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ORIGINAL ARTICLE

Immunomodulatory Effect of d-Carvone in Swiss Albino Mice with Benzo(a)pyrene-Induced Lung Cancer

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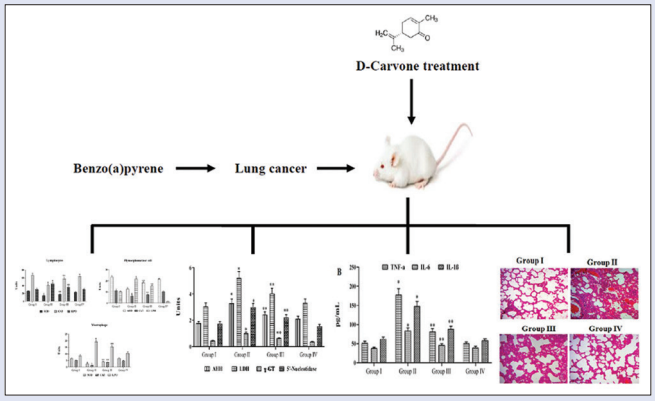
ABSTRACT

Background: Globally, lung cancer is the second most cause of deaths, which accounts for approximately 29% of the cancer cases worldwide. d-Carvone is considered a vital constituent of many essential oils with immense pharmacological benefits. **Objective:** In this study, we examined the immunomodulatory effect of d-carvone in the Swiss albino mice model against benzo(a) pyrene (BaP)-induced lung carcinoma. **Materials and Methods:** BaP was orally administered to the mice (50 mg/kg body weight [bw] for 4 weeks [twice a week]). Post-BaP administration, d-carvone (20 mg/kg bw) was orally administered to the mice (20 mg/kg bw). We calculated the tumor incidence and performed the following measurements: lung and bw, hematological parameters, immune complexes (phagocytic and avidity indexes, nitroblue tetrazolium reduction, soluble immune complex levels, immunocompetent cells (leukocytes, lymphocytes, and neutrophils), immunoglobin (Ig) levels (IgG, IgA, and IgM), level of xenobiotics and enzymes that point toward hepatic dysfunction, carcinoembryonic antigen (CEA), and proinflammatory cytokines (PICs) in experimental and normal mice. The level of oxidative stress in the experimental animals was investigated. The lung tissues of investigational animals were examined for the histopathological changes. **Results:** According to the results, there was an increased level of lipid peroxidation and decreased level of antioxidant activity in the lung tumor samples. The counts of lymphocytes, polymorphonuclear cells, and macrophages were notably decreased and increased, respectively, by the d-carvone post-treatment. Moreover, BaP-induced inflammation that is indicated by the increased in the level of PICs and CEA proteins in lung samples. d-Carvone attenuated the levels of PICs and CEA. **Conclusion:** The results of this study reveal that d-carvone prevented the cells against BaP-induced inflammation in lung cancer without causing adverse effects.

Key words: Benzo(a)pyrene, d-carvone, human erythrocytes, lung cancer, Swiss albino mice, tumor necrosis factor-alpha

SUMMARY

- Lung carcinoma is the second-leading cause of death worldwide among both men and women; it accounts for 29% of cancer cases
- d-Carvone against DMBA-induced skin tumor proliferation in mice model dose dependently by modulating the xenobiotic metabolic and detoxification enzymes and also induces apoptosis.



Abbreviations used: LC: Lung carcinoma; SAM: Swiss albino mice; NBT: Nitrobluetetrazolium, SIC: Soluble immune complex, PIC: Pro-inflammatory cytokines; LPO: Lipid peroxidation; PMN: Polymorphonuclear cells.

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INTRODUCTION

Lung cancer (LC) is the second leading cause of death worldwide in both men and women. It accounts for approximately 29% of the cancer cases globally.[1] Smoking is the primary etiological factor for LC. Polycyclic aromatic hydrocarbons (PAHs) are procarcinogenic chemicals, major reason to tobacco-related disease, which is metabolically activated and converts the benzo[a] pyrene-7,8-dihydrodiol-9,10-oxide (BPDE) highly reactive metabolite synthesis of cytochrome P450 1A1. BPDE is responsible for the formation of DNA adducts, which in turn leads to the initiation of cancer. However, the balance between metabolic activation and detoxification determines if the individual is at cancer risk.[2,3] Nutraceuticals prevent carcinogen-induced oxidative damage by altering the numerous signaling pathways. Antioxidant molecules neutralize the harmful effects of toxic chemicals by scavenging the free radicals.[4]

Cancer chemoprevention is an attractive alternative to various other treatment modalities. It is the foremost research area on cancer, which includes identification, characterization, and production of effective and less toxic chemopreventive agents.[5] Therefore, in this study, we explored the various compounds of the plant origin with possible antitumor effects.[6]

The major role of the liver is to metabolize and detoxify the carcinogens. Measuring the activity of the enzymes responsible for the detoxification

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processes might help us to examine the chemopreventive potential of the natural product.[7] Thus, chemoprevention if a helpful and novel strategy to detect the antitumor action of synthetic agents and natural products. Human diet contains triterpenes, which are one of the natural structural components of a medicinal plant. A previous study has demonstrated the effect of terpenoids against cancer cell growth under *in vitro* conditions.[8]

Carvones are unsaturated monoterpenoid ketones present in the essential oils of dill, caraway, angelica, and spearmint.[9] They show antioxidant, antimicrobial, antihypertensive, and antihyperlipidemic effects. d-Carvone is considered to be an important constituent of many essential oils but is rich in caraway seeds.[9,10]

In this study, we established the association between d-carvone and oxidative stress (OS) specifically in the immune systems as well as aimed to establish its therapeutic effect during immune dysfunction. Benzo(a)pyrene (BaP) induces the production of toxic intermediates of the immune system. We studied the effects of d-carvone in reversing the BaP-induced immunological shortage in antibody synthesis and cellular recognition. The signaling pathway by which BaP induces immunosuppression was also studied.

MATERIALS AND METHODS

Chemicals

Benzo(a)pyrene (BaP) (≥95% purity), d-carvone (≥95% purity, CAS NO: 2244-16-8-5-50), catalase (CAT), bovine serum albumin, reduced glutathione, dithio-bis (2-nitrobenzoic acid), 2-thiobarbituric acid, and corn oil were purchased from Sigma-Aldrich (USA). All other fine chemicals and solvents were obtained from Himedia (USA).

Animals

Adult male Swiss albino mice (SAM) were maintained under the controlled conditions: temperature of 25°C ± 5°C and relative humidity of 55% ± 10% in an atmosphere with 12:12 h light and dark cycles with free access to standard food and water. This experimental protocol was approved by the Institutional Ethical Committee (2019-06). Twenty-four mice were separated into four equal groups: Group 1 animals (control group) received inward corn oil as the vehicle; Group II (positive LC control) mice were administered with BaP (50 mg/kg body weight [bw], dissolved in corn oil, twice a week for 4 consecutive weeks, from the second to sixth week); Group III animals were treated postinitiation with d-carvone (20 mg/kg bw, suspended in corn oil) starting through the 12th week of the experiment (BaP was given as in Group II) throughout the experimental period (18th week); and Group IV animals were treatment with d-carvone only (as above) and continued throughout the duration of the study.

Mice were divided into four groups of 6 mice in each group. The concentration of d-carvone (20 mg/kg bw) and BaP (50 mg/kg bw) was selected based on the previously published literature.[11,12] Experimental mice were assessed weekly in anticipation of the termination of experimental duration. Then, one mouse from each group was sacrificed by cervical decapitation.

LC tissue homogenate (10%) was prepared in phosphate buffer (0.01 M, pH 7.4) and was centrifuged using the refrigerated high-speed centrifuge at 4°C for 10,000 RPM for 15 min. After this, the supernatant was stored at −80°C until further analysis. The protein content in the tissue homogenate was estimated based on the method described by Lowry *et al.*[13]

Body weight, lung weight, and tumor incidence

BW of the mice was recorded throughout the study during the experimental period. Mice were weighed at the initiation of the trial and consequently once in a week to the end of experiment. After the experimental duration, lungs were cut out from the mice, washed with saline, followed by the measurement of weight. Tumor incidence (TI) was determined by manual counting.

Hematological studies

Whole blood cells by counted by the method described by Boyum (1964).[14] Approximately 95% of the neutrophils and lymphocytes were measured by differential counting as the following blood cells were used to analyze the subsequent cycle of immunotherapy: Absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and white blood cell (WBC) count. All the biochemical and hematological parameters exist using semi- and fully automated analyzer.

Immune complex assays

The following immune complex analysis was conducted: Anticoagulant held blood assist for counting IMMC,[15] evaluation of immune function test,[16] nitroblue tetrazolium (NBT) reduction test,[17] and coagulated blood was used to examine soluble immune complex (SIC) activities.

Estimation of serum immunoglobin G, immunoglobin A, and immunoglobin M

Blood levels of IgG, IgM, and IgA from the control and experimental mice are very help to calculation of IgA, IgM, and IgG antibodies and SIC status.[18,19]

Estimation of superoxide dismutase and catalase

Superoxide dismutase (SOD) and CAT levels were examined in the neutrophils, lymphocytes, and macrophages by the method described by Madesh and Balasubramanian (1998)[20] and Maehly and Chance (1954).[21]

Estimation of lipid peroxidation

Lipid peroxidation (LPO) in neutrophils, lymphocytes, and macrophages was determined using the technique of malonaldehyde (MDA) as per Ohkawa *et al.*[22]

Estimation of lactate dehydrogenase, γ-glutamyl transpeptidase, aryl hydrocarbon hydroxylase, and 5′nucleotidase analysis

In this study, the level of aryl hydrocarbon hydroxylase (AHH) was determined by the method described by Buening *et al.*[23] The levels of lactate dehydrogenase (LDH) were determined based on the procedure described by King.[24] The status of γ-glutamyl transpeptidase (γ-GT) was determined based on the procedure described by Orlowski and Meister.[25] Finally, 5′nucleotidase was analyzed based on the technique of Luly *et al.*[26]

Estimation of carcinoembryonic antigen and proinflammatory cytokines analysis

In this study, we quantified the levels of carcinoembryonic antigen (CEA) and interleukin (IL)-1β, IL-16, and tumor necrosis factor-alpha (TNF-α) in cancer tissues. Briefly, tumor homogenate (10%) was prepared in PBS (0.01 M, pH 7.4), and the supernatant was centrifuged at 10,000 × *g* for 20 min. The supernatant was used to estimate the levels of CEA and IL-1β, IL-6, and TNF-α using relevant kits (USA).

Lung tumor histology

Lung tumor tissue was fixed in buffered formalin overnight and then transferred to different concentrations of ethanol, cleared in xylene, and embedded in paraffin to organize the block for the histopathological analysis. Microsections (3–5 mm) of tissues were prepared and stained with hematoxylin and eosin (staining) and inspected under the light microscope.

Statistical analysis

In this study, data are presented as mean ± standard deviation. Differences in means were analyzed by the one-way analysis of variance followed by Tukey's *post hoc* test. Data were considered statistically significant when $P < 0.05$.

RESULTS

Effect of d-carvone on body weight, lung weight, and tumor incidence

Table 1 depicts the effect of the d-carvone in BaP-induced mice on mean their BW, LW, and TI in control and experimental mice. At the end of the experiment (18th week), BaP alone induced mice possessed reduced BW gain, augmented LW and TI 100% formed match up to untreated mice. However, these statistically significant ($P < 0.05$) results of Group II were prevented on post-supplemented d-carvone, to BaP-exposed mice. There were no considerable differences between in d-carvone alone and control mice.

Effect of d-carvone on the hematological study

Figure 1 shows the effects of d-carvone on the levels of IMMC in the experimental mice. The mice treated with BaP alone demonstrated a notably ($P < 0.05$) diminished cell count of WBC, lymphocytes, neutrophils, ALC, and ANC as compared with the control mice. However, posttreated d-carvone showed a notable ($P < 0.05$) improvement in the hematological parameters as compared to the BaP-induced mice. Treatment with d-carvone alone and control mice resulted in no significant differences in the aforementioned parameters.

Table 1: Effect of d-carvone on body weight, lung weight, and tumor incidence in control and experimental animals (n=6)

Groups/ treatments	Bodyweight (g)	Lung weight (mg)	Tumor incidence
Group I	29.55±1.77	247.33±18.84	0
Group II	17.22±0.79*	342.69±26.10*	6
Group III	25.61±1.95#	284.83±21.81#	3
Group IV	30.13±1.97	260.34±19.83	0

Values are expressed as mean±SD for six mice in each group. Data not sharing a common superscript letter (*-#) differ notably at $P<0.05$ (Duncan's multiple range test). SD: Standard deviation

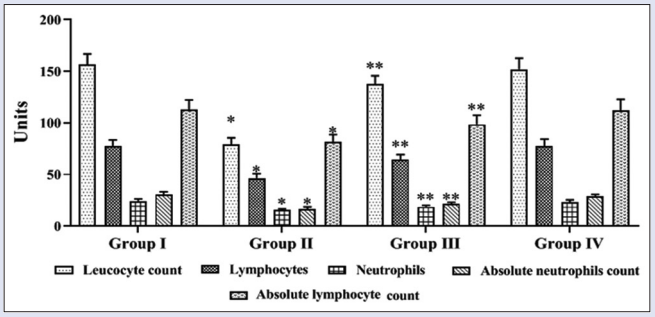

Figure 1: Effect of d-carvone on the hematological counts in control and experimental animals. Values are expressed as mean ± standard deviation for six mice in each group. Data not sharing a common superscript letter (*-**) differ significantly at $P < 0.05$ (Duncan's multiple range test)

Effect on d-carvone on ICs

Figure 2 shows the effects of d-carvone on Immune Complex as suggested by the NBT assay, avidity index, phagocyte index, and SIC levels in experimental mice. BaP-induced mice demonstrated a notably ($P < 0.05$) lower levels of ICs that that of control mice. d-Carvone administration significantly ($P < 0.05$) augmented the status of ICs as compared to the control mice. d-Carvone alone did not reveal significant changes in the aforementioned markers when compared with control mice.

Effect of d-carvone on Ig levels

Figure 3 illustrates the variations in the levels of IgA, IgM, and IgG in the control and treated mice. IgM and IgG levels deceased drastically ($P < 0.05$), whereas the levels if IgA increased significantly ($P < 0.05$) as compared to control mice. The administration of d-carvone considerably improved the levels of IgM and IgG ($P < 0.05$), whereas the levels of IgA decreased ($P < 0.05$). Again, d-carvone supplementation only has no special effects on the standard IG status as compared with the control mice.

Effect of d-carvone on immunological oxidative stress

Figure 4 shows the effect of d-carvone on immunological OS in control and treated mice. BaP administration increased the immunological OS significantly ($P < 0.05$). It increased the level of MDA in the neutrophils, macrophages, and lymphocytes as compared to control mice. It also reduced the activity of CAT and SOD ($P < 0.05$). d-Carvone notably modified the BaP-induced OS as revealed by the decreased levels of MDA in addition to increased activities of CAT and SOD in lymphocytes, macrophages, and neutrophils. No significant differences were recorded in terms of immunological OS levels in the d-carvone alone as compared with control mice.

Effects of d-carvone on the levels of hepatic marker enzymes and xenobiotic agents

Figure 5 shows the activities of hepatic marker enzymes and xenobiotics (LDH, AHH, γ-GT, and 5′-ND) in the tumor samples of the experimental and control mice. These enzymes were notably ($P < 0.05$) increased in the BaP-induced mice when compared to the control mice. d-Carvone increased the activity of these enzyme were lowered drastically ($P < 0.05$) in post-treatment with mice as compared to those

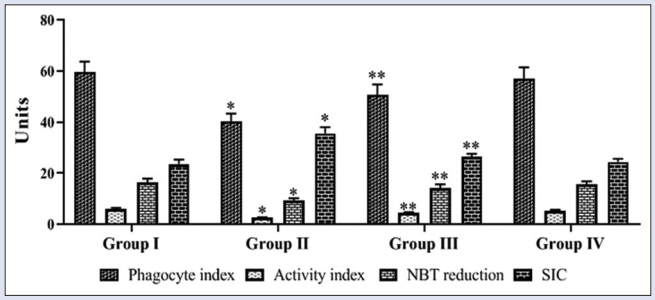

Figure 2: Effect of d-carvone on the phagocyte index, avidity index, nitroblue tetrazolium reduction, and soluble immune complex reduction in control and experimental animals. Values are expressed as mean ± standard deviation for six mice in each group. Data not sharing a common superscript letter (*-**) differ significantly at $P < 0.05$ (Duncan's multiple range test)

in the BaP-induced mice. There is no significant difference between the mice treatment with d-carvone alone and the control mice.

Effects of d-carvone on carcinoembryonic antigen and proinflammatory cytokines

Figure 6 depicts the effect of d-carvone on the levels of CEA and proinflammatory cytokines (PICs) in lung tumor tissue in control and treated mice. The levels of CEA and PICs were drastically ($P < 0.05$) increased in mice with BaP as compared with control mice. However, mice with d-carvone administration demonstrated the decreased expression of IL-6, TNF-α, and IL-1β when compared to BaP-induced mice. Furthermore, d-carvone administration had no effects on the levels of CEA and PICs as in control mice.

Measurement of histological changes in tumor tissue

Figure 7 shows the pathological analysis of carcinogenic adenotumor tissues of control and treatment mice. BaP-induced mice tumor tissue in lung sections exposed progression lesions during hyperplasia as well as showed defeat of architecture among distorted alveoli as seen through augmented number of hyperchromatic nuclei in the cells of alveolar wall during the widespread development of alveolar epithelium. Lung tissue samples of control mice and d-carvone alone treated mice demonstrated normal architecture among the small uniform nuclei and no change in the histopathological appearance. Mice posttreated with d-carvone exhibited slightly diminished alveolar injury with slightly close to normal architecture.

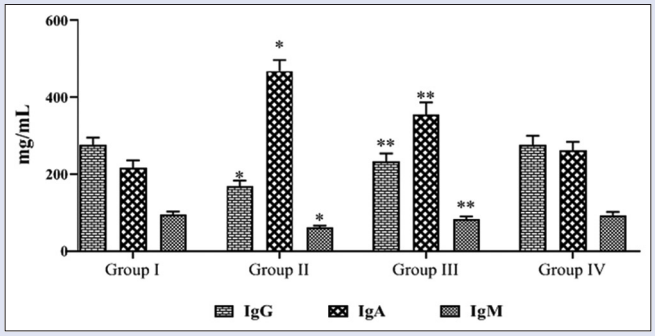

Figure 3: Effect of D-carvone on immunoglobulin levels in the serum of control and experimental animals. Values are expressed as mean ± standard deviation for six mice in each group. Data not sharing a common superscript letter (* - #) differ significantly at $P < 0.05$ (Duncan's multiple range test)

DISCUSSION

Tobacco smoke causes alveolar disorder.[27] In this study, we examined the immunomodulatory effect of d-carvone against BaP-induced tumor in lung cancer in SAM. Recent studies have revealed that BaP decreases BW of the mice which increases the severity of the pulmonary carcinogenesis, which is a general sign of tumorigenesis. The reduction in BW might be due to the cancer anorexia cachexia, which contributes in proliferation decreased of BW and is largely considered through inhibits of final BW compartments like adipose tissue and skeletal muscle.[28,29] Furthermore, an increased BW in BaP-induced mice might be due to the increased incidence of inflammatory nodules. Post-treatment with d-carvone prevented the decrease in BW and decreased the TI. There was a considerable reduction in the average number of tumors in animals treated with d-carvone supplementation. Histological examination of tumor tissue samples revealed a restored (near normal) architecture as compared to augmented tumor nodules in BaP alone treated mice.

The inflammatory constituents of the developing lesions mainly consisted of macrophages, dendritic cells, eosinophils, lymphocytes, neutrophils, and mast cells which secrete various cytokines. These cytokines increase tumor proliferation rate by disturbing metastasis and angiogenesis.[30,31] The results of this study showed that BaP-induced mice were observed the same abnormalities in mice with cancer. A recent study has examined the immune modulatory effects of PAHs by BaP.[32] In this study, decrease in the ATP content in the mice with cancer in the need of adequate cellular energy supply were observed due to a reduced in cell counts, over all leukocyte counts and original neutrophil

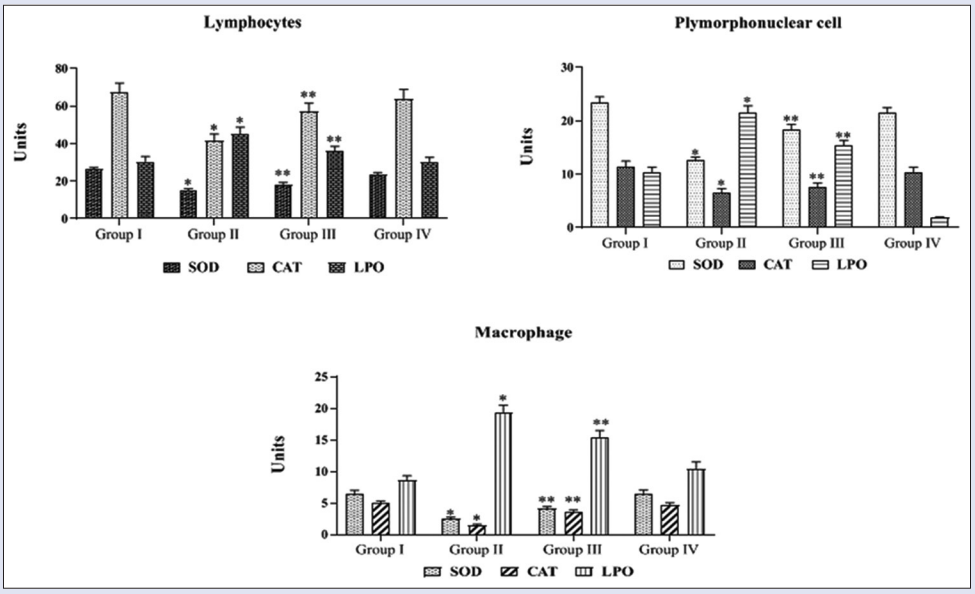

Figure 4: Effect of D-carvone on immunological oxidative stress in control and experimental animals. Values are expressed as mean ± standard deviation for six mice in each group. Data not sharing a common superscript letter (* - **) differ significantly at $P < 0.05$ (Duncan's multiple range test)

and lymphocyte counts, were calculated in the BaP-treated mice. In d-carvone posttreated mice administered with BaP, the lymphocyte, neutrophil, absolute lymphocyte, and absolute neutrophil counts were notably elevated which delayed the process of tumor development.

The NBT reduction, avidity indices, and phagocyte test were significantly decreased in the BaP-alone mice.[33] The NBT reduction and phagocytic ability of neutrophils, as revealed by the phagocytic and avidity indices, were significantly reduced in the tumor-bearing mice. SIC serves as an indicator of ICs due to the constant presence of either antibodies or antigens. These results show that BaP-induced mice markedly increased SIC levels as compared to normal mice. This reduction may have been suitable to the diminished in antibody invention in the carcinogenic in mice lung tissue.

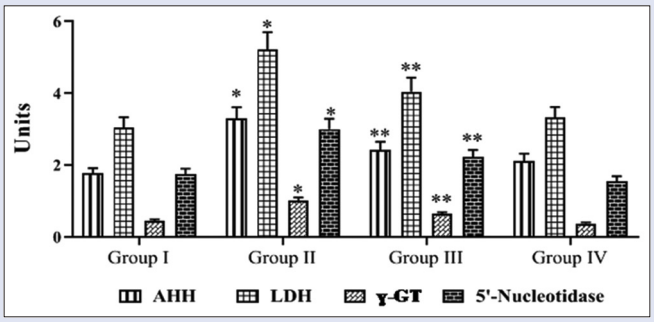

Figure 5: Effect of D-carvone on the activities of xenobiotic and liver dysfunction marker enzymes in the liver of the control and experimental animals. Values are expressed as mean ± standard deviation for six mice in each group. Data not sharing a common superscript letter (* - **) differ significantly at $P < 0.05$ (Duncan's multiple range test)

Immunomodulation might be a better alternative for the prevention and treatment of cancer by the application of chemicals from plant origin or synthetic chemicals.[34] The rate of IG synthesis (IgG and IgM) decreases in cancer, which indicates low humoral immune response, in the IgG, to an enhanced without enzyme glycosylation of IgG. The level of IGs in the serum of people with malignant diseases is highly variable. Rajendran *et al*. demonstrated that the leakage of serum IgA status might be due to the malfunction of the approval mechanism in the injured hepatic.[33] The immunosuppressive effects of BaP is clear in animals with their immune system compromised and the effect of BaP on the status of enzymes for energy metabolism change connecting normal depressed and control mice. The level of IgM and IgG were found to be increased in mice administered with d carvone and BaP, whereas the level of IgA was found to be decreased. Moreover, d-carvone might potentially block the action of free radical, thereby potentiating the cellular antioxidant potential.

However, the mechanism together with the biochemical alterations in the intracellular antioxidant defenses so may be complicated in the abnormal function of immune system connected through BaP immunotoxicity.[35] In addition, the immunoprotective role of d-carvone in stroke is yet to be identified. d-Carvone, a monoterpene, has been shown to have essential scavenging effects on macrophage responsibility during the suppression of phagocytic levels, inhibition

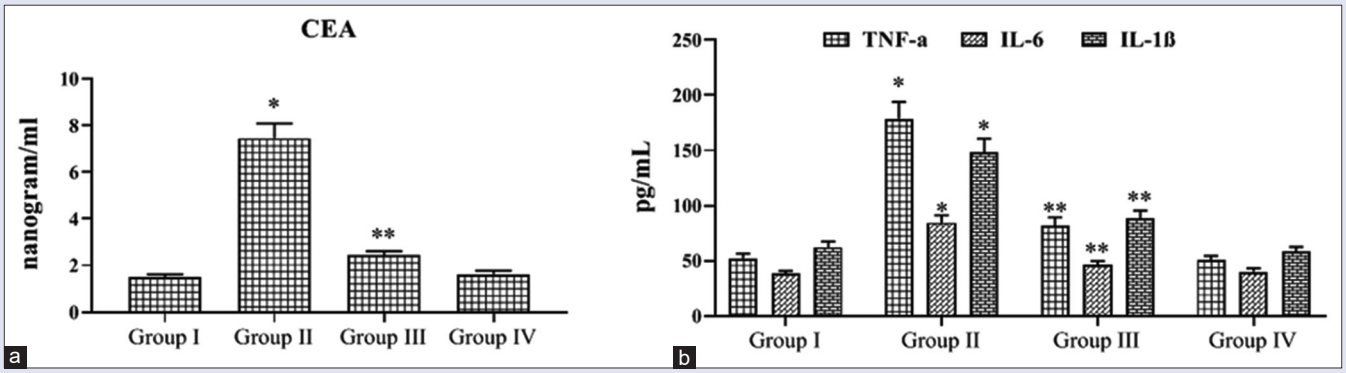

Figure 6: Effect of D-carvone on carcinoembryonic antigen and pro-inflammatory cytokines in the lung tissue of control and experimental mice. Effects of D-carvone on serum carcinoembryonic antigen levels and inflammation response in mice. (a) Activities of serum carcinoembryonic antigen and (b) ELISA was performed for tumor necrosis factor-α, interleukin-6 and interleukin-1β status in mice induced by benzo(a)pyrene. Each value is expressed as mean ± standard deviation for six mice in each group. Values are expressed as mean ± standard deviation for six mice in each group. Data not sharing a common superscript letter (* - **) differ significantly at $P < 0.05$ (Duncan's multiple range test)

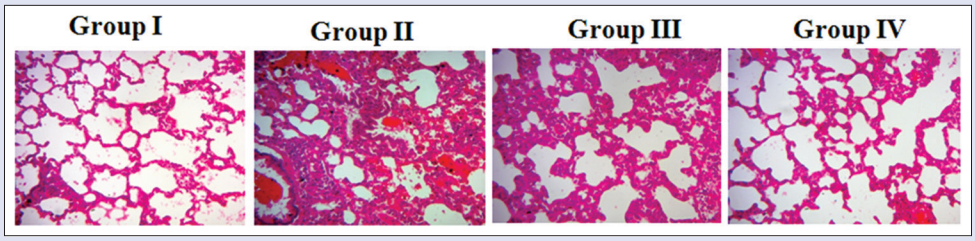

Figure 7: Histological examinations of the lung tumor of control and experimental mice. Control animals (Group I) showing a normal architecture; Benzo(a) pyrene alone treated animals (Group II) showing alveolar damages with more number of pyknoic nuclei; BaP + D-carvone (20 mg/kg bw) post-treated animals (Group III) showing reduced alveolar damage and reduced irregular hyperchromatic cells and D-carvone alone treatment (Group IV) showing no histological abnormalities

of NO creation through macrophages activated among IFNγ, LPS and holding back of intracellular and extracellular invention of ROS in macrophages moved via phorbolmyristate acetate. BaP drastically decreased the levels of SOD and CAT in macrophages, lymphocytes, and polymorphonuclear (PMN) cells, whereas the administration of d-carvone drastically improved these statuses of these enzymes. This result shows that d-carvone imparts immunoprotection through antioxidant system. The type of stroke of BaP-induced immunosuppression as well as complicated the motivation of CAT SOD, thus alleviating the LPO injure from the lymphocytes, macrophages, and PMN. d-Carvone has been demonstrated to have scavenging activity against free radicals and LPOs.[11]

Antioxidants can decrease the OS-induced tumorigenesis. Antioxidants remove toxic-free radicals. Vinothkumar *et al*. have evaluated the effect of oral administration of d-carvone and found that it drastically decreases the TI of polyps/ACF and ACF multiplicity in DMH–treated rats.[36] Further in his studies proved that d-carvone reversed the activity of hepatic and circulatory antioxidants and augmented the levels of LPO. Bodduluru *et al*. have shown that one of the terpenoids of hesperitein protects the lung against BaP-induced oxidative injury by attenuating the formation of LPO and enhancing all the components of antioxidant defense mechanisms.[12] The aforementioned studies support our findings that d-carvone significantly suppressed the formation of LPO in the BaP-induced cancer in mice. This reduction in the formation of LPO decreased the breakdown of DNA.

The histopathological analysis of tumor samples clearly indicates the progressive formation of lesions during hyperplasia as well as the loss of alveolar architecture as seen through augmented amount of hyperchromatic nuclei in the cells of alveolar wall during widespread development of alveolar epithelium in BaP-induced mice tumor tissue in lung section.[37,38] Lung tissue sections of control mice and d-carvone-treated mice showed normal architecture of the small uniform nuclei, and there was no change in the histopathological appearance. Mice posttreated with d-carvone demonstrated slightly decreased alveolar injury with slightly close to normal architecture. These results further support the anticancer effect of d-carvone.

Rajendran *et al*. have reported that the tumor xenobiotic and liver marker enzymes can reveal about hepatic cancer and LC.[39] In this study, BaP-induced LC revealed several changes in the biochemical, immunological, and molecular properties of the aforementioned components. AHH, γ-GT, 5′ND, and LDH are the specific markers of hepatic and LC.[40] These enzymes may be responsible for LC in BaP-treated mice. This leakage is established to reserve upon d-carvone supplementation in postinitiation period. In this study, the decrease in the activity of AHH, γ-GT, 5′ND, and LDH after treatment with d-carvone may have protected against abnormal cell proliferation by changing the permeability of the plasma membrane.

CEAs are the main molecular concern related to inflammation in cancer. Inflammation is responsible for cancer development and progression.[41] CEAs as well as IL-6, TNF-α, and IL-1β stimulate neutrophilia which leads to acute inflammatory processes.[42] In this study, we estimated the amount of CEAs and PICs in the samples of BaP-induced LC in mice. Administration of d-carvone decreased the levels of CEA and PICs in BaP-induced mice, which was close to normal. Our results show that BaP upregulated the expression of IL-6, TNF-α, and IL-1β in LC. Administration of d-carvone significantly downregulated the protein expression of CEA and PICs. Our results clearly demonstrate the immunomodulatory effects of d-carvone.

Carvones are terpenes and are found in many essential oils of plants and herbs such as dill, angelica, spearmint, and caraway.[43-45] Carvone derivatives demonstrate anticancer activity. Carvone suppresses p38–MAPK signaling pathway and induces apoptosis. d-Carvone has demonstrated antitumor activity against colon cancer in the rat by preventing the OS, the formation of preneoplastic lesions, and abnormal stimulation of hepatic enzymes. d-Carvone is present in caraway seeds and is one of the constituent of citrus fruits.[46,47] The LD_{50} for d-carvone has been shown to be 1500 mg/kg bw for mice after intravenous injection.[48] Gopalakrishnan *et al*. have demonstrated the anticancer effects of d-carvone against DMBA-induced skin cancer in mice model and showed that it dose dependently decreases the hepatic detoxification enzymes and also induces apoptosis.[11]

CONCLUSION

The most changes in serum biochemical parameters of mice with lung cancer were found with induced to BaP. We propose that these differences between the BaP and control animal reflect the harmful effects of BaP or might be a general response of the organism against to LC. According to our results, d-carvone administration in BaP-induced LC modulated the antioxidant activity and immune response of the animals. The potential mechanism might be that d-carvone alters the immune cells' response, scavenge/inhibit the formation of reactive oxygen species. Furthermore, d-carvone demonstrated immunomodulatory activity which might be the possible mechanism of action.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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