Research Article

Protective effects of lupeol against benzo[a]pyrene induced clastogenicity in mouse bone marrow cells

Sahdeo Prasad, Vinay Kumar Yadav, Smita Srivastava and Yogeshwer Shukla

Proteomics Laboratory, Indian Institute of Toxicology Research, Mahatma Gandhi Marg, Lucknow, India

Fruits and vegetables contain a variety of ingredients that exhibit chemopreventive effects against an array of xenobiotics. In the present study, the antigenotoxic potential of lupeol, a triterpene, and mango pulp extract (MPE) was evaluated in Swiss albino mice. Benzo[a]pyrene (B[a]P), a well-known mutagen, was given at a single dose of 100 mg/kg body weight intraperitoneally. Pretreatment with lupeol (1 mg/animal) and MPE (1 mL, 20%) was given through oral intubation for 7 days prior to B[a]P administration. Animals from all the groups were killed at sampling time of 24 h and their bone marrow tissue was analyzed for chromosomal damage and micronuclei induction. In B[a]P-treated animals a significant induction of chromosomal aberration and micronuclei was recorded, with a decrease in mitotic index. In lupeol- or MPE-supplemented groups, a significant decrease in B[a]P-induced clastogenicity was recorded. The incidence of aberrant cells and micronuclei was found to be reduced by both lupeol and MPE when compared to the B[a]P-treated group. The anti-cytotoxic effects of lupeol or MPE were also evident, as observed by significant increase in mitotic index. Thus, results of the present investigation revealed that lupeol and MPE have protective effects against B[a]P-induced clastogenic changes in Swiss albino mice.

Keywords: Benzo[a]pyrene / Chromosomal aberrations / Lupeol / Mango pulp / Micronuclei induction

Received: October 12, 2007; revised: December 6, 2007; accepted: December 20, 2007

1 Introduction

Epidemiological and experimental studies have suggested that consumption of cereals, fruits and vegetables reduces the risk of cancers at different organ sites, including the colon, breast, bladder and prostate [1]. Mango (*Mangifera indica* L.), cultivated mainly in tropical countries, is consumed worldwide. Mango extract has been shown to be anti-mutagenic in *Salmonella typhimurium* strain TA1538 [2]. Chemical analysis of mango pulp has shown that it contains vitamins, organic acids, carbohydrates, amino acids [3], polyphenols and volatile compounds [4]. Lupeol [Lup-20(29)-en-3β-ol], a pentacyclic triterpene found in fruits (mango, olive, strawberry, grapes, and figs), vegetables, and several medicinal plants, is used as a traditional native medicine in the treatment of various ailments worldwide [5–7]. Lupeol has been shown to exhibit antioxidant, anti-inflammatory, anti-arthritic, anti-mutagenic, and anti-malarial activities in both *in vitro* and *in vivo* systems [8–11]. It also possesses antitumor-promoting effects in mouse skin carcinogenesis [12]. The oral administration of lupeol changed the tissue redox system induced by cadmium exposure by scavenging the free radicals and by improving the antioxidant status of the rat liver [13]. Recently, lupeol and mango pulp extract (MPE) have been shown to exhibit apoptotic effects in LNCaP human prostate cancer cells and to combat testosterone-induced alteration in mouse prostate by modulating cell-growth regulators [14]. However, there are no reports showing the effect of lupeol or MPE, on initial events of carcinogenesis like mutations caused by mutagenic/carcinogenic chemicals. Therefore, in the present study we studied anti-clastogenic activity of lupeol/MPE in Swiss albino mice using cytogenetic end points.

Correspondence: Dr. Yogeshwer Shukla, Proteomics Laboratory, Indian Institute of Toxicology Research, Mahatma Gandhi Marg, Lucknow 226001, India
E-mail: yogeshwer_shukla@hotmail.com; shukla_y@rediffmail.com
Fax: +91-522-2628227

Abbreviations: B[a]P, benzo[a]pyrene; **MPE**, mango pulp extract; **MNPCE**, micronucleated PCE; **PCE**, polychromatic erythrocytes; **ROS**, reactive oxygen species

2 Materials and methods

2.1 Chemicals

Lupeol, benzo[a]pyrene (B[a]P) and colchicine were purchased from Sigma (St Louis, MO, USA). The rest of the chemicals were of analytical grade of purity and procured locally.

2.2 Preparation of MPE

Fresh mango pulp (20 g) from ripe mango fruits was homogenized with 100 mL 0.1 M PBS (pH 7.0). The resulting homogenate was filtered through four-layered muslin cloth and then centrifuged at 6000 × *g* for 20 min at room temperature. Supernatant was stored at –20°C until further use as MPE.

2.3 Animals and treatment

Adult male Swiss albino mice (*Mus musculus L*), 10–12 weeks old and having a body weight of ~20 g, were used in the study. The animals were maintained in an air-conditioned animal house at a temperature of 25 ± 2°C, relative humidity of 57 ± 2% and photo-cycle of 12:12-h light and dark periods. Water and food pellets were provided *ad libitum*. After a quarantine period of 1 week, animals were randomly divided into six groups of ten animals each. The animals of group I were used as control and no treatment was given. The animals of group II served as positive controls and only a single dose of B[a]P (100 mg/kg body weight) was given intraperitoneally. In groups III and V, lupeol (1 mg/animal) was given through oral intubation for 7 consecutive days. The animals of groups IV and VI received 1 mL 20% MPE through oral intubation for 7 consecutive days. B[a]P (100 mg/kg body weight) treatment was given intraperitoneally to the animals of groups II–IV 1 h after the last dose of lupeol or MPE on day 7. The animals of all groups were analyzed using cytogenetic assays.

2.4 Chromosomal aberration assay

Following treatment with the mutagen (B[a]P), five animals from each group were killed after 24 h by cervical dislocation. Colchicine was given at a dose of 4 mg/kg body weight 2 h prior to sacrificing to arrest the metaphase stage. Cytogenetic analysis was performed according to the protocol of Preston *et al*. [15], Briefly, the bone marrow was flushed out from both femurs using Hanks' buffered salt solution (pH 7.2). The cells were centrifuged at 1000 rpm for 5 min and the pellet was redispersed in a hypotonic solution of 0.56% KCl for 30 min at 37°C to permit osmotic swelling of cells. Swollen cells were fixed in ice-cold Carnoy's fluid, placed onto slides, and stained with phosphate-buffered 5% Giemsa solution. For each animal, 75 well-spread metaphase plates were analyzed for chromosomal aberrations at a magnification of 1006 and the mitotic index was calculated from a scan of 2000 cells per animal. The chromosomal aberrations were classified as breaks, fragments, and exchanges. The incidence of aberrant cells was expressed as the percentage of damaged cells (aberrant metaphases).

2.5 Micronuclei induction assay

The remaining animals were killed at 24 h after treatment with mutagen (B[a]P) and the frequency of micronucleated polychromatic erythrocytes (MNPCE) was evaluated using a modification of the protocol described by Schmid [16]. The bone marrow was flushed from both femurs using Hanks' buffered salt solution, 1% BSA, and 0.15% EDTA (pH 7.2). Evenly spread bone marrow smears were stained using the May-Grünwald and Giemsa protocols. A minimum of 2000 erythrocytes were scored for each treated and control group.

The suppression percentage of chromosomal aberrations was calculated as: $a = 100 - (b/c) \times 100$, where a is % of suppressed aberrant cells, b is% of aberrant cells in lupeol- or MPE-pretreated and B[a]P-post-treated groups, c is % of aberrant cells in B[a]P alone treated group.

2.6 Statistical analysis

Mean values and standard error for all six groups were subjected to statistical comparison using Student's *t*-test; $p < 0.05$ was considered significant.

3 Results

The chromosomal aberration assay showed that the percent incidence of aberrant cells in the B[a]P-treated group was 14.13 (Fig. 1A), compared to 5.25 in the untreated control group I. Administration of the lupeol or MPE prior to B[a]P injection led to significant inhibition in chromosomal aberrations, with the percent incidence declining to 8.14 and 9.25 in groups III and IV, respectively (Fig. 1A). The inhibition of B[a]P-induced incidence of chromosomal aberrations by lupeol and MPE was 42.4% and 34.5%, respectively (Table 1). The mitotic index evaluated as a percentage of dividing cells was found to be decreased in group II treated with B[a]P alone (6.58%) compared to the untreated group I (15.13%), indicating bone marrow cytotoxicity. Supplementation with lupeol and MPE increased the mitotic index to 10.12% and 8.86%, repectively, in group III and IV (Fig. 1B). However, in the groups treated with lupeol or MPE alone the mitotic index was almost equal to that of the untreated group, indicating that they were non-toxic (Fig. 1B).

Similarly, the frequency of MNPCEs/1000 PCEs was 20.35 in the group treated with B[a]P alone, and was

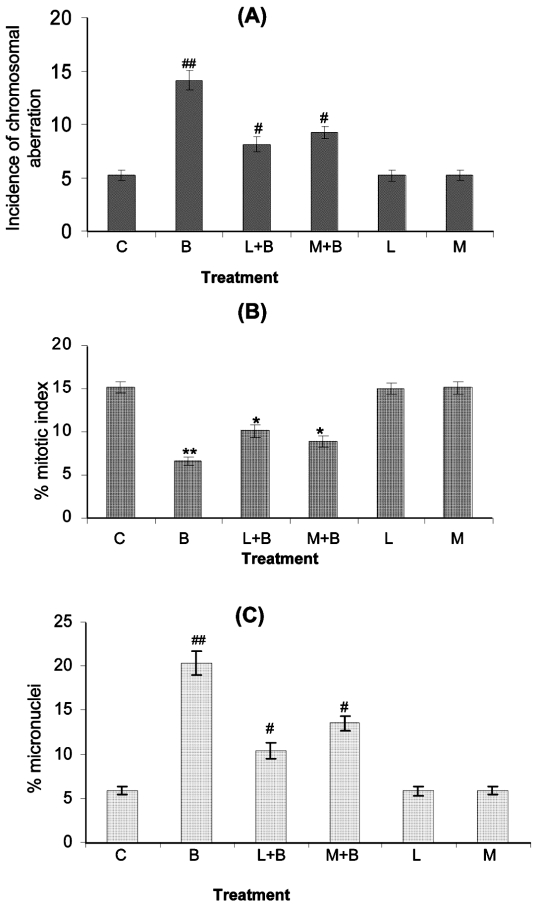

Figure 1. Anti-mutagenic activity of lupeol or MPE in Swiss albino mice. (A) Incidence of aberrant cells. (B) Status of mitotic index. (C) Status of micronuclei induction towards B[a]P-induced clastogenicity (*C*, Untreated control; *B*, B[a]P; *L*, Lupeol; *M*, MPE). ## Significant increase over control group I, # significant decrease over B[a]P-treated group II. ** Significant decrease over control group I, * significant increase over B[a]P-treated group II. Data are mean ± SE of five animals, with significant at $p < 0.05$.

reduced to 10.45 and 13.48 in groups III and V (Fig. 1C). The frequency in the untreated control group was only 5.86. Inhibition in micronucleus induction towards B[a]P-induced clastogenicity was found to be 48.6% and 33.8% by lupeol and MPE, respectively (Table 1).

4 Discussion

There is considerable public and scientific interest in the role of fruits and vegetables in the prevention of genetic diseases [17, 18]. In the present study, lupeol and MPE were found to inhibit the incidence of B[a]P-induced chromosomal damage in mice, suggesting their potential as anti-mutagenic agents. The majority of mutagenic/carcinogenic compounds, *e.g.*, polycyclic aromatic hydrocarbons, act by

Table 1. Suppressive effects of lupeol or MPE against B[a]P-induced clastogenicity in mouse bone marrow

Treatment	Suppression of chromosomal aberration (%)	Suppression of micronuclei induction (%)
Lupeol+ B[a]P	42.4	48.6
MPE+ B[a]P	34.5	33.8

MPE, 1 mL 20% MPE/animal, Lupeol, 1 mg/animal, B[a]P, 100 mg/kg body weight.

generating electrophilic intermediates such as free radicals *via* microsomal enzymatic reactions causing mutations [19, 20]. B[a]P is a potential inducer of reactive oxygen species (ROS), *e.g.*, mitochondrial superoxide anion and hydrogen peroxide [21]. These species can damage DNA, RNA, lipids, and proteins by nitration, oxidation, chlorination, and bromination reactions, leading to increased mutations and altered functions of enzymes and proteins, and thus contributing to the multi-stage carcinogenesis process [22]. Lupeol or MPE supplementation is known to be effective in reducing ROS generation and can also restore antioxidant enzyme activities [8], suggesting a role in the detoxification of free radicals like superoxide anion and hydrogen peroxide due to antioxidant properties. Lupeol, which is present in MPE and other fruits, enhances the level of enzymatic (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and glutathione-S-transferase) and non-enzymatic (reduced glutathione, vitamin C and vitamin E) antioxidants, thereby accelerating the detoxification of mutagens and carcinogens [23].

The anti-carcinogenic potential of lupeol has already been established through various studies [8, 10, 14, 24], which postulate that it as an inhibitory agent of liver, prostate, skin and pancreatic carcinogenesis. In addition, the anti-mutagenic potential of lupeol has also been assessed [25, 26]. Lupeol significantly reduced the free radical-mediated DNA-sugar damage and microsomal lipid peroxidation in an iron/ascorbate-free radical generating system *in vitro* [27]. Since mutations induced at the cytogenetic levels are a probable cause of cancer, the inhibition of chromosomal aberration and micronuclei induction by lupeol or MPE suggest that their anti-mutagenic potential is related to antigenotoxic and anti-carcinogenic activity. The findings of the present investigation revealed that lupeol and MPE have antigenotoxic effect on B[a]P-induced cytotoxic and clastogenic damage. The molecular mechanisms of anti-mutagenesis of lupeol and MPE are yet to be explored to understand the chemopreventive effects.

The authors are thankful to Dr. Ashwani Kumar, Director, Indian Institute of Toxicology Research, Lucknow, for his keen interest in the study. Authors are also thankful to

Indian Council of Medical Research, New Delhi for providing Senior Research Fellowship to Sahdeo Prasad.

The authors have declared no conflict of interest.

5 References

[1] Heggie, S. J., Wiseman, M. J., Cannon, G. J., Miles, L. M. *et al*., Defining the state of knowledge with respect to food, nutrition, physical activity, and the prevention of cancer. *J. Nutr.* 2003, *133*, 3837S – 3842S.

[2] Botting, K. J., Young, M. M., Pearson, A. E., Harrs, P. J., Ferguson, L. R., Antimutagens in food plants eaten by Polynesians: Micronutrients, phytochemicals and protection against bacterial mutagenicity of the heterocyclic amine 2-amino-3-methylimidazo[4,5-f]quinoline. *Food Chem. Toxicol.* 1999, *37*, 95 – 103.

[3] Gil, A. M., Duarte, I. F., Delgadillo, I., Colquhoun, I. J. *et al.,* Study of the compositional changes of mango during ripening by use of nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem*. 2000, *48*, 1524 – 1536.

[4] Pino, J. A., Mesa, J., Muñoz, Y., Martí, M. P., Marbot, R., Volatile components from mango (*Mangifera indica* L.) cultivars. *J. Agric. Food Chem*. 2005, *53*, 2213 – 2223.

[5] Beveridge, T. H., Li, T. S., Drover, J. C., Phytosterol content in American ginseng seed oil. *J. Agric. Food Chem.* 2002, *50*, 744 – 750.

[6] Fournet, A., Angelo, A., Munoz, V., Roblot, F. *et al*., Biological and chemical studies of *Pera benensis*, a Bolivian plant used in folk medicine as a treatment of cutaneous leishmaniasis. *J. Ethnopharmacol.* 1992, *37*, 159 – 164.

[7] Kakuda, R., Iijima, T., Yaoita, Y., Machida, K., Kikuchi, M., Triterpenoids from *Gentiana scabra*. *Phytochemistry* 2002, *59*, 791 – 794.

[8] Prasad, S., Kalra, N., Shukla, Y., Hepatoprotective effects of lupeol and mango pulp extract of carcinogen induced alteration in Swiss albino mice. *Mol. Nutr. Food Res*. 2007, *51*, 352 – 359.

[9] Ziegler, H. L., Staalso, T., Jaroszewski, J. W., Loading of erythrocyte membrane with pentacyclic triterpenes inhibits *Plasmodium falciparum* invasion. *Planta Med.* 2006, *72*, 640 – 642.

[10] Saleem, M., Kweon, M. H., Yun, J. M., Adhami, V. M. *et al*., Novel dietary triterpene Lupeol induces fas-mediated apoptotic death of androgen-sensitive prostate cancer cells and inhibits tumor growth in a xenograft model. *Cancer Res.* 2005, *65*, 11203 – 11213.

[11] Latha, R. M., Lenin, M., Rasool, M., Varalakshmi, P., A novel derivative pentacyclic triterpene and omega 3 fatty acid. *Prostaglandins Leukot. Essent. Fatty Acids* 2001, *64*, 81 – 85.

[12] Saleem, M., Alam, A., Arifin, S., Shah, M. S. *et al*., Lupeol, a triterpene, inhibits early responses of tumor promotion induced by benzoyl peroxide in murine skin. *Pharmacol. Res.* 2001, *43*, 127 – 134.

[13] Sunitha, S., Nagaraj, M., Varalakshmi, P., Hepatoprotective effect of lupeol and lupeol linoleate on tissue antioxidant defence system in cadmium-induced hepatotoxicity in rats. *Fitoterapia.* 2001, *72*, 516 – 523.

[14] Prasad, S., Kalra, N., Shukla, Y., Induction of apoptosis by lupeol and mango extract in mouse prostate and LNCaP cells. *Nutr. Cancer* 2007 (in press).

[15] Preston, R. J., Dean, B. J., Galloway, S., Holden, H. *et al*., Mammalian *in vivo* cytogenetic assays. Analysis of chromosome aberrations in bone marrow cells. *Mutat. Res*. 1987, *189*, 157 – 165.

[16] Schmid, W., The micronucleus test. *Mutat Res*. 1975, *31*, 9 – 15.

[17] Peeters, P., Keinan-Boker, L., van der Schouw, Y., Grobbee, D., Phytoestrogens and breast cancer risk. Review of the epidemiologic evidence. *Breast Cancer Res. Treat*. 2003, *77*, 171 – 183.

[18] Kris-Etherton, P., Hecker, K., Bonanome, A., Coval, S. M. *et al*., Bioactive compounds in foods: Their role in prevention of cardiovascular disease and cancer. *Am. J. Med.* 2002, *113*, 71S – 88S.

[19] Klaassan, C. D., Casarett and Doull's Toxicology, in: Klaassan, C. D. (Ed.), *The Basic Science of Poisons*, 5th edn, McGraw Hill, New York 1996, pp. 269 – 300.

[20] Vainio, H., Magee, P. N., Mc-Gregor, D. B., Mc-Michael, A. J. (Eds.), *Mechanisms of Carcinogenesis in Risk Identification,* Scientific Publication no. 116, WHO-IARC, Lyon 1992.

[21] Huc, L., Tekpli, X., Holme, J. A., Rissel, M. *et al*., c-Jun NH_2-terminal kinase-related Na^+/H^+ exchanger isoform 1 activation controls hexokinase II expression in benzo(a)pyrene-induced apoptosis. *Cancer Res*. 2007, *67*, 1696 – 1705.

[22] Ohshima, H., Tatemichi, M., Sawa, T., Chemical basis of inflammation-induced carcinogenesis. *Arch. Biochem. Biophys*. 2003, *417*, 3 – 11.

[23] Preetha, S. P., Kanniappan, M., Selvakumar, E., Nagaraj, M., Varalakshmi, P., Lupeol ameliorates aflatoxin B1-induced peroxidative hepatic damage in rats. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol*. 2006, *143*, 333 – 339.

[24] Saleem, M., Afaq, F., Adhami, V. M., Mukhtar, H., Lupeol modulates NF-kappaB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice. *Oncogene* 2004, *23*, 5203 – 5214.

[25] Guevara, A. P., Amor, E., Russell, G., Antimutagens from *Plumeria acuminata* Ait. *Mutat. Res.* 1996, *361*, 67 – 72.

[26] Nigam, N., Prasad, S., Shukla, Y., Preventive effects of lupeol on DMBA induced DNA alkylation damage in mouse skin. *Food Chem. Toxicol*. 2007, *45*, 2331 – 2335.

[27] Sultana, S., Saleem, M., Sharma, S., Khan, N., Lupeol, a triterpene, prevents free radical mediated macromolecular damage and alleviates benzoyl peroxide induced biochemical alterations in murine skin. *Indian J. Exp. Biol.* 2003, *41,* 827 – 831.

