ISSN 2278 - 540X EISSN 2278 - 5396

DRUG DISCOVERY

Antioxidant and anticancer efficacy of D-Limonene in Benzo(A)pyrene lung carcinogenesis in mice

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Received 19 April; accepted 12 May; published online 25 June; printed 29 June 2012

ABSTRACT

Chemoprevention is regarded as one of the most promising and realistic approaches in the prevention of cancer, several bioactive compound present in fruit & vegetable have revealed their cancer curative potential on lung cancer. d-Limonene is one such naturally occurring terpenoid widely found in citrus fruits. The aim of the present study is to divulge the chemo-preventive nature of d-Limonene during benzo(a)pyrene induced lung cancer in swiss albino mice. Administration of B(a)P to mice resulted in increased lipid peroxides (LPO), serum marker enzyme aryl hydrocarbon hydroxylases (AHH), gamma glutamyl transpeptidase (GGT), 5'nucleotidase (5'ND) and lactate dehydrogenase (LDH) with concomitant decrease in the levels of tissue antioxidants like superoxide dismutase(SOD), catalase (CAT), glutathione (GPx), reduced glutathione(GSH), vitamin-E & vitamin-C. d-Limonene supplementation significantly attenuated these alternation there by showing potent anticancer effect in lung cancer, further the antiproliferative effect of d-Limonene was confirmed by histopathological analysis & proliferating cell nuclear antigen (PCNA) immunostaining, overall these findings substantiate the chemo-preventive potential of d-Limonene against chemically induced lung cancer in mice.

Key words: Lung cancer; d-Limonene; Antioxidants; Tumor marker.

Abbreviations: PCNA - Proliferating cell nuclear antigen; LPO - lipid peroxides; AHH - Aryl hydrocarbon hydroxylases; GGT - gamma glutamyl transpeptidase; 5'ND - 5' nucleotidase; LDH - Lactate dehydrogenase; SOD - Superoxide dismutase; CAT – Catalase; GPx-glutathione, GSH - Reduced glutathione; BPDE - B(a)P-r-7,t-8-dihydrodiol-t-9,10-ep-oxide; MDA – Malonyldialdehyde; γ-GT - gamma glutamyl transpeptidase; GR - Glutathione reductase; GST - Glutathione-Stransferase; PAH - Polycyclic aromatic hydrocarbon; TBS - Tris buffered saline.

1. INTRODUCTION

Lung cancer is the most common cause of cancer death in developed countries in men (80-90%) and women (40-50%) and 90% are associated with tobacco use. The statistics on lung cancer impose the urge to extend new methods to control this deadly form of cancer. The polycyclic aromatic hydrocarbon (PAH) and N-Nitrosamine are the two major classes of tobacco related inhaled carcinogen the PAH including Benzo(A)pyrene (B(a)P), a potent tobacco carcinogen. It is a significant pro-carcinogenic substance which requires metabolic activity to electrophilic reactive metabolite for its carcinogenic activity (Sticha et al., 2000). B(a)P is metabolized to B(a)P-r-7,t-8-dihydrodiol-t-9,10-ep-oxide (BPDE), the ultimate carcinogen BPDE isomer then bind to the exocyclic nitrogen of deoxyguanosine in DNA via trans-addition of the C-10 position in the epoxide molecule this adduct might also cause activation of protooncogenens. Chemopreventive measures have been introduced substantially to reduce the incidence and mortality due to lung cancer. The search for new compound in food or in plant medicine showing anticancer effect is one realistic and promising approach to prevention (Boone, 1990). Varieties of compound have under gone clinical trials against lung cancer based on this strategy (Galati et al., 1994). Monoterpenoids are naturally occurring molecule abundant in fruits and vegetables (Ratty and Das, 1988; Fujiki et al., 1986; Huang et al., 1983; Anandakumar et al., 2008). d-Limonene belong to a class of terpenoids present in citrus fruits it has several biological function such as antioxidant, immune modulator, isoprenyl transferas inhibitor, the effect of d-Limonene in the prevention and treatment of has been recently received considerable attention

Tumor marker: A tumor marker is a substance found in the blood, urine, or body tissues that can be elevated in cancer, among other tissue types. There are many different tumor markers, each indicative of a particular disease process, and they are used in oncology to help detect the presence of cancer. An elevated level of a tumor marker can indicate cancer; however, there can also be other causes of the elevation.

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with particular use of terpenoids as anticancer compound (Selvendiran et al., 2006). It has been reported to several health beneficial effect including the mammary cancer, liver tumorogenesis, carcinogenesis in colon, breast cancer additional d-Limonene suppresses cell proliferation or apoptosis in induced carcinogenesis. Hence the present study was aimed to elucidate the protective role of d-Limonene on B(a)P induced lung cancer by assessing lipid peroxidation (LPO), antioxidant tissue defense system, tumor marker enzyme. Histopathological study together with immunohistochemical analysis of proliferative marker proliferating cell nuclear antigen (PCNA) in lung tissue was also done to substantiate the anticancer effect of d-limonene against B(a)P induced lung carcinogensis in swiss albino mice.

2. MATERIAL AND METHODS

2.1. Chemicals

Benzo(a)pyrene and d-Limonene were purchased from Sigma Chemicals (St Louis, MO, USA). All other chemicals were of analytical grade and were procured from SRL chemicals Pvt Ltd (Mumbai, India).

Benzo(a)pyrene: It is a polycyclic aromatic hydrocarbon found in coal tar with the formula C20H12. Its metabolites are mutagenic and highly carcinogenic, and it is listed as a Group 1 carcinogen by the IARC. The compound is one of the benzopyrenes, formed by a benzene ring fused to pyrene, and is the result of incomplete combustion at temperatures between 300 °C (572 °F) and 600°C (1,112 °F).

2.2. Animals

Healthy male Swiss albino mice, 20-25 g (8-10 weeks old), obtained from the Veterinary College, Chennai, India, were used in the experiment. The study was approved by the Ministry of Social Justices and Empowerment, Government of India and by the Animal Ethics Committee Guidelines of the University of Madras, India. The animals were housed under conditions of controlled temperature (26̊ C), with a 12-h day-night cycle. They were fed a standard pellet diet (Amrut rat/mice feed; M/s. Hindustan Lever Ltd, Mumbai, India) and were given free access to water *ad libitum*.

2.3. Experimental Protocol

Experimental animals were divided into four groups of six mice each. Group 1 (control) received corn oil throughout the course of the experiment. Group 2 were treated with B(a)P(50mgkg dissolved in corn oil) orally twice a week (Day 1and Day 4) for four successive weeks. This dose of B(a)P has been shown to produce lung cancer. Group 3 received d-Limonene (150mg/kg) orally thrice a week for 16 weeks to assess the cytotoxicity, if any, induced by d-Limonene. Group 4 (d-Limonene) received B(a)P (as for Group 2) along with d-Limonene (150mg/kg) orally treatment was started one week before the first dose of B(a)P and continued for 16 weeks. The dose of d-limonene was chosen based on our previous study. At the end of the experimental period animals were sacrificed by cervical decapitation under ether anesthesia. The serum was separated from the collected blood and lungs were excised immediately and washed with ice-cold saline. A 10% homogenate of the washed tissue (lung) was prepared in 0.01 M phosphate buffer (pH 7.4). The homogenate was centrifuged at a speed of 12,000 g for 30 min in a refrigerated high-speed centrifuge at 4°C. The following biochemical estimations were carried out in the supernatant.

2.4. Biochemical analysis

The serum marker enzyme aryl hydrocarbon hydroxylase (AHH) (Mildred et al., 1981) was estimated by gamma glutamyl transpeptidase (γ-GT) (Orlowski and Meister, 1965), 5'-nucleotidase (5'-ND) (Luly et al., 1972), lactate dehydrogenase (LDH) [17]. The following biochemical estimation was carried out in the supernatant total protein was estimated by the method of LPO (Lowry, 1981) was assayed by the method of in which the malonyldialdehyde (MDA), (Ohkawa et al., 1979) released served as the index of LPO. Superoxide dismutase (SOD) was assayed according to the method (Marklund and Marklund, 1974). Catalase (CAT) activity was assayed by the method of (Sinha, 1974), glutathione peroxidase (GPx) was determined by the method of (Rotruck et al., 1973). Glutathione reductase (GR) was assayed by the method of. Glutathione-Stransferase (GST) was assayed by the method of (Habig, 1974). Reduced glutathione (GSH) was assayed by the method of (Moron et al., 1979), vitamin E was estimated by the method of (Desai, 1984), vitamin C was measured by the method of (Omaye et al., 1971) and vitamin A was determined by the method of (Bayfield and Cole, 1980).

2.5. Lung histology and Immunohistochemistry of PCNA

Histological evaluation was performed on the lung and a portion of specimen was fixed in 10% buffered formalin and embedded in paraffin wax. Sections were cut at 5µmin thicknesses, stained with hematoxylin and eosin and viewed under light microscope for histological changes. Immunohistochemistry was performed following the methods (Ramakrishnan et al., 2008). Tissue sections were deparafinized in two changes of xylene at 60°C for 20 min each and hydrated through a graded series of alcohol, the slides were incubated in citrate buffer (pH 6.0) for three cycles of five min each in a microwave oven for antigen retrieval. The sections were then allowed to cool at room temperature and then rinsed with 1×Tris buffered saline (TBS), and treated with 0.3% H_2O_2 in methanol for 10 min to block endogenous peroxidase activity. Non specific binding was blocked with 3% BSA in room temperature for 1hr. The sections were then incubated with PCNA (Spring biosciences, USA) rabbit polyclonal antibody at a dilution of 1:50 at 4°C overnight. The slides were washed with TBS and then incubated with anti-rabbit HRP labeled secondary antibody (Genei, Bangalore, India) at a dilution 1:500 for 1hr in room temperature. The peroxidase activity was visualized by treating the slides with 3,3′-diaminobenzidine tetrahydrochloride (SRL, Mumbai, India), the slides were then counterstained with Meyer's hematoxylin.

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Table 1 Effect of Limonene on the body weight, lung weight and tumor incidence of control and experimental animals

Particulars	Group 1	Group 2	Group 3	Group 4
Body weight (g)	28.2± 3.70	18±2.12 [a]	29±2.60	25.2±2.18 [b]
Lung weight (mg)	275±27.3	336±37.6 [a]	268.5±32.5	301.5±30.7 [b]
Number of animals examined	6	6	6	6
Tumor incidence	0	6	0	3
No of tumor incidence / mice	0	3.78±0.28 [a]	0	1.18±0.41 [b]

Each value is expressed as mean ± S.D for six mice in each group .Statistical significance: $p<0.05$
[a]group 2 compared with group 1
[b]group 2 compared with group 4

Table 2 Effect of limonene on the activity of marker enzymes in the lung of control and experimental animals

Particulars	Group 1	Group 2	Group 3	Group 4
AHH	0.66± 0.07	0.89±0.09 [a]	0.65±0.06	0.71±0.07 [b]
Γ- GT	1.22±0.15	2.28±0.27 [a]	1.30±0.15	1.76±0.23[b]
5'ND	3.31±0.42	5.77±0.70 [a]	3.36±0.35	3.78±0.52 [b]
LDH	1.47±0.14	2.57±0.28 [a]	1.54±0.12	1.71±0.13 [b]

Each value is expressed as mean ± S.D for six mice in each group . AHH-µmoles of fluorescent phenolic metabolites formed/min/mg protein. γ- GT- nmoles of p-nitroaniline formed /min/mg protein. 5'-Nucleotidase- nmoles of Pi liberated/min/mg protein. LDH-µmoles of pyruvate liberated/min/mg protein.
[a] group 2 compared with group 1.
[b] group 2 compared with group 4.

Negative controls were incubated with TBS instead of primary antibodies. Quantitative analysis was made in a blinded manner under a light microscope. The labeling index was expressed as number of cells with positive staining per 100 counted cells in ten randomly selected fields at high magnification (40×).

2.6. Statistical Analysis

All the grouped data were significantly evaluated with SPSS/10 software. Hypothesis testing methods included one way analysis of variance followed by least significant difference test. p values of less than 0.05 were considered to indicate statistical significance. All these results were expressed as mean±S.D for six animals in each group.

3. RESULTS & DISCUSSION

Table 1 shows the body weight, lung weight and tumor incidence of different groups of mice that were sacrificed at the end of the study. Treatment with d-Limonene increased ($p<0.05$) the final body weight and significantly decreased lung weight and tumor incidence in group 4 animals when compared with group 2 animals, which give the positive index of d-limonene. Table 2 show the effect of d-Limonene on the activities of marker enzymes in serum of control and experimental groups the activities of marker enzymes AHH, γ-

GT, 5′ND and LDH were found to be significantly ($p<0.05$) increased in lung cancer bearing animals (group 2), that were reversed to near normal in d-Limonene in group 4 animals. Analysis of serum marker enzymes serves as an indicator of cancer response to therapy. Distribution of many biochemical, immunological and molecular properties of the host has been observed in B(a)P mediated cancer conditions (Denissenko et al., 1996). Marker enzymes such as AHH, GGT, 5′-ND and LDH are specific indicators of lung damage (Kamaraj et al., 2007; Ferrigno et al., 1994). Chen and Liu (2000) reported that AHH is one of the useful biomarkers in early diagnosis of lung cancer. It is responsible for the activation of B(a)P and other PAHs in cigarette smoke leading to carcinogenesis (Kiyohara and Hirohata, 1997). The AHH activity was increased in lung cancer bearing animals. GGT activity serves as a specific

Table 3 Effect of Limonene on the activities of antioxidant enzymes in the lung of control and experimental animals

Particulars	Group 1	Group 2	Group 3	Group 4
LPO	0.73±0.08	1.39±0.12 [a]	0.74±0.07	0.87±0.08 [b]
SOD	4.20±0.52	2.51±0.29 [a]	4.32±0.56	3.87±0.33 [b]
CAT	254±35.2	125±24.2 [a]	251±34.1	157±21.0 [b]
GPx	41.70±5.52	21.31±3.70 [a]	42.75±5.38	32.86±5.22 [b]
GR	2.59±0.37	1.66±0.15 [a]	2.74±0.31	2.47±0.26 [b]
GSH	1.55±0.15	0.97±0.15 [a]	1.64±0.14	1.16±0.15 [b]
Vitamin E	0.59±0.08	0.36±0.04 [a]	0.55±0.08	0.40±0.04 [b]
Vitamin C	0.48±0.06	0.32±0.06 [a]	0.50±0.05	0.37±0.05 [b]

Each value expressed as mean ± S.D for six mice in each group .LPO = nmol of MDA released/mg prutein,SOD = units/min/mg protein; CAT = µmoles of H_2O_2 consumed/min/mg protein; GPx = µmoles of GSH oxidized/min/mg protein; GR = µmoles NADPH oxidized/min/mg protein. GSH = µg/mg protein; Vitamin E = µg/mg protein; Vitamin C = µg/mg protein.
[a] group 2 compared with group 1.
[b]group 2 compared with group 4.

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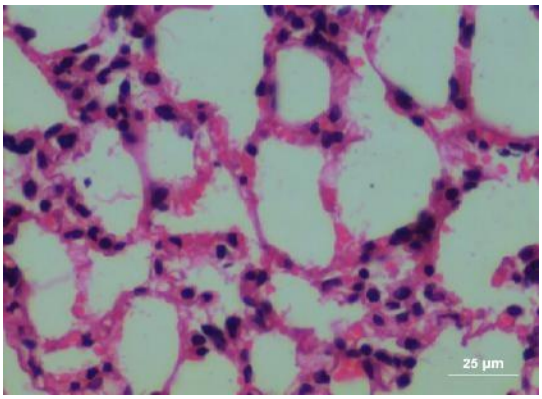

Plate 1

Control group1animals showing lung parenchymal cells with normal alveoli

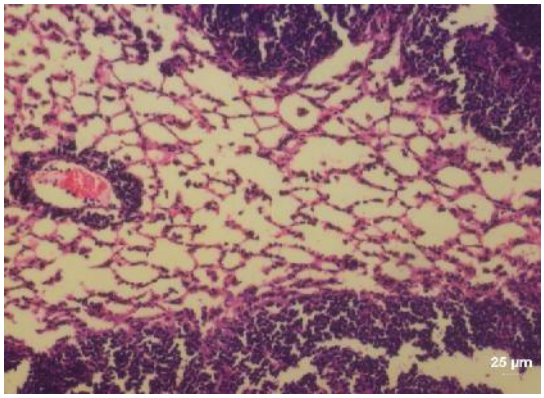

Plate 2

B(a)P induced group2 animals showing proliferation of closely packed alveolar cells with hyperchromatic nuclei

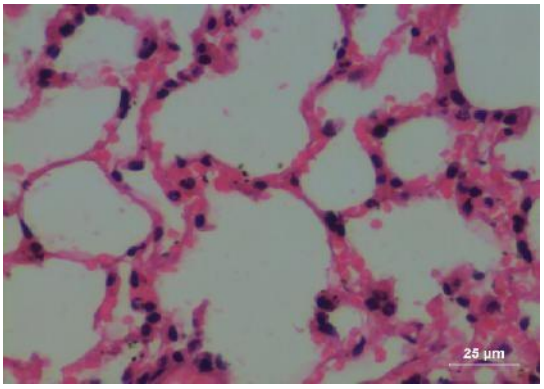

Plate 3

d-Limonene treated group3 animals showing normal architecture

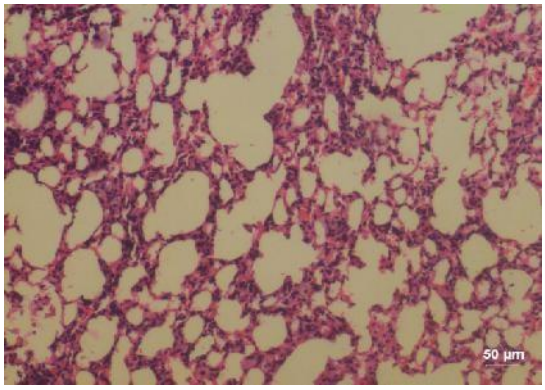

Plate 4

B(a)P with d-Limonene treated group4 animals showingreduced number of hyperchromatic nuclei

Figure 1

Histopathological studies in the lung of control and experimental group of animals (H & E,40x)

marker for the prognosis of carcinogenic events. GGT is not only useful in diagnosis but also has extrapolative value in malignancies such as lung cancer and malignant melanoma. An increased level of GGT was observed in cancer cells (Ngo and Nutter, 1994). This elevation may indicate the basic tumor burden. Increased activity of 5′-ND seems to have originated from the proliferating tumor cells (Ramakrishnan et al., 2007) and a fast moving 5′-nucleotide phosphodiesterase is found to be elevated in metastases to liver from tumor of the lung and breast (Vanisree and Shyamaladevi, 1998). LDH is a fairly sensitive marker for solid neoplasms and elevated activity of the enzyme was reported in serum of lung cancer patient (Anbarasi et al., 2005). The possible reason for elevated levels of LDH may be due to higher glycolysis in cancerous conditions, which is the only energy producing pathway for the uncontrolled proliferating malignant cells (Helmes et al., 1998). Decrease in LDH activity on treatment with d-Limonene protected against abnormal cell growth by changing the permeability of membrane or by affecting cellular growth. The elevated activity of AHH, GGT, 5′-ND and LDH observed in cancer bearing animals was brought down to near normal upon administration of d-Limonene reveals the anticancer potential of the drug.

Table 3 show the levels of lipid peroxides, cellular enzymic antioxidants such as SOD, CAT and GPx and non-enzymatic antioxidants (GSH, vitamin E and vitamin C) in lung tissues of the various experimental groups. A highly significant ($p<0.05$) increase in tissue LPO with concomitant decrease in the activity of enzymic and non-enzymic antioxidants was observed in tumor bearing animals in group 2 animals. These adverse changes were reversed to near normal values in d-Limonene treated group 4 animals. Antioxidant status has been suggested as a useful tool in estimating the risk of oxidative damage induced carcinogenesis. Enzymatic antioxidants like SOD, CAT and GPx synergistically scavenge reactive oxygen species (ROS) and prevent LPO. SOD is the only enzyme that disrupts superoxide radicals and protects the cells against superoxide and hydrogen peroxide-mediated LPO (Ekambaram et al., 2008). Catalase is widely

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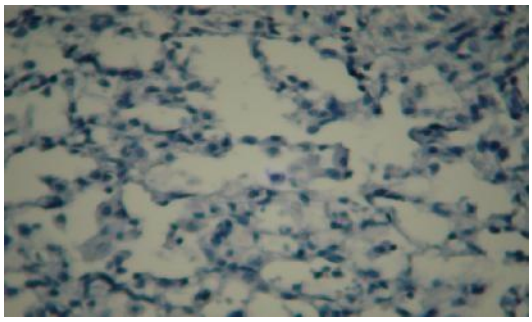

Plate 1
Control group1animals

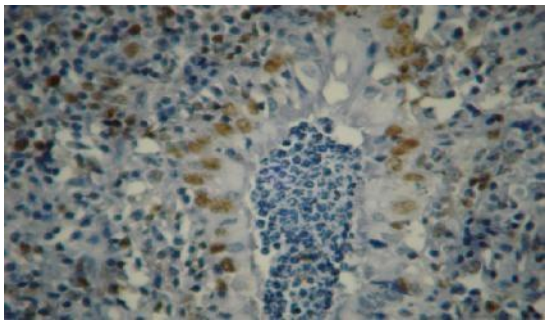

Plate 2
B(a)P induced group2 animals arrows showing the positive nuclei

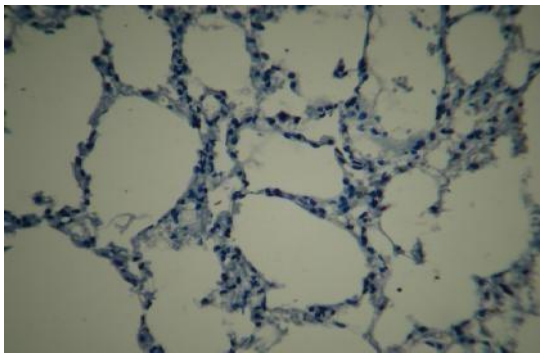

Plate 3
d-Limonene treated group3 animals showing normal architecture

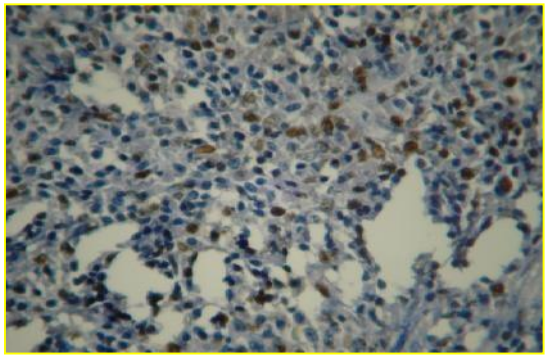

Plate 4
B(a)P with d-Limonene treated group4 animals showing reduced number of positive nuclei.

Figure 2
Immunohistochemical analysis of PCNA in the lung of control and experimental group of animals (40x)

distributed in all tissues and catalyses the breakdown of hydrogen peroxide produced by tumor cells. The source of hydrogen peroxide is mainly SOD mediated dismutation of superoxide radical, which is generated by various enzyme systems as well as by non enzymic pathways. Several reports have cited decreased activities of SOD and catalase in various carcinogenic conditions (Van Driel et al., 1997; Selvendiran et al., 2003; Thirunavukkarasu et al., 2001; Anandakumar et al., 2008; Ramakrishnan et al., 2006). GPx is a well known first line of defense against oxidative stress, it catalyze the transformation of H_2O_2 to harmless byproducts, thereby curtailing the quantity of cellular destruction and several studies have reported the decreased activities of GPx in various cancerous conditions. SOD, CAT and GPx constitutes a mutually supportive team of defense against reactive oxygen species which have been found to be decreased in B(a)P induced lung cancer bearing animals. Neoplastic cells may sequester essential antioxidants from circulation to supply

ANTI-CANCER ACTIVITY OF D-LIMONENE

D-limonene (1-methyl-4-(1-methylethenyl) cyclohexane) is a monocyclic monoterpene with a lemon-like odor and is a major constituent in several citrus oils (orange, lemon, mandarin, lime, and grapefruit). Animal studies have set the stage for further investigation into the chemoprotective activity of d-limonene for several types of cancer. Several experiments demonstrated inhibition of chemically-induced mammary cancer in rodents administered either orange peel oil or pure d-limonene. Inhibition occurs in either the initiation or promotion phases, depending on the chemically-induced medium used. Other animal trials demonstrated d-limonene inhibited development of liver cancer, pulmonary adenoma, and fore-stomach tumors. D-limonene induces phase I and phase II carcinogen-metabolizing enzymes (cytochrome p450), which metabolize carcinogens to less toxic forms and prevent the interaction of chemical carcinogens with DNA. D-limonene has been shown to enhance gastrointestinal UDP-glucuronosyltransferase (UGT) activity in rats. It also inhibits tumor cell proliferation, acceleration of the rate of tumor cell death and/or induction of tumor cell differentiation. Furthermore, d-limonene inhibits protein isoprenylation. Many prenylated proteins regulate cell growth and/or transformation. Impairment of prenylation of one or more of these proteins might account for the antitumor activity of d-limonene. It was found that d-limonene attenuates gastric cancer through increasing apoptosis, while decreasing DNA synthesis and ornithine decarboxylase activity of cancer cells. D-limonene inhibits hepatocarcinogenesis via inhibition of cell proliferation, enhancement of apoptosis, and blockage of oncogene expression. D-limonene may also exhibit immune-modulating properties. One animal study observed increased survival in lymphoma-bearing mice placed on a high d-limonene diet. These mice also demonstrated increased phagocytosis, microbicidal activity, and nitric oxide production.

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the demands of growing tumor (Navarro et al., 1999). GSH, vitamin C and E comprise the non-enzymic antioxidant system that protects the cells against free radicals and ROS. GSH acts directly as a free radical scavenger by donating a hydrogen atom and thereby neutralizing the hydroxyl radical. It also reduces peroxides and maintains protein thiols in the reduced state (Van Poppel and Van den Berg, 1977). Changes in the rate of cancer cell proliferation are accompanied by changes in their intracellular GSH levels and consequently these could be reflected in their antioxidant machineries (Halliwell, 1996). Antioxidant vitamins have a number of biological activities suc as immune stimulation, scavenging the free radicals and alteration in metabolic activation of carcinogens. They can utilize reactive oxygen metabolites, protecting biopolymers and reduce oxidative DNA damage. Vitamin E is the major lipid soluble peroxyl radical scavenger, which can limit LPO by terminating chain reactions initiated in the membrane lipids. It is the most significant antioxidant of its kind in animal cells and it can protect against carcinogenesis and tumor growth. Decreased vitamin E content in lung cancer bearing animals might be due to the excessive utilization of this antioxidant for quenching enormous free radicals produced in this condition. Vitamin E acts as a chain breaking antioxidant by denoting its labile hydrogen atom from phenolic hydroxyl groups to propagation lipid peroxyl and alkoxyl radical intermediates of LPO, thus terminating the chain reaction.

Carcinogenesis: Carcinogenesis or oncogenesis or tumorigenesis is literally the creation of cancer. It is a process by which normal cells are transformed into cancer cells. It is characterized by a progression of changes on cellular and genetic level that ultimately reprogram a cell to undergo uncontrolled cell division, thus forming a malignant mass.

The availability of vitamin C is a determining factor in controlling and potentiating many aspects of host resistance to cancer. Vitamin-C, which prevents oxidative damage to cell membrane induced by aqueous radicals also exists in interconvertable forms and participates in neutralizing free radicals by regenerating the antioxidants from vitamin-E. When there is reduction in the levels of GSH, cellular levels of vitamin E and vitamin C are also lowered. This may be the reason for the observed decrease in vitamin-E and C in cancer bearing mice. d-Limonene supplementation significantly increased all the above enzymic and non-enzymic antioxidants which may be due to its potent free radical scavenging activity. Increased levels of LPO products play a major role in the early phases of tumor growth. 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 LPO. Naturally there is a dynamic balance between the amount of free radicals generated in the body and antioxidant defense system that quench or scavenge them and protect the body against their deleterious effects. Decreased levels of LPO in d-Limonene treated animals might be due to its ability to increase the levels of antioxidants.

Fig.1 shows the histological analysis of lung section of control and experimental groups. Lung from control (group 1) animals revealed normal architecture with small uniform nuclei (Fig.2a). Lung cancer bearing animals (group 2) revealed loss of architecture, alveolar damage as seen from hyperchromatic nuclei in the cells of alveolar wall (Fig. 2b). Cancer bearing animals treated with d-Limonene (group 4) exhibited reduced alveolar damage with near normal architecture (Fig.2c). d-Limonene treated animals (group 3) showed no appreciable change (Fig.2d).

Immunohistochemical is staining for PCNA in the lung of control and experimental group of animals (Fig.2). B(a)P induced group 2 animals showed a significant increase ($p<0.05$) in the number on PCNA positive nuclei when compared with group 1 normal control animals, while d-Limonene treatment significantly decreased ($p<0.05$) the number of PCNA positive nuclei when compared with B (a)P induced animals. PCNA is a 36 KDa auxiliary protein to DNA polymerase-S, which is found in various concentrations within the cell throughout the cell cycle and in greatest quantities during S-phase PCNA is usually regarded as a proliferation marker, it is highly expressed by actively proliferating cells and rapidly degrade as the cell enters the non-proliferative stage. Decreased expression of PCNA upon d-Limonene treatment revealed the anti-proliferative effect of d-Limonene. From these observations it can be concluded that the anticancer effect of d-Limonene against B(a)P induced lung carcinogenesis in mice is due to its ability to increase antioxidants and there by inhibiting proliferation.

4. CONCLUSION

D-Limonene there by significantly showing potent anticancer effect in lung cancer, further , overall these findings substantiate the chemopreventive potential of d-Limonene against Benzo(a)pyrene chemically induced lung cancer in mice.

SUMMARY OF RESEARCH

1. Chemo-preventive nature of d-Limonene during benzo(a)pyrene induced lung cancer in swiss albino mice was revealed.
2. Lipid peroxides (LPO), serum marker enzyme aryl hydrocarbon hydroxylases (AHH), gamma glutamyl transpeptidase (GGT), 5'nucleotidase (5'ND) and lactate dehydrogenase (LDH) with concomitant decrease in the levels of tissue antioxidants like superoxide dismutase(SOD), catalase (CAT), glutathione (GPx), reduced glutathione(GSH), vitamin-E & vitamin-C by the administration of B(a)P.
3. Supplementation of d-Limonene was showing potent anticancer effect in lung cancer, and antiproliferative effect of d-Limonene was confirmed by histopathological analysis & proliferating cell nuclear antigen (PCNA) immuno-staining.

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FUTURE ISSUES

1. How d-Limonene is superior in anticancer effect and anti-proliferative effect when correlated with modern generation drugs?
2. Is d-Limonene has lesser side-effects in cancer chemotherapy while compared with current drugs?

DISCLOSURE STATEMENT

There is no financial support for this research.

ACKNOWLEDGMENTS

We thank the professors and colleague for the enormous support to carry out the research work. We extend a special thanks to our friends and technicians of the Department of Biochemistry, University of Madras, Gunidy Campus, Chennai.

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