per 30 s was measured for 3 min 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 estimated by the method described elsewhere$^{27}$. Assay mixture in a final volume of 3.0 ml contained sodium phosphate buffer (pH 6.5), 1-chloro-2,4-dinitrobenzene (CDNB), enzyme and water. The reaction was initiated by addition of CDNB. The difference in O.D. per 30 s was measured at 340 nm for 3 min against a reference cuvette devoid of the enzyme. The activity was expressed as nmoles CDNB-GSH conjugate/min/mg protein.

Lipid peroxidation (LPO) was estimated by the method described elsewhere$^{28}$. The reaction mixture in a final volume of 3.0 ml contained enzyme, 100 μl of 10% sodium dodecyl sulphate (SDS), 600 μl of 20% glacial acetic acid, 600 μl of 0.8% thiobarbituric acid (TBA) and water. The mixture was placed in boiling water bath for 1 h, immediately shifted to crushed ice bath for 10 min and centrifuged at 2500 g for 10 min. The amount of thiobarbituric acid reactive substances (TBARS) formed was assayed by measuring O.D. of supernatant at 535 nm against a blank devoid of the enzyme. The activity was expressed as nmoles of TBARS/mg of tissue protein using tetramethoxypropane (TMP) as standard.

The protein content of the tissue was determined by the method of Lowry et al$^{29}$ using bovine serum albumin (BSA) as standard.

**Statistical analysis**

All data were evaluated with SPSS ver. 10.0 software. Hypothesis testing methods included One-way Analysis-of-variance (ANOVA), followed by least significant difference (LSD) test. P values of <0.05 were considered statistically significant. All the results were expressed as mean ± SD for six animals in each group.

**Results**

**Chromosomal aberration analysis**

In the pilot study, we used varying doses of CP to induce significant chromosomal aberrations in the animals. A single dose of CP at 40 mg/kg b. w. was found to induce high incidences of chromosomal aberrations in Swiss albino mice. This dose was used in subsequent experiments, where tuftsin pre-treated animals were challenged with same amount of the drug. The exposure with CP resulted 42.5% decrease in mitotic index (p<0.001) in the animals (group II) as compared to normal untreated mice (group I), indicating CP-mediated bone marrow cytotoxicity (Table 1). The tuftsin pre-treated animals, not subsequently exposed to CP (group VI) showed no significant changes in the mitotic index over group I (Table 1). As shown in Table 1, the mitotic index increased by 31% and 61.4% in animals pre-treated

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mitotic index (%)</th>
<th>Incidence of aberrant cells (%)</th>
<th>No. of aberrations/cell</th>
<th>Micronuclei induction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.27 ± 0.09</td>
<td>2.23 ± 0.05</td>
<td>0.02 ± 0.000</td>
<td>0.64 ± 0.023</td>
</tr>
<tr>
<td>II</td>
<td>3.03 ± 0.06*</td>
<td>24.54 ± 0.67**</td>
<td>0.24 ± 0.006*</td>
<td>5.41 ± 0.118*</td>
</tr>
<tr>
<td>III</td>
<td>3.14 ± 0.09</td>
<td>22.10 ± 0.36</td>
<td>0.22 ± 0.003</td>
<td>5.02 ± 0.106</td>
</tr>
<tr>
<td>IV</td>
<td>3.97 ± 0.09**</td>
<td>19.18 ± 0.23**</td>
<td>0.19 ± 0.002*</td>
<td>3.58 ± 0.085**</td>
</tr>
<tr>
<td>V</td>
<td>4.89 ± 0.10***</td>
<td>14.48 ± 0.25***</td>
<td>0.14 ± 0.002***</td>
<td>2.41 ± 0.076***</td>
</tr>
<tr>
<td>VI</td>
<td>5.21 ± 0.13</td>
<td>2.50 ± 0.07</td>
<td>0.02 ± 0.002</td>
<td>0.72 ± 0.021</td>
</tr>
</tbody>
</table>

*Significantly different from untreated controls (group I). **Significantly different from positive control group (group II).
***Significantly different from 25 μg liposomised tuftsin pretreated group (group IV) and positive control group II.
with 25 µg and 50 µg tuftsin (p<0.001) respectively, when compared to CP-exposed control group of animals. No significant difference (only 3% increment) in mitotic index was observed in the animals treated with sham liposomes, when compared to the CP-exposed animals.

Moreover, on pre-treatment with different doses of tuftsin, prior to CP administration (groups III and IV), decreased rates of clastogenic changes were also observed (Table 1). The percentage of aberrant cells was 24.54 ± 0.67 in CP-exposed animals. The aberrant cell count reduced to 19.18 ± 0.23 (p< 0.001) and 14.48 ± 0.25, (p<0.001) in the animals treated with 25 µg and 50 µg liposomised tuftsin, respectively (Table 1). There were 22.10 ± 0.36% aberrant cells in the animals pre-treated with sham liposomes. A decrease in the number of aberrations per cell (both chromosome and chromatic type) was observed in both groups of the animals pretreated with 25 µg as well as 50 µg liposomised tuftsin (Table 1). The calculated suppressive effect of the tuftsin was 21.8% and 41.0% at the administration of 25 µg and 50 µg liposomised tuftsin per animal, respectively (Fig. 1).

**Micronuclei analysis**

The results of micronuclei induction analysis (Table 1) clearly showed the high frequency of micronuclei (5.4% ± 0.118, p<0.001) in the animals exposed to CP, in comparison to normal untreated mice (0.64% ± 0.023). Pre-treatment with various doses of liposomised tuftsin resulted in low frequency of micronuclei induction. As depicted in Table 2, the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Superoxide dismutase (U/mg protein)</th>
<th>Catalase (µmoles/min/mg protein)</th>
<th>Glutathione reductase (nmole/min/mg protein)</th>
<th>Glutathione-s-transferase (nmole/min/mg protein)</th>
<th>Lipid peroxidation (nmole 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>CP</td>
<td>4.3 ± 0.29</td>
<td>161.2 ± 12.2</td>
<td>41.9 ± 2.8</td>
<td>13.1 ± .8</td>
<td>2.62 ± 0.18</td>
</tr>
<tr>
<td>(39.4%)**</td>
<td>(44.5%)**</td>
<td></td>
<td>(44.1%)**</td>
<td>(48.4%)**</td>
<td>(87.1%)****</td>
</tr>
<tr>
<td>CP + Sham liposomes</td>
<td>4.6 ± .24</td>
<td>173.2 ± 8.3</td>
<td>45.7 ± 2.6</td>
<td>14.2 ± .7</td>
<td>2.43 ± .13</td>
</tr>
<tr>
<td>(7 %)</td>
<td>(9 %)</td>
<td></td>
<td>(8.4 %)</td>
<td>(7.25)</td>
<td></td>
</tr>
<tr>
<td>CP + Tufts (25 µg)</td>
<td>5.2 ± 0.28</td>
<td>202.5 ± 11.5</td>
<td>53.7 ± 2.6</td>
<td>17.2 ± 9</td>
<td>1.91 ± 0.1</td>
</tr>
<tr>
<td>(21 %)*</td>
<td>(25.6%)*</td>
<td></td>
<td>(28.2 %)*</td>
<td>(31.3 %)*</td>
<td>(27 %)***</td>
</tr>
<tr>
<td>CP + Tufts (50 µg)</td>
<td>6.7 ± 0.34</td>
<td>268.5 ± 15.5</td>
<td>67.8 ± 4.1</td>
<td>22.9 ± 1.4</td>
<td>1.52 ± 0.8</td>
</tr>
<tr>
<td>(55.8 %)*</td>
<td>(66.5 %)*</td>
<td></td>
<td>(74.8 %)*</td>
<td>(52 %)***</td>
<td></td>
</tr>
<tr>
<td>Tufts (50 µg)</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>

*Significant increase over CP-treated group, **significant decrease over untreated control group, ***significant decrease over CP-treated group (group II), ****significant increase over untreated control group.
frequency of micronuclei observed in the animals pretreated with 25 µg and 50 µg liposomised tuftsin was 3.58% ± 0.085 (p<0.001) and 2.41% ± 0.076 (p<0.001) respectively. There was 5.02 ± 0.106% micronuclei frequency in the group of animals pretreated with sham liposomes (group III). No significant deleterious changes were observed in animals treated with tuftsin alone (group VI). The percentage efficiency of suppression of micronuclei induction was found to be 33.83% (p<0.001) and 55.46% (p<0.001) in the animals pretreated with 25 µg and 50 µg liposomised tuftsin, respectively (Fig. 1).

Activities of antioxidant enzymes

Administration of liposomised tuftsin resulted in significant protection from CP-induced alteration in antioxidant enzymes activities. Table 2 depicts the activities of the enzymatic antioxidants SOD, CAT, GR and GST in the liver of control and experimental animals. The exposure with CP induced oxidative stress in the liver of the treated animals (group II) as evident from the significant reduction in the activities of liver antioxidant enzymes. The activities of SOD, CAT, GR and GST decreased up to an extent of 39.4%, 44.5%, 44.1% and 48.4% respectively over untreated control animals. The tuftsin-pre-treatment at the dose of 25 µg imparted 21%, 25.6%, 28.2%, 31.3% protection in the activity of liver enzymes (SOD, CAT, GR and GST respectively), in comparison to animals treated with CP only (p<0.001) (Table 2). The protection was up to an extent of 55.8%, 66.5%, 61.8%, 74.8% in terms of the activity of SOD, CAT, GR and GST, respectively in the animals pretreated with 50 µg liposomised tuftsin (p<0.001) when compared with animals treated with CP only (group II). In the group of animals pretreated with sham liposomes (group III), the activity of SOD, CAT, GR and GST increased, but was not significantly different from the group of animals exposed to CP only. These results clearly demonstrated that tuftsin could effectively counteract oxidative stress induced by CP at the major site of metabolism i.e., liver.

Lipid peroxidation (LPO) level

Estimation of LPO is a reliable marker for CP-induced oxidative stress. As depicted in Table 2, administration of CP resulted in a significant (p<0.001) increase in the TBARS (up to an extent of 87%) over untreated control (group I). Pre-treatment with liposomised tuftsin resulted in significant reduction in level of TBARS in dose-dependant manner (p<0.001), when compared with animals treated with CP only (group II). Interestingly, pre-treatment with sham liposomes (group III) also reduced level of LPO although not in statistically significant manner.

Discussion

Cyclophosphamide (CP), one of the widely used antitumor agents creates cross-links and strand breaks in the DNA of many cell types, including germ cells30. Such commonly used anticancer agents fail to discriminate normal cells from cancerous cells, thereby killing normally proliferating cells of the body. In fact, usage of most of the available anticancer drugs (including CP) for killing cancer cells is a compromise between necessity and undesirable toxicity to normal host cells. CP is activated by mixed-function oxidases to 4-hydroxycyclophosphamide that spontaneously decomposes to phosphoramide mustard and acrolein31. Phosphoramide mustard binds strongly to DNA, inducing single strand DNA lesions, while acrolein besides depleting GST levels also reacts with DNA to form DNA adducts32. It can also cause protein modification and lipid peroxidation. Further, CP and its metabolites have been reported to induce oxidative stress in mice33. One such metabolite is 4-hydroperoxycyclophosphamide which can cause oxidative damage to cellular as well as isolated DNA34.

Intraperitoneal administration of CP causes an increase in LPO products, on one hand, and lowers the SOD levels on the other32-34. This suggests that the generation of oxidative products is related to the DNA damage caused by CP. In agreement with earlier reports, in the present study, we have also observed depletion of liver antioxidant enzymes with an increase in LPO and DNA damage in animals upon their exposure to CP.

The data of the present study further establish that pre-treatment of animals with liposomised tuftsin can decrease CP-induced oxidative stress and DNA damage. CP causes chromosomal aberrations such as chromosomal or chromatid breaks, chromosome-chromatid exchange and ring chromosome35,36. Interestingly, tuftsin pre-treatment nullifies CP-induced chromatid/chromosome gaps, chromatid breaks as well as chromatid deletions, fragmentations and mitotic disruptional abnormalities (hypoploidy stickness, clumping, pulverization,
polyploidy) in bone marrow cells (Table 1). Moreover, the frequency of micronuclei induction in bone marrow cells is also inhibited (Table 1). Micronuclei assay is widely used to evaluate the property of cytotoxic chemicals to induce chromosome breaks or to damage the mitotic spindle apparatus. The observed dose-related decrease in incidence of micronuclei can be attributed to an inhibitory effect of liposomised tuftsin on CP-induced in vivo chromosomal damage.

ROS generated by CP are capable of damaging macromolecules, including DNA, presumably by depleting antioxidant enzymes. The decrease in the DNA damage and improved status of antioxidant enzymes indicate that liposomised tuftsin is capable of preventing damage to DNA caused by generation of ROS. Some of our preliminary studies demonstrate that tuftsin alleviates CP-induced damage to calf thymus DNA as well (data not shown). However, it is premature to comment upon the possible mechanism(s) by which tuftsin prevents CP induced mutagenicity and requires extensive studies in this regard.

The results of the present study suggest that besides induction of early recovery and abolition of morphological or functional changes in rapidly dividing lymphocytes, tuftsin possesses strong antimutagenic properties as well as ability to correct CP-induced aberration at the chromosomal level. Since mutations induced at cytogenetic level are probable cause of cancer and the fact that tuftsin has both antimutagenic and antitumorogenic properties suggests that it can be exploited in both prophylactic and therapeutic intervention against cancer.

Earlier, we have demonstrated that pre-treatment with liposomised tuftsin leads to the successful elimination of a range of pathogens in mice rendered leukopenic by exposure to CP that induces depletion of neutrophils as well as peripheral blood mononuclear cells. The higher efficacy of tuftsin-bearing liposomal preparations of antibiotics can be attributed to tuftsin-mediated early recovery of immune cells and activation of monocytes and macrophages. Involvement of immunomodulators, such as tuftsin for elimination of cancerous cells seems to offer a novel strategy that can be exploited for treatment of cancer. In this regard, combination of tuftsin and some potent tumoricidal agents may prove to impart improved therapeutic index against treatment of various types of cancer.

Acknowledgements
We are thankful to our Coordinator Prof. M Saleemuddin for allowing us to avail institute’s facilities. Arif Khan acknowledges ICMR for Senior Research Fellowship.

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