

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 anti-oxidative and radical scavenging properties of ME.

Introduction

The use of plant based natural products as chemopreventive agents is drawing a lot of attention and considered to be practically beneficial in certain cell/tissue based systems and animal model systems. It is necessary to provide scientific proof to justify the use of a plant or its active principles for medicinal purposes (1). Modern drugs, plants and plant extracts must be characterized after their pharmacological screening for their pharmacokinetic and pharmacodynamic properties, including toxicity (2). Cancer chemoprevention is defined as the use of chemicals or dietary components to block, inhibit, or reverse the development of cancer in normal or preneoplastic tissue. A large number of potential chemopreventive agents have been identified, and they function by mechanisms directed at all major stages of carcinogenesis (3–5).

Peppermint, *Mentha piperita* Linn (Family, Labiatae) is aromatic and has stimulant and carminative properties. It currently is being used for alleviating nausea, flatulence and vomiting (6). *Mentha* extract (ME) has antioxidant and antiperoxidant properties (7–9). ME and its oil also showed antibacterial and antifungal activities against *Pseudomonas solanaceum* and *Aspergillus niger*, and *Alternaria alternata*

and *Fusarium chlamydosporum*, respectively (10–11). Vokovic-Gacic and Simic (12) showed that MEs could enhance error-free repair of DNA damage. Samman *et al.* (13) reported that *M.piperita* has a chemopreventive effect against the tumorigenicity of shamma and that this activity could be due to antimutagenic properties. It has been reported *M.piperita* (Linn) leaf extract provides protection against radiation-induced alterations in intestinal mucosa (14), chromosomal damage in bone marrow (15) and blood components and modulates values of serum acid and alkaline phosphatase activities in gamma-irradiated mice (16).

Recent focus of cancer chemoprevention is on intermediate biomarkers capable of detecting early changes that can be correlated with inhibition of carcinogenic progression. Short-term tests such as the mouse bone marrow micronucleus assay, chromosomal aberration analysis, assessment of antioxidant status and reactive oxygen species (ROS)-induced lipid peroxidation, are widely used in the detection and evaluation of antimutagens and anticarcinogens (17–18). Therefore, the present investigation was undertaken to evaluate the protective activity of *M.piperita* leaf extract on benzo[a]pyrene (BP)-induced lung carcinogenicity and mutagenicity in Swiss albino mice.

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

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 Tellyesniczky'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 dicentric. 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 fetal calf serum. Smears were made on pre-cleaned, dry slides, and the slides were air dried and fixed in absolute methanol. The slides then were stained with May-Grün-wald's and Giemsa stains. The proportion of polychromatic erythrocytes and normochromatic erythrocytes was estimated from a minimum of 2000 erythrocytes. The micronuclei in these cells were scored and reported as micronuclei per 1000 cells.

Biochemical studies

Sulfhydryl group assay

The level of acid soluble sulfhydryl groups was estimated in liver and lung as total non-protein sulfhydryl groups using the

method described by Moron *et al.* (23). Reduced glutathione (GSH; obtained from Sisco Research Laboratories, Bombay, India) was used as a standard to calculate the micromoles of $-SH/g$ of tissue. The absorbance in the assay was read at 412 nm using a Systronic Spectrophotometer (Systronics Type 108; Naroda, Ahmedabad, India).

Lipid peroxidation (LPO) assay

The LPO level in liver and lung was measured in terms of thiobarbituric acid reactive substances (TBARS) by the method of Ohkhawa *et al.* (24). The absorbance in the assay was read at 532 nm.

DPPH radical scavenging assay

Radical scavenging activity of ME against the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical was determined spectrophotometrically as described by Cuendet *et al.* (25). A stock solution of 1 mg/ml ME was prepared in methanol. Fifty microliters containing different concentration of ME were added to 5 ml of a 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. The assay was carried out in triplicate and the percentage of inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{(AB - AA)}{AB} \times 100,$$

where AB = Absorption of blank, and AA = Absorption of test.

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 μ l 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 χ^2 -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

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

Treatment group	Number of mice	Mean weight of mice (g ± SE)	Mean weight of lungs (g ± SE)	Lung adenoma incidence (%)	Inhibition rate (%)	Tumor multiplicity
Group I: control	30	27.54 ± 0.60	0.28 ± 0.005	0	0	0
Group II: ME	30	28.20 ± 0.58	0.29 ± 0.004	0	0	0
Group III: BP	53	19.3 ± 0.4 ^b	0.20 ± 0.004 ^b	67.92 (36 [†])	Reference	0.83 (44 [‡])
Group IV: BP + ME	76	24.66 ± 0.54 ^c	0.22 ± 0.005 ^a	26.31* (20 [†])	61.26*	0.31 (23 [‡])

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: ^a*P* < 0.05, ^b*P* < 0.001 and ^c*P* < 0.005 (using Student's *t*-test).

*Significant at 0.001 using χ^2 -test.

[†]Number of tumor bearing mice.

[‡]Total number of tumors.

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

Treatment group	Chromatid breaks (%)	Chromosome breaks (%)	Centric rings (%)	Dicentrics (%)	Exchanges (%)	Fragments (%)
Group I: Control	0.16 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.10 ± 0.37
Group II: ME	0.14 ± 0.05 (n.s.)	0.00 ± 0.00 (n.s.)	0.00 ± 0.00 (n.s.)	0.00 ± 0.00 (n.s.)	0.00 ± 0.00 (n.s.)	0.98 ± 0.32 (n.s.)
Group III: BP	11.20 ± 1.32 ^b	6.72 ± 0.70 ^b	1.42 ± 0.28 ^b	2.98 ± 0.62 ^c	1.48 ± 0.28 ^b	128.42 ± 7.60 ^b
Group IV: BP+ ME	1.84 ± 0.42 ^b	1.20 ± 0.0 ^b	1.02 ± 0.02 n.s.	0.94 ± 0.01 ^a	0.22 ± 0.01 ^c	4.80 ± 1.32 ^b

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: ^a*P* < 0.05, ^b*P* < 0.001 and ^c*P* < 0.005; n.s., nonsignificant.

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

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

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: ^a*P* < 0.05 and ^b*P* < 0.001; n.s., nonsignificant.

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 pulverized cells, polyploids, total aberrations and aberrations/damaged cells (Table III). The frequency of micronuclei/1000 cells in the Group III animals was 20.82 ± 1.81 (compared with a frequency of 0.28 ± 0.02 in the Group I control; Table IV). However, treatment with BP followed by ME (Group IV) resulted in a significant decrease in chromosomal aberrations and micronucleus frequencies compared with those found in BP-alone group.

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

Treatment group	Number of micronuclei/1000 cells
Group I: control	0.28 ± 0.02
Group II: ME	0.24 ± 0.02
Group III: BP	20.82 ± 1.81 ^b
Group IV: BP + ME	2.92 ± 0.72 ^b

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

^b*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).

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

Treatment group	Liver		Lung	
	GSH content	TBARS level	GSH content	TBARS level
Group I: control	64.39 ± 1.63	1.14 ± 0.09	48.38 ± 1.08	1.12 ± 0.07
Group II: ME	64.81 ± 3.10	1.01 ± 0.12	45.42 ± 1.10	1.10 ± 0.08
Group III: BP	38.16 ± 1.82 ^b	4.32 ± 0.19 ^b	28.15 ± 1.11 ^b	3.21 ± 0.12 ^b
Group IV: BP + ME	54.22 ± 1.78 ^b	2.48 ± 0.15 ^b	32.82 ± 1.12 ^a	2.18 ± 0.10 ^b

Statistical comparisons: Group I versus Group II; Group III versus Group I; Group III versus Group IV. Level of significance: ^a $P < 0.05$; ^b $P < 0.001$. GSH = $\mu\text{mol/g}$ tissue; TBARS = nmol/mg tissue.

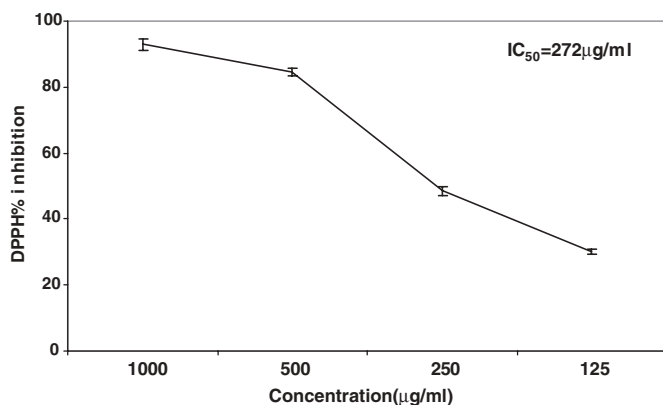


Fig. 1. Inhibition (%) of DPPH radical scavenging activity of *M. piperita* leaf extract at various concentrations. Stock solution of crude ME was prepared as 1 mg/ml in methanol. Fifty microlitres of samples of different concentration were added to 5 ml of 0.004% methanol solution of DPPH*. After 30 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.

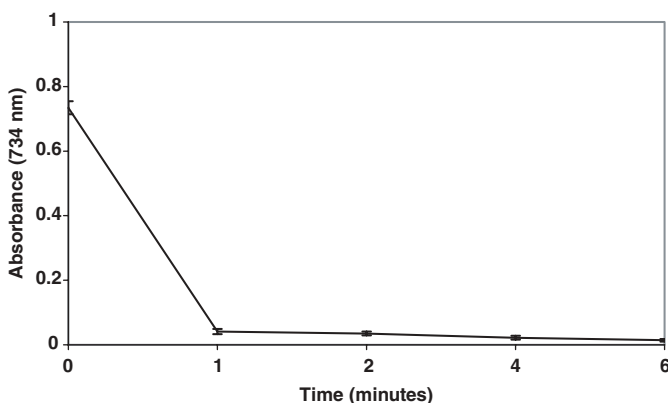


Fig. 2. The effect of time on the suppression of absorbance of $\text{ABTS}^{\bullet+}$ by *M. piperita* leaf extract. After addition of 1 ml of diluted ABTS solution ($A_{734\text{ nm}} = 0.700 \pm 0.020$) to 10 μl of plant extracts the absorbance reading was taken at 30°C exactly 1 min., after initial mixing and up to 6 min. All determinations were carried out in triplicates.

The radical scavenging activity of ME was evaluated using the DPPH \bullet and $\text{ABTS}^{\bullet+}$ assays. In the present investigation ME displayed strong radical scavenging activity in both the assays. The ME had an IC_{50} value of 272 $\mu\text{g/ml}$ in the DPPH \bullet scavenging assessment (Figure 1). The $\text{ABTS}^{\bullet+}$ 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 *Mentha cordifolia* Opiz. A dose of 0.01 mg/20 g mouse inhibited 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 papiloma incidence, and showed that DMBA also significantly increased the frequency of micronuclei and other chromosomal anomalies in keratinocytes. Furstenberger *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.

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 extrahepatic 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 IC₅₀ = 2.53 µg/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 monoterpene 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 qualitative 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.

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References

- Ammon, H.P. and Wahl, M.A. (1991) Pharmacology of curcuma longa. *Planta Med.*, **57**, 1–7.
- Kelloff, G.J., Boone, C.W., Crowell, J.A., Steele, V.E., Lubet, R. and Sigman, G.C. (1994) Chemopreventive drug development: perspectives and progress. *Cancer Epidemiol. Biomarkers Prev.*, **3**, 85–98.
- Tanaka, T. (1994) Cancer chemoprevention by natural products. *Oncol. Rep.*, **1**, 1139–1155.
- Morse, E.C. and Stoner, G. (1996) Cancer chemoprevention principles and prospects. *Carcinogenesis*, **14**, 1737–1746.
- Pezzuto, J.M. (1997) Plant-derived anticancer agents. *Biochem. Pharmacol.*, **53**, 121–133.
- The Wealth of India (1962) A dictionary of Indian Raw Materials and Industrial Products. Vol. VI L-M, New Delhi: Publication and Information Directorate, CSIR, pp. 337–346.
- Rastogi, R.P. and Mehrotra, B.N. (1991) Compendium of Indian medicinal plants, Vol. 3 (1980–81). CDRI and PID, New Delhi, pp. 420–422.
- Krishnaswamy, K. and Raghuramulu, N. (1998) Bioactive phytochemicals with emphasis on dietary practices. *Indian J. Med. Res.*, **108**, 167–181.
- Al-Sereiti, M.R., Abu-Amer, R.M. and Sen, P. (1999) Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. *Indian J. Exp. Biol.*, **37**, 124–130.
- Lirio, L.G., Hermano, M.L. and Fontanilla, M.Q. (1998) Antibacterial activity of medicinal plants from the Philippines. *Pharmaceut. Biol.*, **36**, 357–359.
- Aqil, F., Beg, A.Z. and Ahmad, I. (2001) In vitro toxicity of plant essential oils against soil fungi. *J. Med. Aro. Plant Sci.*, **22/23**, 177–181.
- Vokovic-Gacic, B. and Simic, D. (1993) Identification of natural antimutagens with modulating effects on DNA repair. *Basic Life Sci.*, **61**, 269–277.
- Samman, M.A., Bowen, I.D., Taiba, K., Antonius, J. and Hannan, M.A. (1998) Mint prevents shamma-induced carcinogenesis in hamster cheek pouch. *Carcinogenesis*, **19**, 1795–1801.
- Samarth, R.M., Saini, M.R., Maharwal, J., Dhaka, A. and Kumar, A. (2002) *Mentha piperita* (Linn.) leaf extract provides protection against radiation induced alterations in intestinal mucosa of Swiss albino mice. *Indian J. Exp. Biol.*, **40**, 1245–1249.
- Samarth, R.M. and Kumar, A. (2003) *Mentha piperita* (Linn.) leaf extract provides protection against radiation induced chromosomal damage in bone marrow of mice. *Indian J. Exp. Biol.*, **41**, 229–237.
- Samarth, R.M. and Kumar, A. (2003) Radioprotection of Swiss albino mice by plant extract *Mentha piperita* (Linn.). *J. Radiat. Res.*, **44**, 101–109.
- Locigno, R. and Castronovo, V. (2001) Reduced glutathione system: role in cancer development, prevention and treatment (review). *Int. J. Oncol.*, **19**, 221–236.
- Ohyama, W., Gonda, M. and Miyajima, H. (2002) Collaborative validation study of the *in vivo* micronucleus test using mouse colonic epithelial cells. *Mutat. Res.*, **518**, 39–45.
- Yun, T.K., Kim, H.S. and Lee, Y.S. (1995) Trial of new medium term model using benzo (a) pyrene induced lung tumor in newborn mice. *Anticancer Res.*, **15**, 839–845.
- Savage, J.R.K. (1975) Classification and relationships of induced chromosomal structural changes. *J. Med. Genet.*, **12**, 103–122.
- Bender, M.A., Awa, A.A., Brooks, A.L., Evans, H.J., Groer, P.G., Littlefield, L.G., Pereira, C., Preston, R.J. and Wachholz, B.W. (1988) Current status of cytogenetic procedures to detect and quantify previous exposures to radiation. *Mutat. Res.*, **196**, 103–159.
- Schmid, W. (1975) The micronucleus test. *Mutat. Res.*, **31**, 9–15.
- Moron, M.A., Depierre, J.W. and Mannervick, B. (1979) Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochem. Biophys. Acta*, **582**, 67–78.
- Ohkawa, H., Ohishi, N. and Yogi, K. (1979) Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Analyt. Biochem.*, **95**, 351–358.
- Cuendet, M., Hostettmann, K. and Potterat, O. (1997) Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Hel. Chim. Acta*, **80**, 1144–1152.
- Re, R., Pellegrini, N., Protagente, A., Pannala, A., Yang, M and Rice-Evans, C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.*, **26**, 1231–1237.
- IARC (1973) Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, Vol. 3. IARC, Lyon.

28. IARC (1983) Polynuclear aromatic compounds, part 1: chemical, environmental and experimental data. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Human*, Vol. 32. IARC, Lyon.
29. Yang,S.K., McCourt,D.W., Leutz,J.C. and Gelboin,H.V. (1977) Benzo[a]pyrene diol-epoxides: mechanisms of enzymatic formation and optically active intermediates. *Science*, **196**, 1199–1201.
30. Kaina,B. (1989) Chromosomal aberrations as a contributing factor for tumor promotion in the mouse skin. *Teratog. Carcinog. Mutagen*, **9**, 331–348.
31. Villaseñor,I.M., Echegoyen,D.E. and Angelada,J.S. (2002) A new anti-mutagen from *Mentha cordifolia* Opiz. *Mutat. Res.*, **515**, 141–146.
32. Hayashi,M., Tice,R.R., Macgregor,J.T., Anderson,D., Blakey,D.H., Kirsch-Volders,M., Oleson,F.B., Jr, Pacchierotti,F., Romaya,F., Shimada,H. *et al.* (1994) *In vivo* rodent erythrocyte micronucleus assay. *Mutat. Res.*, **312**, 293–304.
33. Hayashi,M., Mac Gregor,J.T., Gatehouse,D.G., Adler,I.D., Blakey,D.H., Dertinger,S.D., Krishna,G., Morita,T., Russo,A. and Sotou,S. (2000) *In vivo* rodent erythrocyte micronucleus assay. II some aspects of protocol design including repeat treatments, integration with toxicity testing and automated scoring. *Environ. Mol. Mutagen.*, **35**, 234–252.
34. Steinel,N.H., Bonin,A.M., He,S. and Baker,R.S. (1993) Cytogenetic damage and tumor incidence in mouse skin after single, topical applications of 7,12-dimethylbenz (a) anthracene. *Mutat. Res.*, **285**, 19–26.
35. Furstenberger,G., Schurich,B., Kaina,B., Petrusevska,R.T., Fwenig,N.E. and Mark,F. (1989) Tumor induction in initiated mouse skin by phorbol esters and methyl methanesulfonate: correlation between chromosomal damage and conversion (Stage I of tumor promotion) *in vivo*. *Carcinogenesis*, **10**, 749–752.
36. Haesen,S., Timmermans,M. and Kirsch-Volders,M. (1993) Induction of micronuclei and karyotype aberrations during *in vivo* mouse skin carcinogenesis. *Carcinogenesis*, **14**, 2319–2327.
37. Das,U.N. (2002) A radical approach to cancer. *Med. Sci. Monit.*, **8**, 79–92.
38. Lu,S.C. (1999) Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB J.*, **13**, 1169–1173.
39. Mayer,C., Schmezer,P., Freese,R., Mutanen,M., Hietanen,E., Obe,G., Basu,S. and Bastsch,H. (2000) Lipid peroxidation status, somatic mutations and micronuclei in peripheral lymphocytes: a case observation on a possible interrelationship. *Cancer Lett.*, **152**, 169–173.
40. Ketterer,B. (1998) Glutathione *S*-transferase and prevention of cellular free radical damage. *Free Radic. Res.*, **28**, 647–658.
41. Mimica-Dukic,N., Bozin,B., Sokovic,M., Mihajloric,B. and Matavulj,M. (2003) Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Med.*, **69**, 413–419.
42. Yu,T.W., Xu,M. and Dashwood,R.H. (2004) Antimutagenic activity of spearmint. *Environ. Mol. Mutagen.*, **44**, 387–393.
43. Dorman,H.J., Kosar,M., Kahlos,K., Holm,Y. and Hiltunen,R. (2003) Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties and cultivars. *J. Agric. Food Chem.*, **51**, 4563–4569.
44. Romero-Jimenez,M., Campos-Sanchez,J., Analla,M., Munoz-Serrano,A. and Alonso-Moraga,A. (2005) Genotoxicity and anti-genotoxicity of some traditional medicinal herbs. *Mutat. Res.*, **585**, 147–55.

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