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ORIGINAL ARTICLE

Management of Early Lung Tumorigenesis by Corilagin via Modulation of Proliferating Cell Nuclear Antigen and Apoptotic Pathway

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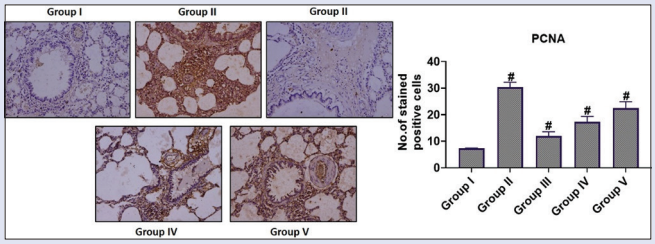
ABSTRACT

Background: Corilagin is vastly found in plants and incorporated in countless traditional medicinal systems to treat various ailments including cancer disease. However, till now, no scientific study has been done to validate its efficiency with appropriate experimental evidence. Resulting, this study examined the ameliorative effects of corilagin in benzo(a)pyrene (B[a] P)-induced lung cancer *in vivo*. **Methods:** Experimentally, the B(a)P (50 mg/kg body weight [b. wt.]) was administered orally to initiate lung cancer in mice, and the supplementation of prophylactic and therapeutic treatment by corilagin (30 mg/kg b.wt.) was observed for changes in b.wt. and lung weight, tumor incidence, oxidative stress marker (lipid peroxidation), tumor markers (carcinoembryonic antigen, neuron-specific enolase, aryl hydrocarbon hydroxylase, lactate dehydrogenase, γ-glutamyl transpeptidase, 5′-nucleotidase, alpha-keto dehydrogenase, citric acid cycle enzymes isocitrate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase), histological and immunohistochemical analysis (proliferating cell nuclear antigen), and apoptotic mRNA gene expression (Bax, Bcl-2, caspase-3, caspase-9, and cytochrome-c). **Results:** B(a)P-induced animals exhibited unusual changes in b.wt. and lung weight, tumor incidence, oxidative stress marker, enzymatic antioxidants, tumor markers, histological lung tissue damage, number of proliferating cell nuclear antigen, and apoptotic mRNA gene expression. On prophylactic and therapeutic treatment of corilagin, the animals showed reinstatement of all the above alterations to the near normal. **Conclusion:** These findings of all the analyzed data demonstrate that the B(a)P-induced animals of pre- and post-treated with corilagin may prevent initiation of lung cancer, hence establishing its chemopreventive effect against lung tumorigenesis.

Key words: Benzo(a)pyrene, chemoprevention, corilagin, lung cancer, medicinal plants, tannin

SUMMARY

- Reactive free radical-induced oxidative stress initiates lipid peroxidation in all phases of cancer progression including lung cancer
- Corilagin-rich antioxidant content allows the maintenance of the tissue enzymic and non-enzymic antioxidant levels to defend against oxidative damage and various tumor markers caused by benzo(a)pyrene induction.



Abbreviations used: PBS: Phosphate-buffered saline; TBARS: Thiobarbituric acid reactive substances; BCA: Bicinchoninic acid; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GST: Glutathione-S transferase; GR: Glutathione reductase; GSH: Reduced glutathione; CDNB: 1-Chloro, 2,4-dinitrobenzene.

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INTRODUCTION

Cancer of the lung is the most typical cancer type in developing nations. Currently, at least 1.5 million people are diagnosed with lung cancer annually, which represents 15% of the total incidence of all cancer types. Adding, lung cancer is the deadliest among all cancers. Alone, it accounts for at least 30% of all cancers' mortality in 2010.[1] Although numerous advances in the conventional therapeutic strategies (surgery, radiotherapy, and chemotherapy) have been applied in the lung cancer treatment, the prevalence of lung cancer has not dramatically improved in recent times. Regardless of positive response to chemotherapy at initial stage, it has a poor prognosis, due to frequent malignancy dissemination and disease reoccurrence. Despite unceasing research to improvise the therapeutic index, the overall 5-year survival rate is still below 15% for lung cancer.[2]

Amplified carcinogenic risk, therapeutic resistance, and poor survival outcomes are robustly associated with genetic instability and environmental factors. Contact with asbestos, arsenic, and radon has been reported to stimulate predominant genetic mutations, which may be responsible for their strong carcinogenicity incidence, regardless of smoking habit.[3] Furthermore, individuality dissimilarities may add to fluctuating degrees of susceptibility to the carcinogenicity. For

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an example, nonsmoking women tend to have a greater chance of developing lung cancer, possibly attributed to higher chances of contact to radon and polyaromatic hydrocarbons, for being present at the home more. Hence, it is important to determine the events associated with carcinogenesis for risk valuation, observation, and follow-up treatment.[4]

Ultimately, lung cancer prevention is more relevant because as for now the treatment for lung cancer remains disappointingly insufficient and signifies an enormous social and financial hindrance on healthcare systems. This is merely due to the fact that, in the coming years, the number of patients diagnosed with lung cancer may not be a smoker or have ceased smoking. Following, preventive strategies have gained more interest recently, for the purpose of halting cancer progression, because carcinogenic process can be more efficiently interrupted at the initial phase of cancer treatment. Nevertheless, improvement of synthetic drug for cancer treatment is laborious, expensive, and overlong process, which may not guarantee the drugs therapeutic response without concerning the adverse effects. Hence, chemoprevention has shown its prominence to be done instantly.[5]

Chemoprevention has been demarcated as the usage of specific natural agents to reverse, curb, or avert the carcinogenic process of a tumor. Medicinal plants and their active phytochemicals have been used for the prevention and treatment of cancer in folklore medicine for ages. In this current year, tannin has been reported to possess cytotoxic activities toward countless human cancers, especially in lung cancer cell line. Corilagin is an ellagitannin, present in various plants, and has been reported to exert numerous therapeutic beneficial activities such as antioxidant, antidiabetic, anti-inflammatory, antihypertensive, antimicrobial, and antitumor.[6-10] However, the antitumor activity of corilagin on lung cancer has not been scrutinized yet. For that reason, the ability of corilagin against benzo(a)pyrene (B[a] P) lung tumorigenesis and the role of corilagin in chemoprevention are investigated.

MATERIALS AND METHODS

Chemicals and reagents

Corilagin ($C_{27}H_{22}O_{18}$) of an analytical standard was purchased from Sigma Aldrich for the present investigation. The following chemicals and kits were also acquired from Sigma-Aldrich: B(a)P (B1760), phosphate-buffered saline (PBS), formaldehyde solution, bicinchonic acid (BCA) protein assay kit, thiobarbituric acid reactive substances colorimetric assay kit, commercial enzyme-linked immunosorbent assay (ELISA) test kit for lung cancer biomarker, real-time polymerase chain reaction (RT-PCR) (iTaq Universal Probes Supermix), and hematoxylin and eosin (H and E) staining dye. Other reagents and solvents incorporated in this experiment were of analytical grade.

Animal model

The animal models (male Swiss albino) of 22–28 g were maintained under controlled laboratory environment and allowed access to tap water and rodent pellets. This study was ethically authorized by the Institutional Animal Ethics Committee to minimize suffering of the animals during experimentation. The acclimatization to experimental requirement was implemented for a week before commencement of the analysis in animal house.

EXPERIMENTAL DESIGN

Thirty animals were separately categorized into groups of five and each had six animals ($n = 6$). The dosage selection for corilagin (dissolved in dissolved in corn oil) was done according to the previous study.

- Group I (Control): Animals were fed with corn oil (vehicle) by oral gavage (for 16 weeks)
- Group II (B[a] P): Animals were given B(a)P (50 mg/kg body weight [b.wt.] in corn oil) by oral gavage twice/week for 4 weeks (2nd–6th week)
- Group III (corilagin alone): Animals were fed with corilagin (30 mg/kg b.wt. in corn oil) by oral gavage twice a week for 16th week
- Group IV (corilagin pretreatment): Animals were fed with corilagin (30 mg/kg b.wt. with corn oil) by oral gavage continuously, starting a week before induction of B(a)P – similar schedule as Group II
- Group V (corilagin posttreatment): Animals were fed with corilagin (30 mg/kg b.wt. with corn oil) by oral gavage continuously, starting at 8th week after induction of B(a)P – similar schedule as Group II, till the end of experiment.

The animal's b.wt. was recorded weekly during the course of the experimentation. The food consumption on daily basis of each animal was recorded every day. All animals were inspected for tumors weekly after 4 weeks of B(a)P induction and observed daily for clinical sign of illness. Animals were sacrificed at the end of 16th week by cervical dislocation, under anesthesia with ketamine/xylazine (90/10 mg/kg). Lung and blood samples were also collected for histological and biochemical estimations.

Assessment of organ indexes (body weight and lung weight) and tumor incidence

The cleansed lung organs were measured and blotted on filter paper for complete dryness. The organs were then weighed again. The organ indexes (lung weight and b.wt.) were calculated statistically. To achieve a tumor incidence index, percentage (%) of tumor contained mice/total mice in each group was analyzed. The number and size of each tumor were recorded on necropsy sheet before histological diagnosis. Then, each lung was stored for further analysis.

Assessment of tissue homogenate and serum oxidative stress marker, lipid peroxides

Serum collection

Blood samples collected from each experimental animal were permitted to sit at room temperature for clotting process. The blood was centrifuged at 5000 × *g* for 20 min (4°C). The formed serum was separated and collected in fresh tubes for biochemical analysis.

Tissue homogenate

Lung samples were separated into two symmetrical parts. One part was stored in 10% saline for biochemical analysis. Another part was fixed in 4% formaldehyde for histological and immunochemistry study. For biochemical study, the tissues were homogenized in PBS solution (pH 7.4) and centrifuged at a high speed for 20 min (4°C). The resulting supernatant was subjected to protein concentrations estimation by using BCA protein assay kit.

Lipid peroxides

The tissues lipid peroxide (LPO) concentration was assessed according to Jain *et al.*[11] Concisely, the homogenate solutions were mixed with PBS, BHT, and 30% TC in a falcon tubes. Subsequent to 1 h incubation at room temperature, the reaction solution was centrifuged at 3000 × *g* for 20 min. Then, the supernatant was mixed with EDTA and 1% TBA. This mixture was incubated in a water bath at 80°C for at least 20 min. The absorbance reading was recorded at 532 nm for malondialdehyde (MDA).

Assessment of tissue homogenate biomarker enzymes

The supernatants of tissue homogenate were incorporated to determine the protein concentration using Bradford method.[12] Following, the supernatant was used to determine the tissue marker enzymes, aryl hydrocarbon hydroxylase (AHH),[13] lactate dehydrogenase (LDH),[14] γ-glutamyl transpeptidase (GGT),[15] and 5′-nucleotidase (5′-NT)[16] colorimetrically by using ELISA kits according to the manufacturer's protocols.

Assessment of serum biomarker enzymes

The blood samples were drawn using syringes and placed into anticoagulant tubes. After clotting process, the blood was centrifuged at 4000 × *g* for 10 min to obtain the serum. The serum was separated using EDTA-treated Pasteur pipettes. The remaining blood cells were discarded, and the serum was processed further for the preparation of serum biochemical analysis for quantification of carcinoembryonic antigen (CAE) and neuron-specific enolase (NSE) using commercial ELISA kit.

Assessment of mitochondrial enzymes

The supernatant of lung homogenate was used to access the following mitochondrial enzymes by commercial ELISA kit; alpha-keto dehydrogenase (alpha-KDH) by Reed and Mukherjee,[17] citric acid cycle enzymes isocitrate dehydrogenase (ICDH) was analyzed according to King,[18] succinate dehydrogenase (SDH) by Slater and Bonner,[19] and malate dehydrogenase (MDH) by Mehler and Kornberg.[20]

Assessment of proliferating cell nuclear antigen

The lung tissue sections were immersed in xylene for 1 h at 60°C. The entire sample was hydrated via series of graded alcohol and followed by incubation in citrate buffer for 5 min for antigen retrieval. Then, the sections were allowed to stand at room temperature and washed with buffered saline before treating with H_2O_2. The section was blocked with 3% bovine serum albumin for 1 h at room temperature and then incubated overnight with proliferating cell nuclear antigen rabbit polyclonal antibody. The slides were washed with buffered saline, incubated with antirabbit HRP-labeled secondary antibody and 3,3′-diaminobenzidine tetrahydrochloride before counterstaining with Meyer's hematoxylin. The slides were viewed at high magnification (×400), and the proliferating cell nuclear antigen-positive cells/100 tumors cells were documented.

Assessment of histopathological alteration

A section of lung tissue was fixed flat on filter papers using 10% formaldehyde for 48 h. The tissues were then processed sequentially in graded alcohol for dehydration. Further, the tissues were impregnated with paraffin wax for half an at least 1 h at 56°C and formed into a block. The blocks were sliced into 5 µm by rotary microtome, dewaxed, rehydrated, and stained with H and E stain solution. Finally, the slides were viewed under light microscope for histological significance.

Assessment of apoptotic-related gene expression

RT-PCR assay was performed to analyze the mRNA expression of, Bcl-2, Bax, caspase-3, caspase-9, and cytochrome-c. Total RNA from tissue homogenate was extracted using RNAzol reagent conferring to the manufacturer's manual. The purity of RNA extract was determined at 260 nm using a spectrophotometer and was stored at −70°C. One microgram of total RNA was used to synthesize cDNA. The transcription of all the genes was performed using a Maxima SYBR Green/ROX qPCR Master Mix. β-actin was used as housekeeping gene to normalize expression levels. Changes in the expression of the target genes were determined using the $2^{-\Delta\Delta C}$ method. The primers details are shown in Table 1.

Statistical data

All values are presented mean ± standard error of at least triplicate experiments and calculated using SPSS 17.0 software (SPSS, Chicago, IL). Assessments between experimental groups were scrutinized.

RESULTS

Response of corilagin to body and lung weight with tumor incidence in benzo(A)pyrene-induced lung cancer

In Figure 1, the alteration of B(a)P induction and the treatment of corilagin on animal body and lung weight with tumor incidence were illustrated. A significant decrease in b.wt. and increase in lung weight was observed in the B(a)P-induced animals (Group II) when associated with their control animals in Group I. There were no considerable alterations in both b.wt. and lung weight of animals administered with corilagin alone in Group III. However, prophylactic (Group IV) and therapeutic (Group V) treatment of corilagin to B(a)P-induced animals improved the b.wt. and lung weight significantly. As for tumor manifestation, in B(a)P-induced animals (Group II), the tumor incidence was markedly noted when compared to animals from control group (I) and corilagin-treated group (Group III). Remarkably, both prophylactic (Group IV) and therapeutic (Group V) corilagin treatment in B(a)P-induced animals showed significant reduction in the tumor incidence gradually, which increases the percentage of tumor inhibition. Comparatively, prophylactic (Group IV) treatment by corilagin showed enhanced antitumor activity than the therapeutic treatment (Group V).

Response of corilagin to tissue and serum lipid peroxidation in benzo(A)pyrene-induced lung cancer

In Figure 2, the alteration of B(a)P induction and the treatment of corilagin on animal tissue and serum LPO were illustrated. A significant elevation in the extent of LPO was observed in both tissue and serum sample of B(a)P-induced animals (Group II) compared to their control animals in Group I. A comparable pattern was observed between animals from Group I and animals administered with corilagin alone (Group III). The adverse changes induced by B(a)P-induction in animals from Group II were significantly overturned to near-normal values by prophylactic Group IV and to some extent by therapeutic group V in B(a)P-induced animals.

Response of corilagin to tissue marker enzymes in benzo(A)pyrene-induced lung cancer

In Figure 3, the alteration of B(a)P induction and the treatment of corilagin on tissue marker enzymes (AHH, GGT, 5′-NT, and LDH) were illustrated. The activities of all the tissue marker enzymes were observed to be significantly elevated ($P < 0.05$) in B(a)P-induced animals (Group II) when associated to their animals from control group (I) as well as the animals administered with corilagin alone (Group III). When B(a)P-induced animals (group II) were linked with animals in prophylactic (Group IV) and therapeutic (Group V) corilagin treatment, there were significant reduction in all the tissue marker enzymes level. Again, animals in prophylactic (Group IV) corilagin treatment showed slight improvement than therapeutic (Group V) corilagin treatment.

Table 1: Primer details

Primers	Forward	Reverse
Bax	5'-AGTAACATGGAGCTGCAGAGG-3'	5'-ATGGTTCTGATCAGTTCCGG-3'
Bcl-2	5'-GTGACTTCCGATCAGGAAGG-3'	5'-CTTCCAGACATTCGGAGACC-3'
Caspase-3	5'-AGGGGTCATTTATGGGACA-3'	5'-TACACGGGATCTGTTTCTTTG-3'
Caspase-9	5'GCTCTTCCTTTGTTCATCTCC -3'	5 CATCTGGCTCGGGGTTACTGC -3'
Cytochrome-C	5'-CGTGTCGACCTAATATGGGTGATGTTGAAAAGG- 3'	5'-ACAGATCTTTCTCATTAGTAGCCTTTTTAAG -3'
β-actin	5'-GTGAAAAGATGACCCAGA-3'	5'TACGACCAGAGGCATACAG-3'

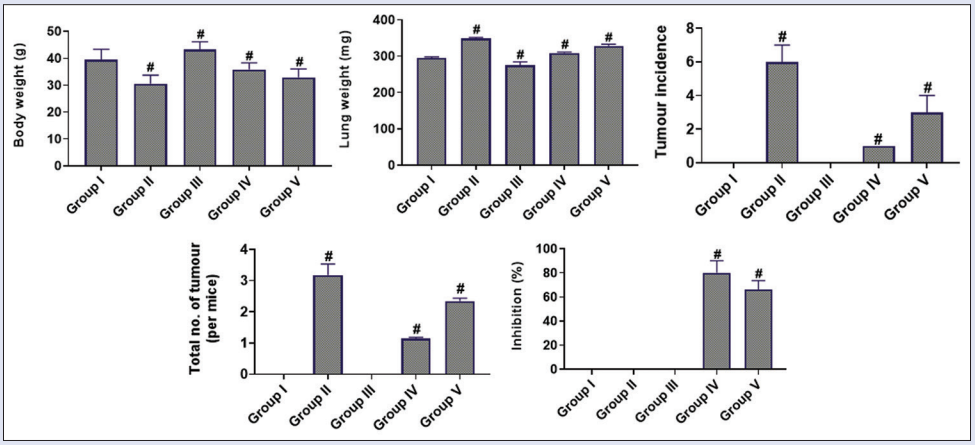

Figure 1: Effect of corilagin on body and lung weight with tumor incidence in benzo (a) pyrene-induced experimental animals. Outcomes are stated in the mean ± standard deviation of five liberated data (*.**$P < 0.05$)

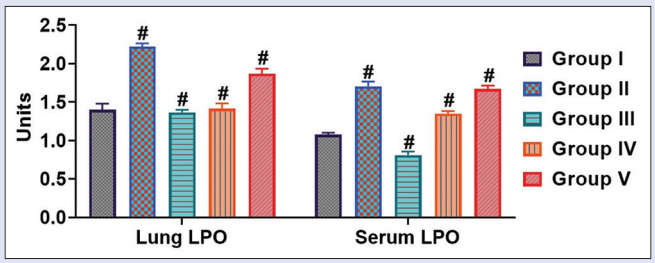

Figure 2: Effects of corilagin on lipid peroxidation level in benzo (a) pyrene-induced lung cancer. The tissue and serum oxidative stress parameter (lipid peroxidation) were done by ELISA method. Outcomes are stated in the mean ± standard deviaton of five liberated data (#$P < 0.05$)

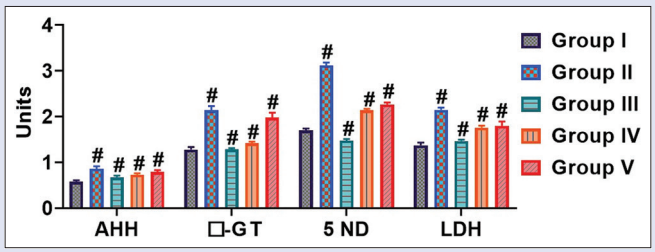

Figure 3: Effects of corilagin on tissue marker enzymes in benzo (a) pyrene-induced lung cancer. The marker enzymes parameter (AHH, GGT, 5′-NT, and LDH) were done by ELISA method. Outcomes are stated in the mean ± standard deviation of five liberated data (#$p < 0.05$). AHH: Aryl hydrocarbon hydroxylase; LDH: Lactate dehydrogenase; GGT: γ-glutamyl transpeptidase; 5′-NT: 5′-nucleotidase

Response of corilagin to serum marker enzymes in benzo(A)pyrene-induced lung cancer

In Figure 4, the alteration of B(a)P induction and the treatment of corilagin on serum marker enzymes (CAE and NSE) were illustrated. The marker enzymes activities of CAE and NSE in serum were found to be markedly ($P < 0.05$) elevated in B(a)P-induced animals (Group II) when associated to their animals in control group (I) and in animals administered with corilagin alone (Group III). On corilagin prophylactic (Group IV) and therapeutic (Group V) treatment, the enzyme concentration was markedly ($P < 0.05$) engaged to near-normalcy. Relatively, prophylactic (Group IV) treatment by corilagin showed enhanced antitumor activity than the therapeutic treatment (Group V).

Response of corilagin to mitochondrial enzymes in benzo(A)pyrene-induced lung cancer

In Figure 5, the alteration of B(a)P induction and the treatment of corilagin on mitochondrial enzymes (α-ketoglutarate dehydrogenase (KGDH), SDH, ICDH, and MDH) were illustrated. The activities of the enzymes KGDH, SDH, ICDH, and MDH were found to be markedly ($P > 0.05$) depleted in B(a)P-induced animals (Group II) when associated to their animals in control group (I). The effects of corilagin treatment alone in animals of Group III were comparable with animals from control group (I). On corilagin prophylactic- and therapeutic-treated animals (Group IV and Group V), a significant increase ($P < 0.05$) in the activities of the mitochondrial enzymes was found when related with B(a)P-induced animals (Group II). However, the animals from prophylactic group (IV) showed small difference in the mitochondrial enzyme activities from animals in therapeutic group (V).

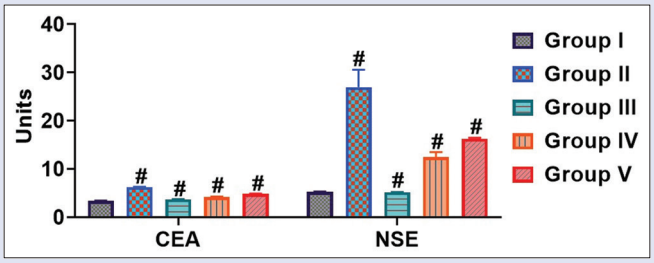

Figure 4: Effects of corilagin on serum marker enzymes in benzo (a) pyrene-induced lung cancer. The marker enzymes parameter (CEA and NSE) were done by ELISA method. Outcomes are stated in the mean ± standard deviation of five liberated data (#$P < 0.05$). CAE: Carcinoembryonic antigen; NSE: Neuron-specific enolase

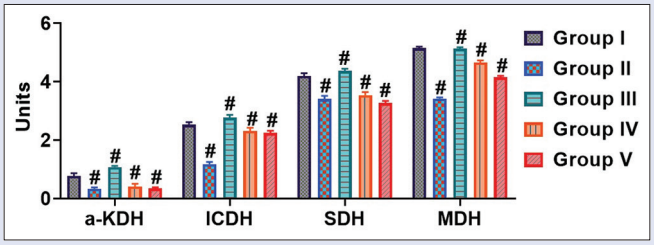

Figure 5: Effects of corilagin on mitochondrial marker enzymes in benzo (a) pyrene-induced lung cancer. The marker enzymes parameter (α-KDH, SDH, ICDH, and MDH) were done by ELISA method. Outcomes are stated in the mean ± SD of five liberated data ($P < 0.05$). α-KDH: Alpha-keto dehydrogenase; ICDH: Isocitrate dehydrogenase; SDH: Succinate dehydrogenase; MDH: Malate dehydrogenase

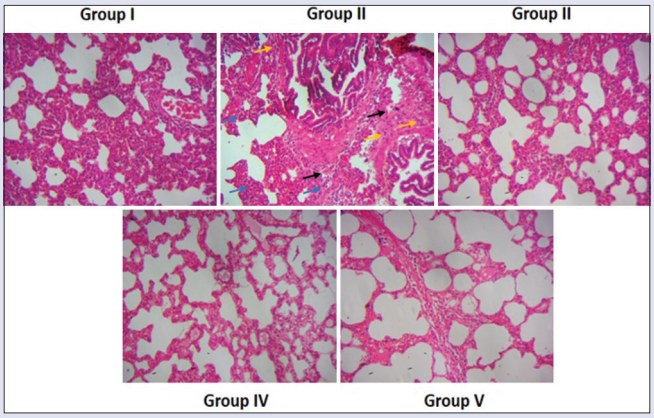

Figure 6: Effects of corilagin on lung histology in benzo (a) pyrene-induced lung cancer. The histological changes in the lung were done by H and E staining method. In benzo (a) pyrene-induced animals (Group II), loss of architecture and alveolar injury (black arrows), increased proliferative lesions with hyperplasia (blue arrows), and higher hyperchromatic nuclei (yellow arrows) were noted. Both control (Group I) and corilagin alone treated animals (Group III) exhibited normal architecture. The corilagin pre- and post-treated animals (Group IV and V) showed to some extent, reduced alveolar injury, reduced hyperchromatic cells and hyperplasia with near-normal architecture. H and E: Hematoxylin and eosin

Response of corilagin to lung histopathology in benzo(A)pyrene-induced lung cancer

In Figure 6, the alteration of B(a)P induction and the treatment of corilagin on the histology of animal lung tissues were illustrated. In B(a)P-induced animals (Group II), an architecture loss and injury in the alveolar pattern were observed. The lung tissues of B(a)P animals also demonstrated the increased proliferative lesions with focal bronchial and alveolar epithelial hyperplasia. B(a)P animals exhibited higher numbers of hyperchromatic nuclei with widespread alveolar epithelium proliferation. Comparatively, both control animals (Group I) and corilagin treatment alone in animals of Group III exhibited normal architecture with unaltered alveoli patent. However, corilagin prophylactic treatment (Group IV) and prophylactic treatment (Group V) showed, to some extent, an abridged alveolar injury, reduced hyperchromatic cells and hyperplasia with near-typical architecture which signifies reinstatement in the histopathological structures.

Response of corilagin to immunohistochemical analysis of proliferating cell nuclear antigen in benzo(A)pyrene-induced lung cancer

In Figure 7, the alteration of B(a)P induction and the treatment of corilagin on the proliferating cell nuclear antigen of animal lung tissues were illustrated. Animals from B(a)P-induced group (II) showed a marked elevation ($P < 0.05$) in the number of proliferating cell nuclear antigen (PCNA)-positive nuclei when associated with animals from control group (I) and animals treated with corilagin alone from Group III. Following, both prophylactic (Group IV) and therapeutic (Group V) corilagin-administered animals significantly decreased ($P < 0.05$) the number of PCNA-positive nuclei with prophylactic treatment displayed slightly better efficiency than therapeutic treatment in B(a)P-induced animals.

Response of corilagin to apoptotic mRNA gene expression in benzo(A)pyrene-induced lung cancer

In Figure 8, the alteration of B(a)P induction and the treatment of corilagin on the apoptotic associated mRNA gene expression of animal lung tissues were illustrated. Animals from B(a)P-induced group (II) amplified the Bcl-2 expression (antiapoptotic proteins) with concomitant suppression in the expression levels of proapoptotic (caspase-3, caspase-9, and Bax) proteins when associated with both control group (I) animals and corilagin-treated (Group III) animals ($P < 0.05$). A considerable amelioration was found when opposing changes induced by B(a)P induction in animals from Group II were significantly reversed by both prophylactic (Group IV) and therapeutic (Group V) corilagin-administered animals, emitting a significant antitumor activity in B(a)P-induced lung cancer.

DISCUSSION

In the study of cancer, animal models of tumor are permitted to evaluate the efficacy of new chemotherapeutic drugs extracted from natural products. Animal model connects the observation established in *in vitro* studies to determine which compounds are qualified to progress to human clinical trials. Animal models that are organ specific are selected to determine the most effective non-toxic drugs to halt the formation of cancer.[21] Rodents provide an excellent choice in animal

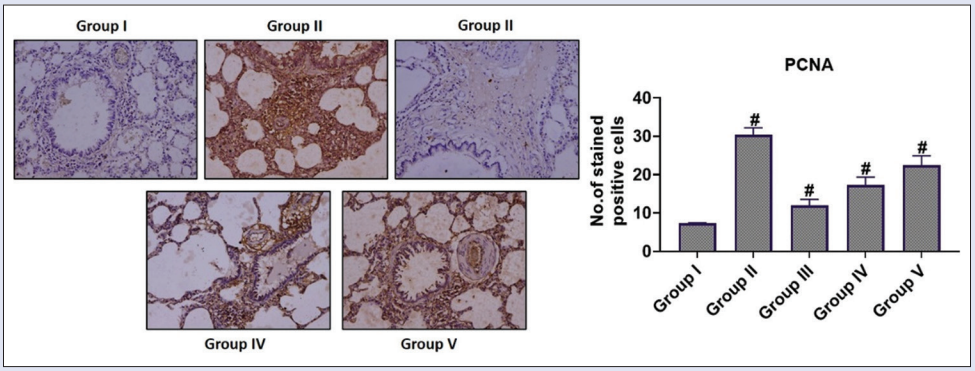

Figure 7: Effects of corilagin on proliferating cell nuclear antigen in benzo (a) pyrene-induced lung cancer. The proliferating cell nuclear antigen in the lung was done by immunohistochemical analysis method. Outcomes are stated in the mean ± standard deviation of five liberated data (#$P < 0.05$)

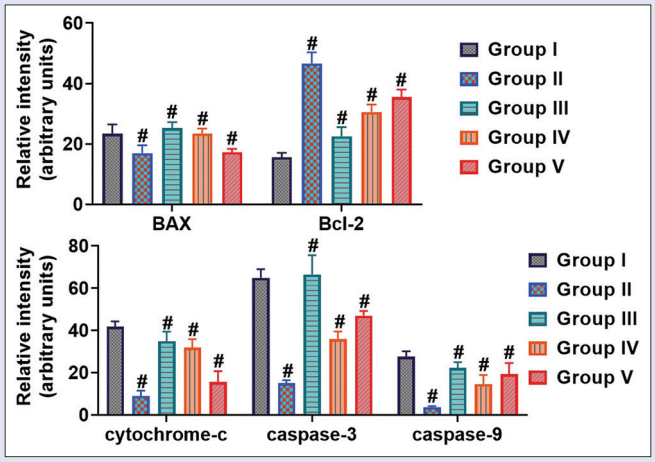

Figure 8: Effects of corilagin on apoptotic mRNA gene expression in benzo (a) pyrene-induced lung cancer. The apoptotic mRNA gene expression in the lung was done by RT-PCR analysis method. Outcomes are stated in the mean ± standard deviation of five liberated data (#$P < 0.05$)

model, especially mouse, because mouse genome represent a close resemblance with the human genome which allows the possibility of the chemoprevention and early detection. For lung cancer, animal tumor models for chemoprevention investigation studies are either established by carcinogen-induced or by transgenic/mutant animals. A clear validation of animal tumor models, however, requires clinical trials on human to predict the effectiveness of with chemopreventive drugs. Validated or not animal model studies remain the crucial the decision-making process to the clinical trial.[22]

In this investigation, it was opted to use the oral administration of the diluted B(a)P in rats as the experimental model. B(a)P is a part of PAH family, a potent carcinogen in animal models of lung cancer. Polycyclic aromatic hydrocarbons (PAHs) are the main group of environmental carcinogenic pollutants used for toxicity studies and induce selective carcinogenesis in the lung as a result of numerous biochemical factors, such as DNA repair, metabolism, and absorption.[23] The sequential activation of B(a)P metabolism by cytochrome-p450 produce 7,8-diol-9,10-epoxide-benzo(a)pyrene, the final product responsible for the carcinogenesis. Although the development of lung tumor may not be identical to human lung cancer, induction with B(a)P shares the same pathways for the initiation of lung cancer. Hence, combination of mouse model induced by B(a)P represents a very important preclinical tool for the study of tumor development, promotion, and treatment in primary lung carcinogenesis.[24]

Subsequently, this experiment was anticipated to assess the effect of corilagin against lung cancer through induction of B(a)P in Swiss albino mice. The dosage of B(a)P utilized in this experiment is 50 mg/kg b.wt. *in vivo*. The dosage is ecologically pertinent concentration and is frequently used in animal studies.[25] A continuous proliferation of the lung cancer cells leads to several abnormalities which is indicated by a considerable fall in b.wt. and distended lung weight. Hence, it can be used as analytical parameter to study the toxic effects of the therapeutic agent involved. Several reports that have incorporated the administration of B(a)P demonstrated parallel results with reduced b.wt. and enlargement of animal's lung. Conversely, prophylactic and therapeutic administration of corilagin averts this alteration in b.wt. and lung weight of B(a)P-induced lung cancer in mice. In addition, antitumor response of corilagin was monitored on the tumor incidence basis where corilagin successfully reduced the extent of tumor incidence when associated to B(a)P-induced animals. A previous study supported that these data which showed various plant extracts were effective in decreasing the lung tumor incidence induced by B(a)P in mice.[26]

Reactive free radical-induced oxidative stress initiates LPO in all phase of cancer progression including lung cancer. During the progression of lung carcinogenesis, the generated reactive free radical leads to progressive cellular architectural damage. Antioxidants interrupt the free radicals within the cells against its toxic effects and protect the cells from free radical assault. Hence, the balance between free radicals and antioxidant is vital, because an inequity would result in the free radicals stimulated toxic accumulation which obviously leads to cell damage.[27] The main purpose of incorporating plant bioactive compounds is to combat ROS-induced damage through antioxidant effect, and from this assay, it was noted that corilagin possesses free radical scavenging capabilities through rich antioxidant content. Corilagin effectively combats against oxidative injuries induced by B(a)P and upholds the levels of enzymic and nonenzymic antioxidant in mice. These data were evidently justified with decreased mitochondrial enzyme activities of ICDH, SDH, MDH, and KGDH in B(a)P animals, and corilagin administration elevates the level of these enzymes. This may be credited to corilagin capacity to elevate the antioxidant status and preserve the integrity of the cell. Similarly, a previous study led by Velli *et al.*[28] demonstrated that vanillic acid protects against B(a)P-induced lung cancer in mice by their antioxidant activities.

Tissue damage induced by oxidative stress is one of the vital features in carcinogenesis as this condition leads to leakage of certain enzymes which function as biomarker in cancer chemoprevention and therapeutic

investigation. AHH, LDH, GGT, and 5′-NTs are positively associated to cytotoxicity of B(a)P-induced carcinogenesis. Elevated levels of these prognostic markers are induced in both pulmonary tissues and serum of animals administered with B(a)P. Hence, AHH, LDH, GGT, and 5′-NTs are acknowledged as a standard biomarker enzyme in evaluating the tumor cell proliferation, and amplification of this enzyme activity has been stated in previous lung cancer investigations.[29] In the current study, a marked elevation in all mentioned tissue marker enzymes was noted in B(a)P-induced animals. Corilagin of both prophylactic and therapeutic administration brought down the marker enzymes levels close to normality signifying its antitumor effect. This study was effectively correlated with a similar work done on silymarin-treated B(a)P-induced animals.[30]

Inflammation plays a critical part in cancer progression including lung cancer. A latest study suggested that CEA and NSE, a malignant serological tumor marker, may be connected to inflammation. An increase of CEA and NSE serum levels is an indicative of carcinogenesis associated inflammation.[31] Here, the elevated CEA and NSE levels in B(a)P-induced animals were significantly ameliorated by prophylactic and therapeutic corilagin treatment to rear-normal level. This was evidently justified with histopathological analysis where, in experiments using B(a)P-induced animals, the pulmonary inflammatory process is directly related to the incidence of lung cancer by revealing inflammatory alterations by reduced emphysema areas and thickness of lung alveolar upon treatment with corilagin. This result emphasizes the need of the inhibiting capacity against the inflammatory process in the carcinogenesis induction, further suggesting the corilagin's anti-inflammatory activity against experimental B(a)P-induced lung carcinogenesis.

Uncontrollable cell proliferation is strongly correlated with carcinogenesis. In fact, in the lung, an increased proliferation of epithelial cell was documented to induce the liability of tumor progression. During carcinogenesis, abnormal apoptosis (programmed cell death which eliminates damaged DNA in cells from the body to maintain cell function) is one of the distinctive features of uncontrolled cell proliferation.[32] This current study showed that the abnormally high level of pulmonary PCNA during lung carcinogenesis was suppressed by corilagin administration. The alteration in the level of PCNA was in agreement with the apoptotic mRNA gene expression results, indicating the activation of intrinsic apoptosis, which is indicated by the upsurge of cytochrome-c, caspase-3, and caspase-9. Thus, it can be concluded that the improvement done by corilagin in B(a)P-induced lung cancer strengthens its capability as a chemopreventive agent.

CONCLUSION

Conclusively, the outcome of this work clearly demonstrates that corilagin plays an imperative protective role in lung carcinogenesis and can play the chemopreventive role against B(a)P-induced lung cancer. Corilagin-rich antioxidant content allows the maintenance of the tissue enzymic and nonenzymic antioxidant levels to defend against oxidative damage and various tumor markers caused by B(a)P induction. In addition, its proapoptotic activities illustrate its antiproliferative and protective activity during lung cancer development. However, additional research is required in the upcoming years to explicate the likely molecular mechanisms fundamental to the perceived corilagin antitumor activity.

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Conflicts of interest

There are no conflicts of interest.

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