Protective effects of *Mentha piperita* Linn on benzo[a]pyrene-induced lung carcinogenicity and mutagenicity in Swiss albino mice

R.M. Samarth*, Meenakshi Panwar, Madhu Kumar and Ashok Kumar

Radiation and Cancer Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur-302 004, India

The chemopreventive and antimutagenic effects of an aqueous extract of *Mentha piperita* leaves were evaluated by using 9 week medium term model of benzo[a]pyrene (BP)-induced lung tumors. Lung tumors were induced by a single subcutaneous injection in the scapular region with BP in newborn Swiss albino mice (<24 h old). The oral administration of *Mentha* extract (ME) showed a significant reduction in the number of lung tumors from an incidence of 67.92% in animals given only BP to 26.31%. The inhibition rate was 61.26% in ME treated group with respect to reference group (BP-alone). However, tumor multiplicity was reduced from 0.83 in the BP-alone group to 0.31 in the BP + ME group. Also, ME treatment reduced the frequency of BP-induced chromosomal aberrations and micronuclei in bone marrow cells and decreased the levels of lipoperoxides and increased sulfhydryl groups in liver as well as lung. In cell-free assays, ME showed strong scavenging activity for both the DPPH* and ABTS* radicals. ME had an IC₅₀ value of 272 µg/ml in the DPPH* assay. The chemopreventive action and antimutagenic effects observed in the present study is attributed to the antioxidative and radical scavenging properties of ME.

**Materials and methods**

**Animals**

Swiss albino mice (*Mus musculus*) 6–8 weeks old were procured from Hamadard University, Delhi and maintained as an inbred colony. Newborn mice (<24 h old) of both the sexes were used for the experiments. The animals were maintained at a temperature of 24 ± 3°C and housed in polypropylene cages. After weaning at 3 weeks of age, the animals were fed standard mouse feed (Hindustan Lever, Delhi, India), and provided tap water ad libitum.

**Mentha extract**

Plant material *M. piperita* Linn. was collected locally and identified, and the specimen was placed at Herbarium, Department of Botany, University of Rajasthan, Jaipur (Voucher number-RUBL-19443). Freshly collected leaves of *M. piperita* were air-dried, powdered and extracted with double-distilled water (DDW) by refluxing for 36 h (12 h × 3) at 80°C. The extract was vacuum evaporated to a powder. The extract was dissolved in DDW just before oral administration. A preliminary drug tolerance study and the selection of the optimum dose were described in an earlier report (16).

**Benzo[a]pyrene**

BP (purity 97% high-performance liquid chromatography), CAS Number 50-32-8, B-1760 Lot Number 043K3524 obtained from Sigma-Aldrich, St Louis, MO was used in the present investigation.

**Yun's medium-term anticarcinogenicity test model**

Newborn Swiss albino mice (<24 h old) were given a single subcutaneous injection in the scapular region with 0.02 ml of BP (0.5 mg/mouse using a suspension of BP in 1% aqueous gelatin). After weaning, the test material (ME) or control (DDW) was administered in a volume of 0.1 ml for 6 weeks by oral gavage once daily. All mice were sacrificed at the ninth week after birth (19).

*To whom correspondence should be addressed. Tel: +91 9414458883/+91 141 2711158; Fax: +91 141 2701137; Email: rmsamarth@yahoo.co.in

© The Author 2006. Published by Oxford University Press on behalf of the UK Environmental Mutagen Society.

All rights reserved. For permissions, please email: journals.permissions@oxfordjournals.org
Experimental design

Group I (Control): The animals of this group were given DDW for 6 weeks (after weaning) by oral gavage.

Group II (ME): After weaning, the animals in this group were given ME (1 gm/kg body weight once daily) for 6 weeks by oral gavage.

Group III (BP): This group of animals was subcutaneously injected once in the scapular region with 0.02 ml of BP (0.5 mg/mouse using a suspension of BP in 1% aqueous gelatin).

Group IV (BP + ME): The animals in this group were subcutaneously injected once in the scapular region with 0.02 ml of BP as in Group III. After weaning, ME was administered for 6 weeks by oral gavage as in Group II.

All mice were sacrificed at the ninth week after birth, the lungs were excised and fixed in Telyesniczky’s solution (100 ml of 70% ethanol, 3 ml formalin and 5 ml glacial acetic acid), and the numbers of tumors on surface were counted by visual inspection using a lens. To obtain an index of tumor incidence, the percentage of tumor-bearing mice was calculated for each group. Tumor multiplicity was defined as the average number of tumors per mouse and was obtained by dividing the total number of tumors by the total number of mice per group, including non-tumor-bearing animals (19).

Cytogenetic studies

Chromosomal aberration analysis

Chromosomal aberration analysis in bone marrow cells was performed at the end of the experiment. The animals were injected i.p. with 0.1 ml of 0.025% colchicine and sacrificed 2 h later by cervical dislocation. Both femurs were dissected out, and metaphase plates were prepared by the air drying method (20). Briefly, bone marrow from the femur was aspirated, washed in saline, treated hypotonically (0.6% sodium citrate), fixed in 3:1 methanol:acetic acid and dried. Slides were prepared and stained with 4% Giemsa (Sigma, St Louis, MO). Chromosomal aberrations were scored under a light microscope. A total of 400 metaphase plates were scored per animal for chromatid breaks, chromosome breaks, fragments, rings, exchanges and dicentrics. Breaks that involved both the chromatids were termed ‘chromosome-type’ aberrations, while ‘chromatid-type’ aberration involved only one chromatid. If the deleted portion had no apparent relation to a specific chromosome, it was called a fragment (21).

Micronucleus assay

The method of Schmid (22) was employed for the micronucleus assay. After the 9 week treatment protocol, the femurs were dissected out and the bone marrow was flushed out, mixed with a vortex mixer, and the cells pelleted by centrifugation. The pellet was resuspended in a few drops of citrate), fixed in 3:1 methanol:acetic acid and dried. Slides were added to 5 ml of a 0.004% methanol solution of DPPH*.

ABTS radical cation decolorization assay

The 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS**) decolorization test was also used to assess the antioxidant activity of ME. The ABTS assay was carried out using the improved assay of Re et al (26). In brief, ABTS** was generated by oxidation of ABTS with potassium persulphate. ABTS was dissolved in deionized water at a concentration of 7 mM, and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left at room temperature overnight (12–16 h) in the dark before use; the ABTS** solution then was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 1 ml of the diluted ABTS solution to 10 μl of ME and mixing, absorbance readings were taken at 30°C at intervals of exactly 1–6 min later. All determinations were carried out in triplicate.

Statistical analysis

Statistical comparison was made using χ²-test for tumor study. Results of cytogenetic and biochemical studies were evaluated using Student’s t-test.

Results

Newborn Swiss albino mice (<24 h old) which were subcutaneously injected once in the scapular region with 0.02 ml of benzo[a]pyrene (0.5 mg suspension of BP in 1% aqueous gelatin) showed a significant reduction in body weight and weight of lungs at the termination of experiment (9 weeks). A single subcutaneous injection of BP resulted in a lung tumor incidence of 67.92% (Table I).

When ME was administered after the BP treatment, the lung tumor incidence was significantly reduced to 26.31%. There was a significant increase in the average weight of mice and in...
the weight of the lungs of animals that were treated with BP and ME in comparison with the average weight of mice and lungs in animals treated with BP alone. Also, the tumor multiplicity was 0.83 in mice treated with BP alone, whereas, ME treatment resulted in a tumor multiplicity of 0.31. The inhibition rate was increased in extract treated group as it was observed as 61.26% with respect to reference (BP alone). Thus the results of the present investigation indicate that ME has chemopreventive activity against BP-induced lung carcinogenicity in Swiss albino mice.

A single subcutaneous injection of BP in the scapular region of newborn Swiss albino mice resulted in significantly increased chromosomal anomalies in bone marrow cells 9 weeks later. In Group III, significant increases were observed for chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments (Table II). Significant increases for this group were also observed in the percentage of pulzerized cells, polyploids, aberrant cells and aberrations/damaged cell in BP-treated newborn Swiss albino mice.

Table I. Protective effect of M.piperita extract (ME) on BP-induced lung carcinogenicity in Swiss albino mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of mice</th>
<th>Mean weight of mice (g ± SE)</th>
<th>Mean weight of lungs (g ± SE)</th>
<th>Lung adenoma incidence (%)</th>
<th>Inhibition rate (%)</th>
<th>Tumor multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: control</td>
<td>30</td>
<td>27.54 ± 0.60</td>
<td>0.28 ± 0.005</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group II: ME</td>
<td>30</td>
<td>28.20 ± 0.58</td>
<td>0.29 ± 0.004</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group III: BP</td>
<td>53</td>
<td>19.3 ± 0.4a</td>
<td>0.20 ± 0.004b</td>
<td>67.92 (36)</td>
<td>Reference</td>
<td>0.83 (444)</td>
</tr>
<tr>
<td>Group IV: BP + ME</td>
<td>76</td>
<td>24.66 ± 0.54a</td>
<td>0.22 ± 0.005a</td>
<td>26.31* (20)</td>
<td>61.26a</td>
<td>0.31 (23)</td>
</tr>
</tbody>
</table>

Weight of mice and weight of lung: Statistical comparisons: Group I versus Group II; Group III versus Group I; Group III versus Group IV; Level of significance: *P < 0.05, †P < 0.001 and ‡P < 0.005 (using Student’s t-test).

*Significant at 0.001 using χ²-test.

†Number of tumor bearing mice.

Table II. Protective effect of ME on BP-induced chromosomal aberrations in newborn Swiss albino mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Chromatid breaks (%)</th>
<th>Chromosome breaks (%)</th>
<th>Centric rings (%)</th>
<th>Dicentrics (%)</th>
<th>Exchanges (%)</th>
<th>Fragments (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Control</td>
<td>0.16 ± 0.06</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>1.10 ± 0.37</td>
</tr>
<tr>
<td>Group II: ME</td>
<td>0.14 ± 0.05 (n.s.)</td>
<td>0.00 ± 0.00 (n.s.)</td>
<td>0.00 ± 0.00 (n.s.)</td>
<td>0.00 ± 0.00 (n.s.)</td>
<td>0.00 ± 0.00 (n.s.)</td>
<td>0.98 ± 0.32 (n.s.)</td>
</tr>
<tr>
<td>Group III: BP</td>
<td>11.20 ± 1.32b</td>
<td>6.72 ± 0.76b</td>
<td>1.42 ± 0.28b</td>
<td>2.98 ± 0.62b</td>
<td>1.48 ± 0.28b</td>
<td>128.42 ± 7.60b</td>
</tr>
<tr>
<td>Group IV: BP + ME</td>
<td>1.84 ± 0.42b</td>
<td>1.20 ± 0.0b</td>
<td>1.02 ± 0.02 n.s.</td>
<td>0.94 ± 0.01b</td>
<td>0.22 ± 0.01c</td>
<td>4.80 ± 1.32b</td>
</tr>
</tbody>
</table>

Each value represents mean ± SE/cell. 400 metaphases were scored/animal. Statistical comparisons: Group I versus Group II; Group III versus Group I; Group III versus Group IV. Level of significance: aP < 0.05, bP < 0.001 and cP < 0.005; n.s., nonsignificant.

Table III. Protective effect of ME on the frequency of pulzerized cells, polyploids, aberrant cells and aberrations/damaged cell in BP-treated newborn Swiss albino mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Pulverized cells (%)</th>
<th>Polyploids (%)</th>
<th>Aberrant cells (%)</th>
<th>Total aberrations (%)</th>
<th>Aberrations per damaged cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: control</td>
<td>0.00 ± 0.00</td>
<td>0.12 ± 0.02</td>
<td>0.52 ± 0.05</td>
<td>0.72 ± 0.05</td>
<td>1.22 ± 0.01</td>
</tr>
<tr>
<td>Group II: ME</td>
<td>0.00 ± 0.00 (n.s.)</td>
<td>0.11 ± 0.01 (n.s.)</td>
<td>0.53 ± 0.04 (n.s.)</td>
<td>0.68 ± 0.06 (n.s.)</td>
<td>1.01 ± 0.01 (n.s.)</td>
</tr>
<tr>
<td>Group III: BP</td>
<td>4.32 ± 0.08b</td>
<td>3.18 ± 0.08b</td>
<td>42.62 ± 6.20b</td>
<td>152.02 ± 10.20b</td>
<td>3.56 ± 0.46b</td>
</tr>
<tr>
<td>Group IV: BP + ME</td>
<td>0.42 ± 0.02b</td>
<td>0.31 ± 0.01b</td>
<td>6.12 ± 0.84b</td>
<td>10.02 ± 1.58b</td>
<td>1.63 ± 0.49a</td>
</tr>
</tbody>
</table>

Each value represents mean ± SE/cell. 400 metaphases were scored/animal. Statistical comparisons: Group I versus Group II; Group III versus Group I; Group III versus Group IV. Level of significance: aP < 0.05 and bP < 0.001; n.s., nonsignificant.

Table IV. Protective effect of ME on BP-induced micronucleus frequency in Swiss albino mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of micronuclei/1000 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: control</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td>Group II: ME</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Group III: BP</td>
<td>20.82 ± 1.81b</td>
</tr>
<tr>
<td>Group IV: BP + ME</td>
<td>2.92 ± 0.72b</td>
</tr>
</tbody>
</table>

Statistical comparisons: Group I versus Group II; Group III versus Group I; Group III versus Group IV. 

*P < 0.001

Modulatory effects of ME treatment on the levels of sulfhydryl groups and TBARS in the livers and lungs of the Swiss albino mice were evaluated in the present study. The Group III (BP alone) animals had significantly (P < 0.001) decreased hepatic and lung SH-group content and a significantly increased extent of hepatic and lung LPO as compared with Group I and Group II. However, Group IV (BP + ME) animals had a significantly (P < 0.001) decreased liver and lung LPO level and increased sulfhydryl content compared with those of Group III. The Group IV values approached those seen in Groups I and II (Table V).
The radical scavenging activity of ME was evaluated using the DPPH* and ABTS**+ assays. In the present investigation ME displayed strong radical scavenging activity in both the assays. The ME had an IC_{50} value of 272 µg/ml in the DPPH* scavenging assessment (Figure 1). The ABTS**+ assay showed effect was complete within 1 min (Figure 2).

Discussion
Polycyclic aromatic hydrocarbons comprise an important class of environmental genotoxins, and the most extensively studied member of this class of chemicals is BP. This compound is ubiquitous in the environment, is mutagenic in both prokaryotic and eukaryotic test systems, and is an animal carcinogen (27–28). BP itself is unreactive, but it is converted to a highly-reactive electrophile by enzymes involved in drug metabolism. The routes of metabolism of BP are complex, but the ultimate genotoxic metabolite produced by the cytochrome P450 (CYP) system has been identified as the anti isomer of BP 7,8-diol-9,10-epoxide. Three enzymatic reactions are required for its formation: initial epoxidation to yield the 7,8-epoxide, hydrolysis of this epoxide to yield the (−)-trans-7,8-diol, and finally a second epoxidation of the diol to produce BP-7,8-diol-9,10-epoxide (anti isomer) (29). The present study demonstrated that oral administration of ME has chemopreventive and antimutagenic effects against BP in Swiss albino mice. The ME produced significant reductions in the lung tumor incidence and tumor multiplicity and significant increases in body weight and the weight of the lungs in Swiss albino mice treated with BP as newborns.

The action of tumor promoters and the process of tumor promotion in the mouse skin system have been discussed, such as the frequency, reversibility and transient nature of conversion of initiated cells, the dependence of tumor promotion on DNA synthesis, the induction of DNA breaks by tumor promoters, and the protective effect of free radical scavengers. Kaina (30) evaluated chromosomal aberrations as a contributing factor for tumor promotion in the mouse skin. However, the timing of ME treatment in present investigation suggests that it is affecting in the tumor promotion phase of carcinogenesis.

ME treatment in the present investigation resulted in a significant decrease in the BP-induced lung tumor incidence. Also, corresponding decreases in frequencies of chromosomal aberrations and micronuclei were observed in the animals of this group. Villasenor et al. (31) have reported on a new anti-mutagen from Menhia cordifolia Opiz. A dose of 0.01 mg/20 g mouse poisoned the genotoxicity of tetracycline by 68.7%. Micronuclei arise as a consequence of clastogenic or aneugenic action and this end-point is widely used to evaluate the genotoxic potential of test agents (32–33). However, because micronucleus and chromosome aberration analysis in the present investigation was conducted 9 weeks after BP treatment, it very unlikely that the increased frequencies reflect the direct genotoxic effects of BP. Steinel et al. (34) evaluated the effect of single topical applications of 7,12-dimethylbenz[a]anthracene (DMBA) that result in significant increase in papilloma incidence, and showed that DMBA also significantly increased the frequency of micronuclei and other chromosomal anomalies in keratinocytes. Furstemberger et al. (35) also demonstrated a correlation between tumor induction and chromosomal damage in the conversion stage (stage I of tumor promotion) in mouse skin. Haesen et al. (36) studied the induction of micronuclei and karyotype aberrations during mouse skin carcinogenesis. A complete carcinogenic protocol produced significant increases in the frequency of micronuclei but the time of micronucleus appearance differed, indicating that the accumulation of aberrations could be more important than the order of appearance. Results of the present study support the concept that the induction of chromosomal aberrations plays an important role in tumor development.

Table V. Protective effect of ME on reduced sulfhydryl (measured as GSH) and lipid peroxidation levels (measured as TBARS) in the liver and lung of BP-treated Swiss albino mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Liver</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH content</td>
<td>TBARS level</td>
</tr>
<tr>
<td>Group I: control</td>
<td>64.39 ± 1.63</td>
<td>1.14 ± 0.09</td>
</tr>
<tr>
<td>Group II: ME</td>
<td>64.81 ± 3.10</td>
<td>1.01 ± 0.12</td>
</tr>
<tr>
<td>Group III: BP</td>
<td>38.16 ± 1.82^b</td>
<td>4.32 ± 0.19^b</td>
</tr>
<tr>
<td>Group IV: BP + ME</td>
<td>54.22 ± 1.78^b</td>
<td>2.48 ± 0.15^b</td>
</tr>
</tbody>
</table>

Statistical comparisons: Group I versus Group II; Group III versus Group I; Group IV: BP-treated Swiss albino mice and lipid peroxidation levels (measured as TBARS) in the liver and lung of Swiss albino mice. GSH = μmol/g tissue; TBARS = nmol/mg tissue.

Fig. 1. Inhibition (%) of DPPH radical scavenging activity of *M. piperita* leaf extract at various concentrations. Stock solution of crude ME was prepared as 1 mg/ml in methanol. Fifty microlitres of samples of different concentration were added to 5 ml of 0.004% methanol solution of DPPH*. After 30 min incubation in dark at room temperature, the absorbance was read against a blank at 517 nm. IC_{50} value was calculated by linear regression analysis.

Fig. 2. The effect of time on the suppression of absorbance of ABTS**+ by *M. piperita* leaf extract. After addition of 1 ml of diluted ABTS solution (A734 nm = 0.700 ± 0.020) to 10 μl of plant extracts the absorbance reading was taken at 30°C exactly 1 min., after initial mixing and up to 6 min. All determinations were carried out in triplicates.

Polycyclic aromatic hydrocarbons comprise an important class of environmental genotoxins, and the most extensively studied member of this class of chemicals is BP. This compound is ubiquitous in the environment, is mutagenic in both prokaryotic and eukaryotic test systems, and is an animal carcinogen (27–28). BP itself is unreactive, but it is converted to a highly-reactive electrophile by enzymes involved in drug metabolism. The routes of metabolism of BP are complex, but the ultimate genotoxic metabolite produced by the cytochrome P450 (CYP) system has been identified as the anti isomer of BP 7,8-diol-9,10-epoxide. Three enzymatic reactions are required for its formation: initial epoxidation to yield the 7,8-epoxide, hydrolysis of this epoxide to yield the (−)-trans-7,8-diol, and finally a second epoxidation of the diol to produce BP-7,8-diol-9,10-epoxide (anti isomer) (29). The present study demonstrated that oral administration of ME has chemopreventive and antimutagenic effects against BP in Swiss albino mice. The ME produced significant reductions in the lung tumor incidence and tumor multiplicity and significant increases in body weight and the weight of the lungs in Swiss albino mice treated with BP as newborns.

The action of tumor promoters and the process of tumor promotion in the mouse skin system have been discussed, such as the frequency, reversibility and transient nature of conversion of initiated cells, the dependence of tumor promotion on DNA synthesis, the induction of DNA breaks by tumor promoters, and the protective effect of free radical scavengers. Kaina (30) evaluated chromosomal aberrations as a contributing factor for tumor promotion in the mouse skin. However, the timing of ME treatment in present investigation suggests that it is affecting in the tumor promotion phase of carcinogenesis.

ME treatment in the present investigation resulted in a significant decrease in the BP-induced lung tumor incidence. Also, corresponding decreases in frequencies of chromosomal aberrations and micronuclei were observed in the animals of this group. Villasenor et al. (31) have reported on a new anti-mutagen from Menhia cordifolia Opiz. A dose of 0.01 mg/20 g mouse poisoned the genotoxicity of tetracycline by 68.7%. Micronuclei arise as a consequence of clastogenic or aneugenic action and this end-point is widely used to evaluate the genotoxic potential of test agents (32–33). However, because micronucleus and chromosome aberration analysis in the present investigation was conducted 9 weeks after BP treatment, it very unlikely that the increased frequencies reflect the direct genotoxic effects of BP. Steinel et al. (34) evaluated the effect of single topical applications of 7,12-dimethylbenz[a]anthracene (DMBA) that result in significant increase in papilloma incidence, and showed that DMBA also significantly increased the frequency of micronuclei and other chromosomal anomalies in keratinocytes. Furstemberger et al. (35) also demonstrated a correlation between tumor induction and chromosomal damage in the conversion stage (stage I of tumor promotion) in mouse skin. Haesen et al. (36) studied the induction of micronuclei and karyotype aberrations during mouse skin carcinogenesis. A complete carcinogenic protocol produced significant increases in the frequency of micronuclei but the time of micronucleus appearance differed, indicating that the accumulation of aberrations could be more important than the order of appearance. Results of the present study support the concept that the induction of chromosomal aberrations plays an important role in tumor development.

Discussion
Polycyclic aromatic hydrocarbons comprise an important class of environmental genotoxins, and the most extensively studied member of this class of chemicals is BP. This compound is ubiquitous in the environment, is mutagenic in both prokaryotic and eukaryotic test systems, and is an animal carcinogen (27–28). BP itself is unreactive, but it is converted to a highly-reactive electrophile by enzymes involved in drug metabolism. The routes of metabolism of BP are complex, but the ultimate genotoxic metabolite produced by the cytochrome P450 (CYP) system has been identified as the anti isomer of BP 7,8-diol-9,10-epoxide. Three enzymatic reactions are required for its formation: initial epoxidation to yield the 7,8-epoxide, hydrolysis of this epoxide to yield the (−)-trans-7,8-diol, and finally a second epoxidation of the diol to produce BP-7,8-diol-9,10-epoxide (anti isomer) (29). The present study demonstrated that oral administration of ME has chemopreventive and antimutagenic effects against BP in Swiss albino mice. The ME produced significant reductions in the lung tumor incidence and tumor multiplicity and significant increases in body weight and the weight of the lungs in Swiss albino mice treated with BP as newbor
Because of the timing of ME administration, the modulation of xenobiotic enzyme activity may be more likely to act on reactive species produced during the promotion phase of carcinogenesis rather than metabolites of BP itself. ROS formed during BP metabolism or secondarily during tumor formation can diffuse from the site of generation to other targets within the cells or even propagate the injury to other intact cells. These ROS produce deleterious effects by initiating lipid peroxidation directly or by acting as second messengers for the primary free radicals that initiate lipid peroxidation (37). Thus, the enhanced hepatic LPO in BP-treated animals may be due to the generation of ROS exacerbated by decreased efficiency of host antioxidant defense mechanisms. The liver, which is rich in GSH, supplies this antioxidant to various extracellular tissues via a distinct GSH transport system (17). GSH maintains the integrity of the liver when the organ is challenged by a wide variety of xenobiotics, ROS and toxic compounds (38). The depletion of GSH resulting from increased utilization to scavenge lipid peroxides may shift the redox status towards oxidative stress. The GSH content in liver and lung tissues observed in present study was significantly elevated suggesting a protective role of ME.

The potential for oxidative stress due to the ROS overproduction coupled with a deficiency in host antioxidant defense mechanisms that was observed in the present study might be important factors contributing to the increase in bone marrow micronuclei and chromosome aberrations. Mayer et al. (39) demonstrated a positive correlation between LPO status and genotoxicity as reflected by increased micronucleus formation in lymphocytes. Treatment with ME effectively reduced the frequency of BP-induced bone marrow micronuclei as well as the extent of hepatic LPO and enhanced the antioxidant status. Elevated levels of GSH protect cellular proteins against oxidation through the glutathione redox cycle and also detoxify ROS directly and/or neutralize reactive intermediate species generated from exposure to xenobiotics, including chemical carcinogens (40).

The present study also demonstrated that ME has a strong radical-scavenging activity in both the DPPH* and ABTS** assays. Mimica-Dukic et al. (41) observed that the essential oils of M. piperita and Mentha longifolia were more active than that of Mentha aquatica in these assays and reported that M. piperita oil had a IC_{50} = 2.53 \mu g/ml in the DPPH assay. This study also found that M. piperita oil exhibited OH-radical scavenging activity, reducing OH-radical generation in the Fenton reaction by 24%. The most powerful scavenging compounds in M. piperita oil were monoterpenic ketones. Yu et al. (42) suggested that spearmint tea protects against IQ and possibly other heterocyclic amines through inhibition of carcinogen activation and via direct effects on the activated metabolites. The total phenol content and quantitative compositional analysis of aqueous extracts from other Mentha species, hybrids, varieties and cultivars also were studied (43–44). M. piperita extract had greater radical scavenging activity than the related extracts and the activity was strongly associated with the phenolic content. Thus, the chemopreventive action and antigenotoxic effect of ME may be due to its antioxidative and radical scavenging properties.

Acknowledgement

Financial support to R.M.S. (Research Associateship) from Council of Scientific and Industrial Research [CSIR], New Delhi, is gratefully acknowledged.

References


Received September 30, 2005; revised December 4, 2005; accepted December 15, 2005