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Rehabilating Activity of Mangiferin in Benzo(a) Pyrene Induced Lung Carcinogenesis*

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Abstract: Cancer chemoprevention involves prevention, delay, or reversal of the process of carcinogenesis through ingestion of dietary or pharmaceutical agents. A large number of potential chemopreventive agents are known, some of which have proven effective in clinical trials. These agents may function by a variety of mechanisms, directed at all major stages of carcinogenesis. One mechanism of particular note involves the inhibition of biosynthesis; of polyamines such as spermine, spermidine and putrescine are promising drug for chemoprevention. In the present study we evaluate chemopreventive efficacy of mangiferin against Benzo(a)Pyrene (B(a)P) induced lung carcinogenesis. Male Swiss albino mice strains were selected for the present investigation. Lung carcinoma was induced with B(a) P (50 mg kg^{-1} body weight, orally) and the treatment was started by the oral administration of mangiferin (100 mg kg^{-1} body weight). The modulatory effect of the mangiferin was examined on lung and liver to evaluate the level of polyamines, protein carbonyl, nucleic acid content and lipid peroxidation. Mangiferin significantly decreased the levels of polyamines, protein carbonyl, nucleic acid content and lipid peroxidation that were found to be increased in lung cancer bearing animals. Mangiferin could effectively inhibit B(a)P-induced lung carcinogenesis in albino mice by offering protection from protein damage and also by suppressing cell proliferation.

Key words: Benzo(a)Pyrene, mangiferin, polyamines, lipid peroxidation chemoprevention

INTRODUCTION

Lung cancer is the most common cause of cancer related deaths in both men and women accounting for 29% of all cancer deaths (Perin and Notani, 2001). Scientific evidence suggests that free radicals and electrophiles play an important role in all stages of chemical carcinogenesis and tumorigenesis (Sun, 1990). The oxidative inactivation of enzymes by free radicals and accumulation of oxidized proteins may play a critical role in the alteration of cellular function and cell death. Attack by free radicals upon proteins can damage several amino acid residues. Oxidative damage of residues of protein can generate large amounts of carbonyl products and hence measurement of protein carbonyls has been used as a sensitive assay for oxidative protein damage (Sundari *et al*., 1997). Synthesis of polyamines such as histamine, putrescine, spermine and spermidine is one of the essential factors for regulation of protein, RNA and DNA synthesis. They are the best markers of cell proliferation and tumour destruction. In addition, the ability of polyamine to alter DNA-protein interactions may be disruptive to cellular function in cancer conditions (Thomas and Thomas, 2001; Yano *et al*., 2001).

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A large number of medicinal or dietary plants have been shown to exhibit substantial inhibitory effects on experimentally induced carcinogenesis and in many cases their chemopreventive activities are associated with antioxidative and/or anti-inflammatory properties. Mangiferin-enriched extract obtained from the stem bark of *Mangifera indica* L. (mango), which shows potent anti-inflammatory and antioxidant activities (Martinez *et al*., 2000; Sanchez *et al*., 2000; Garrido *et al*., 2004). Mangiferin have been shown to prevent bowel carcinogenesis induced by chemical carcinogen (Yoshimi *et al*., 2001). Mangiferin has been reported to show antioxidant action in experimental conditions both in vivo and in vitro through its radical quenching effect (Prabhu *et al*., 2006). In addition, mangiferin has recently been reported to show protective effect against glycated protein-iron chelate induced toxicity (Venugopal *et al*., 2007).

The purpose of our present research is to study the effect of mangiferin on lung carcinogenesis induced by B(a)P in albino mice and to explore the possible protection mechanisms by measuring the levels of protein damage and polyamine synthesis.

MATERIALS AND METHODS

Chemicals

Benzo(a)pyrene (B(a)P) and Mangiferin from *Mangifera indica*, bovine serum albumin were purchased from Sigma chemicals, St. Louis, MO, USA. All other chemicals used were of analytical grade.

Animals

Male Swiss albino mice (7-8 weeks old) weighing about 23-26 g were purchased from King Institute of Preventive Medicine, Chennai. The animals were maintained under standard conditions of humidity, temperature (25±2°C) and light (12 h light/12 h dark). They were fed standard rat pellet diet and had free access to water. The animal experiments were conducted according to the guidelines for the care and use of Laboratory Animals in our University.

Experimental Design

The animals were divided into five groups and each group consists of 6 animals. Group 1 served as Control animals received corn oil as vehicle. Group 2 animals were treated with Benzo(a)pyrene (50 mg kg^{-1} b.wt., given orally twice a week for 4 consecutive weeks, from 2nd to 6th week). Group 3 animals were subjected to Mangiferin pretreated animals (100 mg kg^{-1} b.wt., dissolved in corn oil, given orally) from the 1st week to 18th week. B(a)P was administered to the animals simultaneously from the 2nd week to 6th week for the induction of lung cancer. Group 4 animals were subjected to Mangiferin post treated animals (as in group 3) for 12 weeks after the induction of lung cancer upon administration with B(a)P (as in group 2). The induction of lung cancer was conformed by histopathological study. Group 5 animals were treated with mangiferin alone treated animals (as above) for 18 weeks, to study the cytotoxicity of mangiferin if any. The pre and post-treatment of Mangiferin were used to study the chemopreventive and/or chemotherapeutic efficacies of Mangiferin in the experimental animals.

Biochemical Analysis

At the end of the experimental period (18 weeks), the animals were sacrificed by cervical decapitation. Lung and liver tissues were immediately excised, weighed and then homogenized in Tris-HCl buffer 0.1 mol L^{-1} (pH 7.4). The Lipid Peroxidation (LPO) was estimated by the method of Ohkawa *et al.* (1979), reduced glutathione was determined by the method of Moron *et al.* (1979). The oxidative protein damage was determined by the estimation of carbonyl content by method of Levins *et al.* (1990). Protein content was estimated by the method of Lowry *et al.* (1971). The DNA and RNA contents were estimated in the homogenate by the methods of Burton (1956) and

Rawal *et al.* (1977), respectively. Separation of various polyamine fractions such as histamine, putrescine, spermine and spermidine and their subsequent estimation was done by the method of Endo (1978).

Statistical Analysis

For statistical analysis, one-way analysis of variance (ANOVA) was used, followed by the Newman-Keuls multiple comparison test. Treatment mean differences with $p<0.05$ were considered significant.

RESULTS

There was found to be a significant ($p<0.05$) increase in the extent of LPO and protein carbonyl content and a significant ($p<0.05$) decrease in GSH content in lung cancer bearing mice (group 2) when compared with the control (group 1) animals (Fig. 1). Treatment with mangiferin (group 4) caused a significant ($p<0.05$) decrease in the LPO and protein carbonyl contents and also a significant increase ($p<0.05$) in the GSH levels when compared with B(a)P-induced group (group 2). However, mangiferin alone treated animals (group 5 did not show any significant changes when compared with the control animals (group 1).

Cancer bearing animals (group 2) showed a significant increase ($p<0.05$) in nucleic acid levels in both tissues when compared with the control animals (group 1). On treatment with mangiferin (group 3 and group 4), there was found to be a significant ($p<0.05$) decrease in the levels of nucleic acids in both tissues when compared with cancer-induced animals (group 2). However, mangiferin alone treated animals (group 5) did not show any significant changes when compared with control animals (group 1) (Fig. 2).

There was found to be a significant ($p<0.05$) increase in the extent of polyamines content and a significant ($p<0.05$) in lung cancer bearing mice (group 2) when compared with the control (group 1) animals. On treatment with mangiferin (group 3 and group 4), there was found to be a significant ($p<0.05$) decrease in the levels of polyamines in both the tissues when compared with cancer-induced animals (group 2). However, mangiferin alone treated animals (group 5) did not show any significant changes when compared with the control animals (group 1) (Table 1).

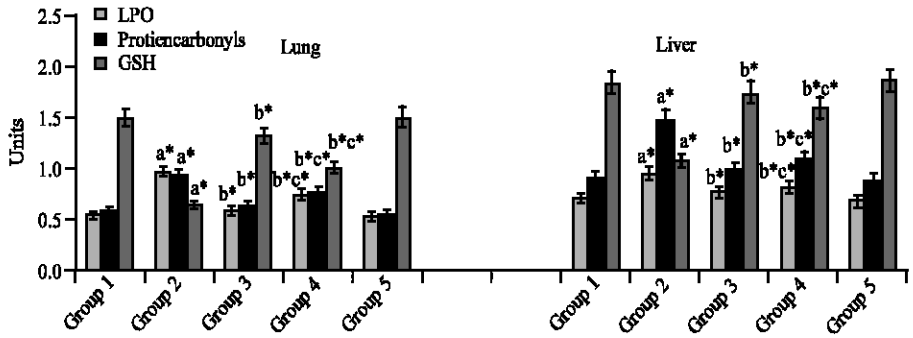

Fig. 1: Effect of mangiferin on oxidative damage to lipidperoxidation, protein carbonyl and its effect on GSH levels in lung and liver of control experimental animals, Each value is expressed as means±SD (n = 6). LPO: nmol of MDA released/mg protien; reduced GSH: Ag/mg protien carbonyls: nmol of DNPH incorporated/mg protien, (a) As compared with group 1, (b) As compared with group 2 and (c) As compared with group 3. Statistical significance*: $p<0.05$ Group 1: Control animals, Group 2: Lung carcinogenesis, Group 3: Pre-treated, Group 4: Post-treated and Group 5: Magniferin alone

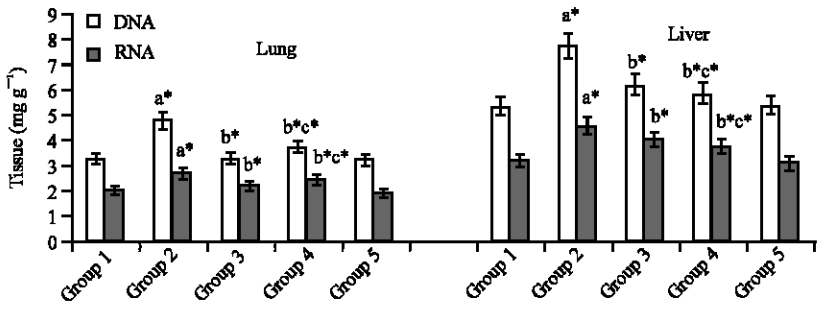

Fig. 2: Effect of mangiferin on the levels of nucleic acids in lung and liver of control experimental animals, Each value is expressed as means±SD (n = 6). (a) As compared with group 1, (b) As compared with group 2 and (c) As compared with group 3. Statistical significance*: $p<0.05$ Group 1: Control animals, Group 2: Lung carcinogenesis, Group 3: Pre-treated, Group 4: Post-treated and Group 5: Magniferin alone

Table 1: Effect of mangiferin on the levels of polyamines in lung and liver of control and experimental animals

Particulars	Group 1	Group 2	Group 3	Group 4	Group 5
Lung tissue					
Putrescine	18.32±2.15	34.58±4.61^{a†}	22.34±2.54^{b†}	26.41±4.28$^{b\dagger c\dagger}$	18.14±2.13
Histamine	5.16±0.43	9.84±0.87^{a†}	5.82±0.63^{b†}	6.78±0.71$^{b\dagger c\dagger}$	5.47±0.42
Spermidine	851.00±94.25	1438.00±209.46^{a†}	967.00±94.64^{b†}	1076.00±201.34$^{b\dagger c\dagger}$	915.00±97.5
Spermine	412.34±34.21	759.00±51.23^{a†}	464.00±34.22^{b†}	527.00±63.57$^{b\dagger c\dagger}$	418.27±36.44
Liver tissue					
Putrescine	21.51±1.67	42.56±5.34^{a†}	31.25±4.35^{b†}	28.64±51$^{b\dagger c\dagger}$	22.89±3.43
Histamine	7.21±0.84	12.49±0.93^{a†}	8.34±0.91^{b†}	7.54±0.54$^{b\dagger cNS}$	6.67±0.85
Spermidine	978.00±82.64	1562.00±254.61^{a†}	1169.00±221.52^{b†}	1014.00±82.43$^{b\dagger c\dagger}$	972.00±81.36
Spermine	475.00±34.15	812.00±71.46^{a†}	561.00±65.65^{b†}	467.00±42.86$^{b\dagger c\dagger}$	476.00±32.77

Each value is expressed as mean±SD for six mice in each group. Statistical significance, †: $p<0.05$, NS: Not Significant. a: as compared with group 1; b: as compared with group 2; c: as compared with group 3. Polyamines: nmol g^{-1} tissue. Group 1: Control animals, Group 2: Lung carcinogenesis, Group 3: Pre-treated, Group 4: Post-treated and Group 5: Mangiferin alone

DISCUSSION

Benzo(a)pyrene a ubiquitous genotoxicant was used to induce DNA damage. This carcinogen is present in high concentrations in environmental tobacco smoke and is known to produce deleterious effects (Claxton *et al*., 1989). B(a)P and its diol epoxides are known to bind to cellular macromolecules (Heidelberger, 1975; Phillips, 1983). This event is one of the key factors in the process of carcinogenesis (Dipple, 1983). It is a multi step process, it seems logical to postulate that if we are able to induce apoptosis of cells that have accumulated only few genetic changes and have just initiated the journey that will lead to malignancy, it will be possible to prevent the development of cancer. A great strength of chemoprevention is that a large number of compounds can prevent the occurrence of cancer and a variety of mechanisms exist for producing such protection (Belinsky and Jaiswal, 1993). Numerous experimental studies shows that chemoprevention has the potential of providing an important means for cancer prevention, for both the general population and even more importantly for individuals at high risk (Hakama, 1998; Sporn and Suh, 2000). So it is becoming increasingly important to screen natural products, which can block or reverse the process of carcinogenesis and can be a promising cancer preventive agent (Kellen, 1999). Regardless of this protection, the toxicity of the reactive oxidation products emerging on account of this antioxidant action might be substantial; this aspect should be considered when mangiferin/vimang or other antioxidants (Kennedy *et al*., 1999) are employed as nutritional supplements. Nevertheless, while this effect is the opposite of the beneficial effect of mangiferin antioxidant activity, it could be beneficial for certain cancer cells exposed to an over-production of ROS. This condition, by triggering mitochondrial permeability transition mediated

apoptosis, may represent an important defense factor. It is conceivable that Vimang/mangiferin, by stimulating such a mechanism, could act as a prodrug, whose toxicity may be restricted to ROS-damaged cells (Gilberto *et al*., 2006). Among the various oxidative modifications in proteins, carbonyl formation may be an early marker for protein oxidation (Liggins and Furth, 1997) and B(a)P has been shown to cause oxidative protein damage in lung carcinogenesis (Mikhail *et al*., 1996). However, we found that tissue carbonyl levels in mangiferin supplemented groups were decreased when compared with those induced with cancer.

The polyamines comprise a family of aliphatic cations that occur ubiquitously in nature (Tabor and Tabor, 1984). They are critical for cell proliferation, differentiation and transformation and are involved in DNA, RNA and protein synthesis, as well as in stabilizing membrane and cytoskeletal structures (Janne *et al*., 1978; Scalabrino and Ferioli, 1981; Pegg, 1988). Ornithine Decarboxylase (ODC) is a key enzyme in polyamine biosynthesis, catalyzing the conversion of L-ornithine into PUT. It is found in very limited amounts in quiescent cell, although its activity rapidly and markedly increases in response to many trophic stimuli (hormones, growth factors and tumor promoters) and during tissue regeneration (Russell, 1985). Many different studies from animal models have shown that polyamines accumulate in cancer cells and that the use of inhibitors of polyamine biosynthesis or polyamine analogues has a remarkable potential to block tumor growth and prevent metastases (Sunkara *et al*., 1987; Marton and Pegg, 1995). Polyamines are essential to ensure successful completion of the replication process, with failure to maintain the individual polyamine concentrations leading to cell-cycle arrest, transformation which protects cells from oxidative stress (Fairlamb *et al*., 1987; Bailey *et al*., 1993). Studies have shown the importance of polyamines in chemoprevention by demonstrating depleted levels of polyamines upon treatment with anticancer agents. Earlier reports demonstrate the potential of polyphenolic to inhibit ODC in various cancer conditions (Pegg *et al*., 1995; Edurdo *et al*., 1999). Hence, mangiferin, one of the polyphenolic, may inhibit ODC activity and reduce cell proliferation in lung carcinogenesis through depleting the levels of polyamines.

The B(a)P is a very effective carcinogen with a capability to induce enormous amounts of free radicals, which in turn reacts with lipids causing release of lipid peroxides (Sikkim and Mulee, 2000). Involvement of free radicals in B(a)P induced lung carcinogenesis, was confirmed by the over production of 8-hydroxyguanine in lung and liver of B(a)P administrated mice (Mikhail *et al*., 1996). Mangiferin is an effective anti-oxidant, mainly on account of its catechol moiety (Prabhu *et al*., 2006), which is present also in numerous flavonoids and other polyphenols. During protection afforded against free radical production, the catechols are oxidized, generating products like semiquinone radicals and quinones. These compounds have been described to be potentially toxic, especially in virtue of their ability to arylate protein thiol groups (Ito *et al*., 1988; Monks *et al*., 1992). Indeed, a reference quinone, i.e., 4-methylortho-benzoquinone, is able to react with protein thiols as efficiently as the synthetic sulfhydryl-alkylating agent NEM. This observation suggests that the oxidation products of catechol- containing anti-oxidants like mangiferin shift the damage provoked by oxidative stress to sulfhydryl arylation (Boots *et al*., 2002). Mangiferin significantly reduced the lipid peroxides in cancer bearing animals, which could be due to its capability to enhance antioxidant enzymes. Mangiferin also enhanced the GSH activity in cancer bearing animals. It is suggested that it offers protection by maintaining high pool of GSH that is involved in protecting the cells from oxidative damage. Mangiferin inhibits ROS generation in U-937 cells (Abira Sarkar *et al*., 2004). Thus, it is possible that the effects are mediated through quenching of reactive oxygen intermediates. GSH is an important cellular reductant involved in detoxifying reactive oxygen intermediates and we observed an increase in the GSH level by mangiferin pre and post treatment animals.

CONCLUSION

Present study displays the protective effect of mangiferin against lung carcinogenesis, which may be due to its inhibitory effect on cell proliferation. Therefore, mangiferin may be explored as a chemopreventive agent for humans at high risk of lung cancer.

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