Effect of bioactive compound derived from *Calophyllum inophyllum* on Benzo(a)pyrene induced lung cancer

*Accepted 12th November, 2018*

**ABSTRACT**

The aim of this study is to isolate bioactive compounds and analyze its effect on the cigarette carcinogen benzo(a)pyrene induced lung cancer. The crude extract of root bark of *Calophyllum inophyllum* using CHCl₃-MeOH and most powerful reducing agent was purified and identified by spectral studies. The effect of partially purified compound was investigated against benzo(a)pyrene induced lung cancer in male white albino rats and protective effect of purified metabolite was analyzed by morphological, histological and antioxidant and haematological variations. The purified compound induced modulation of particular apoptosis and tumor related genes bcl-2, bax, p53, NF-κB were examined in lung tissue. From this study, a new bioactive compound 2-ethyl-1, 2, 3, 4-tetrahydro-N-(methoxy(methylperoxy)-3-acetate, methyl)-3, 4- bis(methylperoxy)naphthalen-1-amine, named *innophyllamine* was identified as potent antioxidant compound. The compound greatly reduced the B(a)P induced lung tumor volume and its curative effect was confirmed by histology of lung section. Alterations were noticed through haematological parameters and antioxidant level in lung cancer bearing animal was restored by innophyllamine-1 treatment. The compound did not exhibit any deleterious effect on normal animal group. Innophyllamine-1 was up-regulated in the p53 mediated bax expression and concordantly decreased the transcription of bcl-2 and NF-κB. This investigation concluded that the newly identified Innophyllamine-1 reported for the first time with strong radical scavenging activity and chemoprotective effect on B(a)P induced lung cancer in white albino rats.

**Key words:** *Calophyllum inophyllum*, Benzo(a)pyrene, Lung carcinoma, Antioxidant, haematology, innophyllamine-1.

**INTRODUCTION**

Our industrialized society generates a variety of environmental pollutants that contribute to various cancers (Coyle, 2004). Benzo(a)pyrene [B(a)P] is an important toxic polycyclic aromatic hydrocarbon mainly found in cigarette smoke (ATSDR, 1995). Metabolites of B(a)P act as carcinogenic by binding with DNA; the event results in mutation which leads to formation of a range of cancers in physiology of the human system. The B(a)P of tobacco smoking causes genetic damage particularly in lungs tissue and pertaining to effect of lung cancer. B(a)P is a powerful and major carcinogen in tobacco smoking, a type of polycyclic aromatic hydrocarbon (PHAs). The metabolites of B(a)P forms DNA adducts by rigorous ROS generation to facilitate the process of carcinogenesis (Kamaraj et al., 2010).

Lung cancer is a leading cause of cancer mortality in most of the countries accounting for more than a million annual death worldwide (Leong et al., 2009) and tobacco smoking act as a predominant risk factor, approximately 87% of lung cancer cases are associated with cigarette smoking (Hecht, 2002).

Chemotherapy is the standard method of treatment for
wide array of diseases including cancer (Patil et al., 2012). The number of anti-cancer drugs used includes vinblastine, vincristine, camptothecin derivatives-topotecan, etoposide and paclitaxel and these are directly related to the regulation of gene expression to induce apoptosis, cell cycle arrest or DNA fragmentation and inhibition of cellular enzymes (Patil et al., 2011). Generally, clinically available drugs were recommended for apoptosis. Though the selective therapeutic agents to induce apoptosis are available, cancer cells develop drug resistance mechanism to chronic exposure of available therapeutic agents which results in poor survival rate. Hence, it is in urgent to find the novel product for effective treatment of cancer.

Recently, marine based chemotherapy was well recognized for its anti-tumor studies through various cancer signaling pathways (Bhatnagar and Kim, 2010). Researchers reinvigorated the discovery of novel natural products from marine flora and fauna to challenge the obstacles in cancer treatment.

Coastal plants are rich source of biologically diverse active compounds with unique functional groups that has explored great interest of pharmacological activity. Calophyllum inophyllum [Guttiferae (Clusiaceae)] is an evergreen large shrub found near the coastal region, traditionally used in treating various diseases including eye sickness, wounds, rheumatism and inflammations (Dai and Mei, 2007); it is well known that the plant C. inophyllum have cancer chemo-protective agents (Dai et al., 2010; Masataka, 2001). Ample scientific reports has been documented on C. inophyllum for various activity which made an impact in the present study to isolate anti-lung cancer compound from C. inophyllum against B(a)P induced lung cancer.

MATERIALS AND METHODS

Extraction and purification

Root bark materials of C. inophyllum were collected from Portonovo, east coast of India. The material was thoroughly washed, air dried and grounded to 500 g of fine powder. The powder was extracted with 9:1 CHCl₃-MeOH (v/v) and the solvent condensed by vacuum to obtain a dark brown gummy extract. The crude extract (12.5 g) mixed with CHCl₃-silica gel slurry was loaded into a silica gel 100 to 200 mesh (E-Merck, Darmstadt, Germany) column and packed in chloroform (450 × 30 mm dimension). The column was eluted with stepwise gradient of CHCl₃/MeOH (100:0; 90:10; 80:20; 70:30; 50:50; 30:70 and 10:90, v/v) solvent system. The collected (32) fractions were then tested for their anti-oxidant activity using photometric based 2,2′-diphenyl-1-picryl hydrazyl (DPPH) assay (Soler-Rivas et al., 2000). Fractions showed potent radical scavenging activity with same Rf value on thin layer chromatography pooled together and was further purified by Sephadex LH-20 column using the same solvent gradient as applied in silica gel. The active product was further taken for compound identification and anti-lung cancer studies.

Spectroscopic analysis

The purified active metabolite was subjected to IR spectral study (recorded on a Perkin–Elmer 1600 using KBr pellets). Flow by NMR spectrum was performed and ¹H NMR of purified compound was recorded in deuterated DMSO with tetramethylsilane (TMS) as internal standard solution using 400 MHz Bruker machine.

Carcinogen

The carcinogen Benzo(a)pyrene [B(a)P] used in the present experiment is a polycyclic aromatic hydrocarbon (Sigma chemical company, USA).

Experimental animal model

Male white albino rats (70 to 90 g, 6 to 8 weeks old) were obtained from Central animal facility house, Raja Muthiah Medical College, Annamalai University. The animals were housed in the same place as six animals per polypropylene cage and fed with standard pellet feed (Mysore Snak feed Ltd., Mysore, India) and water ad libitum. The animals were maintained at 28 ± 2°C with an alternating 12 h light/12h dark cycle. The norms of animal ethical committee of Annamalai University (Ethical number 160/1999/CPCSEA) were followed.

Experimental design

The animals were sorted into control and experimental groups, which was thereafter divided into four groups with each group containing 6 animals. Group I served as control and was fed with olive oil. Group II animals were treated with B(a)P along with olive oil as vector (50 mg/kg body weight), while group III animals received B(a)P inophyllamine-1 (10 mg/kg body weight) and group IV animals administered only inophyllamine-1 to assess its toxicity. B(a)P was given to groups II and III twice a week for two successive weeks to induce the lung cancer. Treatment with inophyllamine-1 continued up to 16 weeks. (Fixation dosage of inophyllamine-1 was based on our laboratory experiment, data not shown here). Finally, the experimental animals were sacrificed by cervical dislocation at the end of 16th week after an overnight fasting and the fresh blood and lung tissue taken for further analysis.
Analysis of animal weight, tumor volume and cancer burden

Animal weight was calculated by subtracting the initial weight from the final weight of the animals. The tumor volume was calculated using the water displacement method and tumor burden calculated as the numbers multiplied with volume of the tumor.

Histopathological studies

The lungs were carefully dissected, fixed in 10% buffered formalin and embedded in paraffin. After routine processing, 5 µm thick sections were cut with each slide containing the whole cross-sectional area of the lungs and lymph nodes of each animal was stained with Hematoxilin-Eosin (HE). The entire lungs were kept for histological analysis.

Estimation of hematological parameters

Hemoglobin (Hb) content, Red blood cell (RBC) count, white blood cell (WBC) and differential count of WBC was done on vector normalized second derivatives of average spectra. The alteration in enzymatic and non-enzymatic antioxidant level was estimated in serum of control and experimental group. Superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck et al., 1973), glutathione-S-transferase (GST) (Moron et al, 1979) and glutathione reductase (GR) (Horn and Burn, 1978) reduced glutathione (GSH) (Moron et al., 1979), vitamin E (Desai et al., 1984) and vitamin C (Omaye et al., 1979).

Enzymatic and non-enzymatic antioxidant levels

The Fourier-transform infrared (FT-IR) spectra

A dark brown colored compound was purified from the root bark of C. inophyllum by column chromatography based on radical scavenging assay. The Fourier-transform infrared (FT-IR) spectra (4000 to 400 cm\(^{-1}\)) were obtained as KBr pellets on a Nicolet 210 FT-IR spectrophotometer. The KBr pellets were prepared by grinding 15 mg of each sample with 300 mg of solid KBr (previously dried at 120°C for at least 2 h). The mixture was transferred to a punch and dye system and a pressure of 15,000 pounds applied for 10 min using a hydraulic press, where a transparent pellet was obtained. OMNIC software was employed for data analysis and the number of scans and resolution were 120 and 8 cm\(^{-1}\), respectively. FT-IR spectroscopy identifies the molecular structures that are present in a substance based on their respective absorption bands in the infrared spectrum. Characterization of the chemical structure of any extracted compound is done by using Fourier transform infra-red (FTIR) technique. The specific chemistries and orientation of the structure will be known from the IR spectrum. The extracted compound infrared spectra were measured using the Perkin Elmer FTIR model 2000 spectrophotometer. The absorption spectrum was recorded in the wave number range from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\). Background spectra, which were collected under identical conditions were automatically subtracted from the sample spectra.

Total RNA was extracted from lung tissue using TRIzol kit according to the manufacturer’s instructions. RNA preparations were carefully checked by gel electrophoresis and found to be free from DNA contamination. For the RT reaction, total RNA (3 µg) was primed with oligo (dT) and reverse-transcribed into cDNA with 30 units of M-MLV reverse transcriptase in a final volume of 20 µL. The mixture was incubated at 37°C for 2 h. The generated cDNA was used for the semi-quantitative RT-PCR analysis to assess mRNA expression. The expression profile used in the following primers in RT-PCR were bd-2: Fwd 5'-CTGGTGGACAACATCGCTCTG-3', Rev 5'-GGTCTCTGACCTCACTTGTG-3', bax: Fwd 5'-TTCATCCAGTCAGACCAGA-3', Rev 5'-AACATAGGCAACAG-3', p53 Fwd 5'-CCTGGTTTTTTCCTAGAGCCC-3', Rev 5'-CTACCCGGGACAAGAGGAG-3', NF-κB: Fwd 5'-AAGATCAATGTGCCAACGGGG-3' and Rev 5'-CCTCAATGTCTCTTCTTGCG-3'. Expression of β-actin (house keeping gene) was used as internal control to compare the expression level of target genes. Initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 30 s (bcl-2: 64°C for 80 s; bax: 60°C for 80 s) and extension at 72°C for 30 s and final extension at 72°C for 5 min. The RT-PCR product was analyzed by 1% agarose gel electrophoresis and visualized under UV light and images photographed.

RESULTS

Structural identification

The semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR)
components. Second derivative spectra were vector normalized at 3500 to 3000 cm\(^{-1}\), 1500 to 1800 cm\(^{-1}\), 1200 to 1600 cm\(^{-1}\) and 800 to 1200 cm\(^{-1}\).

Fourier self-deconvolution and second derivative resolution enhancement were used to narrow the width of infrared bands and increase the separation of the overlapping components with software Peak Fit Version 4.12. The resolution enhancement resulting from self-deconvolution and the second derivative was such that the number and the position of the bands to be fitted were readily determined. Curve fitting was accomplished during a curve-fitting process with Peak Fit software (version 4.121) for the ester and amide carbonyl I, and II band region. The program iterated the curve-fitting process by adjusting the peak height and width to achieve the best Gaussian-shaped curves that fit the original spectrum. In the observed IR-frequency (vcm\(^{-1}\)) 3539.38 assigned the primary amine N-H; similarly, in IR-frequencies 2748.56 vcm\(^{-1}\) consigned alkane C-H stretching and stretching frequencies was Methoxy C-O given 2748.56 stretching frequencies and frequencies 2642.48, 2528.68 shown as alkane C-H stretching in cyclo-pentane, and 2198.85, 2079.26, 1969.32 IR frequencies given as Alkene C=C symmetry reduces intensity and the carbonyl stretching frequencies are shown respectively as Ester C=O 1732.08 and Amide C=O 1624.06, 1512.19

\[
\text{Figure 1: Structure of inophyllamine-1 isolated from root bark of } C. \text{ inophyllum.}
\]

**General and morphological observation**

The significant morphological parameters like breathing difficulties (dyspnea), risky tiredness (fatigue), swollen lymph nodes, shortness of breath, extreme weight loss, and breathless (wheezing), droopy eyelids (hanging down), face and neck swelling, fever, muscle weakness and watery skin was noticed during the experimental period. Significant loss of body weight was noted in B(a)P treated animals. Innophyllamine-1 was effective in preventing lung tumor when administered with carcinogen and showed significant reduction in cancer burden. There was no sign of behavioral change and malaise from innophyllamine-1 treatment. No obvious changes occurred between control and inophyllamine-1 treated control group (Table 1).

**Histopathological observation**

The histological examination of lung section revealed the
### Table 1: Effect of inophyllamine-1 on B(a)P induced lung carcinoma in experimental animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Animal weight</th>
<th>Animal weight</th>
<th>Tumor volume (ml)</th>
<th>Tumor incidence</th>
<th>Tumor incidence (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>120±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>B(a)P</td>
<td>55±0.3</td>
<td>24±0.03</td>
<td>0.43±0.01</td>
<td>100%</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>III</td>
<td>B(a)P + inophyllamine-1</td>
<td>99±0.01</td>
<td>0</td>
<td>0</td>
<td>Hyperplasia</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>inophyllamine-1</td>
<td>124±0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD for 6 animals in each group. Values not sharing a common superscript are differ significantly at P>0.05. Tumor burden was calculated by multiplying tumor volume and the number of tumor/animals. Tumor volume is calculated by water displacement method.

**Figure 2:** The histopathological studies of lung viewed under light microscope in control and experimental animals. H and E staining (10x). (a) Control animals showing normal architecture appearance of lung section indicated by arrow marks; (b) B(a)P treated animals showing disoriented tissue section with alveolar damage and irregular-chromatin containing nuclei of cells (arrow mark); (c) Inophyllamine-1 treated cancer induced animals showed near normal lung architecture indicated by arrow marks; (d) Arrow marks of Inophyllamine-1 control animals showing normal architecture similar as of control animals.

The chemopreventive effect of inophyllamine-1 against B(a)P induced lung cancer (Figure 2). Lung tissue of control animal showed normal architecture with uniformly intact nuclei (Figure 2a). The cross section of B(a)P rendered lung carcinoma was clearly evidenced by distorted alveoli with aggressive cell proliferation (Figure 2b). The cells were disoriented with condensed hyper chromatin nuclei. Re-established normal cellular arrangement and nuclei of the cells appeared uniformly as normal lung tissue by the treatment of inophyllamine-1 (Figure 2c). Figure 2d shows the architecture of lung tissue in inophyllamine-1 treated animal appeared similar to the control group.

**Effect of innophylamine-1 on haematology of experimental animals**

B(a)P induced lung cancer bearing (Group II) animals showed significantly (p<0.001) decreased levels of haemoglobin, RBC, lymphocytes and monocytes count with
increased levels of WBC and neutrophil count when compared to (group I) animals. These changes were significantly altered in inophyllamine-1 treated group III animals (p<0.05) when compared with cancer induced group II. However, inophyllamin-1 alone treated animals (Group IV) did not show any significant changes in their haematological levels when compared with control animals (Group I). Table 2 showed the effect of inophyllamine-1 on haematology of control and experimental animal group.

### Effect of innophyllamine-1 on enzymatic and non-enzymatic antioxidant

Quenching of free radicals in cancer disease boost chemotherapeutic effect and antioxidant assay has been suggested as a useful tool in estimating the risk of oxidative damage. Table 3 represents activities of both enzymatic SOD, CAT, GPx GST, and GR and non-enzymatic antioxidants GSH, vitamins E and C significantly (P < 0.05) decreased in B(a)P induced tumor bearing animal group II due to excessive generation of free radicals. Inophyllamine-1 treated group III animal normalized the level of antioxidant to control animals by quenching the free radicals level. The animal group IV almented only with inophyllamine-1 did not show any significant alteration from control animal.

### Gene expression profile by RT-PCR

Expression of apoptosis related genes bcl-2 and bax, and tumor suppressor gene p53 and critical cellular regulator gene NF-κB were studied by RT-PCR (Figure 3). The transcription level of bcl-2 was found to be positive and up-regulated in B(a)P treated animal tissue and interestingly, the same gene was down-regulated in inophyllamine-1 treated animal. The control animal group showed minimum or insignificant expression of bcl-2. Likewise p53 gene expression decreased in B(a)P induced cancer tissue and it was found highly expressed in inophyllamine-1 treated cells of animal. The expression level of p53 in cancer treated and control animal tissue was comparatively same. NF-κB is a regulator of cell growth and stress stimulation; its expression was triggered in B(a)P induced lung cancer cell and in inophyllamine-1 treated cancer tissue the expression level was normalized to control level. Gene expression study clearly demonstrated that B(a)P rendered lung cancer normalized by inophyllamin-1 treatment and promote cell death by down regulating anti-apoptotic bcl-2 gene. All the tested genes were conditioned to normal level.
by the isolated compound treatment. The house keeping gene β-actin served as control and no modification was observed in its expression during different expression condition.

**DISCUSSION**

Plants act as source for various molecule entities which are used as template for synthesizing efficacious drugs for a multitude of disease including cancer. Plant derivatives play an important role in the development of several clinically useful anticancer agents (Cochrane et al., 2008) such as vinblastine and vincristine (Catharanthus roseus), epipodophyllotoxin, an isomer of podophyllotoxin (Podophyllum peltatum roots) and paclitaxel (Taxus baccata, T. brevifolia and T. canadensis) (Boopathy and Kathiresan, 2010). Several countries including India and China practiced preparation of herbal drugs from bioactive natural products of plant material, which dates back thousand years ago, and currently so many modern drugs have been developed from natural product of plants (Shoeb, 2006).

Mangroves are salt-tolerant evergreen forests found along the coastal region with tremendous medicinal values. The evidence of several earlier reports exhibited strong anticancer activity of isolated compounds of different mangrove species such as Acanthus illicifolius, Bruguiera sexangula, Morinda citrifolia, Terminalia catappa and Ecteinacida turbinate (Bandaranayake, 2002).

Based on traditional therapeutic value and scientific reports, *C. inophyllum* was chosen for the present investigation and the solvent extraction of its root bark material showed significant antioxidant activity purified and identified as inophyllamin-1 by spectral studies. The identified compound is a newly derived structure that act as potent reducing agent and not reported elsewhere. It showed marked chemotherapy activity against B(a)P induced lung cancer in male albino rats. Mangrove plants act as source of unique metabolites with promising pharmacological activity.

Xiao et al. (2008) reported a new prenylated xanthone (1), named caloxanthone N, with cytotoxic effect against chronic myelogenous leukemia cell line (K562). The mangrove species of *Bruguiera sexangula* barks were recorded for its significant activity against Sarcoma 180 and Lewis Lung cancer cells (Loder and Russell, 1969). Shi et al. (2010) observed potent cytotoxicity in several tumor cell lines by Chinese mangrove plant *Laguncularia racemosa* L. extract. Many researchers carried out investigation on phytochemical distribution of *C. inophyllum*, but no report was made available on its phytochemical action against lung cancer.

Recently, lung cancer considered as a global health problem due to drug resistance mechanism to the available therapeutic agents leads to mortality and morbidity, and over all five year survival rate remains less than 15% (Shanker et al., 2010). Despite the attempts made in understanding resistance mechanism at molecular level, it is necessary to find an effective molecule to thwart the drug resistance by targeting a particular molecule for successive treatment. It is hoped that identification of new therapeutic agents for target locking treatment and understanding their contribution will provide opportunities for innovative therapies in overcoming drug resistance.

In the present investigation, B(a)P induced lung cancer bearing animals showed hematological variation normalized to control the level by inophyllamin-1 alimentary. The reduction of RBC and hemoglobin caused by B(a)P metabolites, a state of anemia was thwarted by our compound treatment. The increase in WBC and neutrophil was encountered in cancer patients in response to inflammation, an embedded event of carcinogenesis. The B(a)P generated stress condition may reduce the lymphocytes count level. The metabolites of B(a)P is a potent radical generator and experimentally it was documented here in B(a)P administered animal group, the homeostatic level of enzymatic and non-enzymatic
substances were disturbed and radical levels were increased to promote tumor formation. The increased level of ROS mediated stress condition may attribute to reduction in lymphocytes, RBC and hemoglobin in cancer induced animal group II. In our observation, the ROS level was decreased in inophyllamine-1 treated animal and antioxidant enzyme level was equalized to control group I. Also, the compound inophyllamine-1 cured the tumor in carcinogen induced animal as raveled by histological observation of lung tissue. Hence, the histological observation clearly supported the result of haematology coupled with antioxidant assay.

Inophyllamin-1 up-regulated the expression on tumor suppressor gene p53, the most mutated or inactivated gene in half of the tumors which sense the DNA damage to either arrest the cell cycle for DNA repairing or induce apoptosis. Cadwell and Zambetti (2001) documented that repression of p53 is capable of immortalizing which leads to uncontrolled cell proliferation. The activation of p53 is involved in many pathways of apoptosis, a type of programmed cell death. The induction of apoptosis related cell death in cancer cell is a major objective for the clinicians and pharmacologists to effectively treat the disease by chemotherapeutic agents.

The compound rendered the activation of bax gene, whose protein is pro-apoptotic, transcribed by p53, since its promoter contains a p53 recognition motif (Breen, 2005). In addition, the expression of the bcl-2 protein found to be down-regulated in inophyllamine-1 administered animal, which is anti-apoptotic, is repressed by wild-type p53 (Gallagher and Brown, 1999). The over-expression of bcl-2 can block p53-mediated apoptosis (Levine, 1997), which tend the cells to drug resistance mechanism was interestingly crossed in B(a)P induced lung cancer cells by inophyllamine-1 treatment. Generally, bcl-2 can protect the cell from various apoptotic stimuli by preventing release of cytochrome-c from mitochondria which is a key molecule involved in activation of cascade event of apoptotic enzyme capase (Fernald and Kurokawa, 2013). bax gene transcription was affected in lung cancer tissue and modulated in inophyllamine-1 treated tissue. The balance between pro- and bcl-2 family protein is a fundamental determinant for the initiation of mitochondrial mediated apoptosis. In the present study, the pro-apoptotic protein was expressed markedly higher than anti-apoptotic gene bcl-2 expression.

The successive treatment of cancer does not only depend on apoptosis induction. Generally, cancer progression and surpass the cell death is depend on balance between cell survival and death signal (Chen et al., 2011). NF-κB is a major survival signaling factor; it was established as a factor for drug resistant obstacle in recent studies (Lin et al., 2010). In the present study, inophyllamine-1 inhibited the expression level of NF-κB and conversely up-regulated the p53 mediated bax transcription. Similarly, a new diterpinoid agallochoas from mangrove plant, *Excoecaria agallocha* exhibited the suppression of NF-κB expression (Li et al., 2010). The higher expression of NF-κB hijack the death signals to facilitate cell division to cancerous state (Hanahan and Weinberg, 2000). The p53 activation was suppressed in B(a)P induced cancer tissue and the same p53 activation was restored concomitantly; NF-κB was suppressed in our compound treated animal model. Our result was supported by Meylan et al. (2009). He stated that inhibition of NF-κB led to restoration of p53 activity and repressed the cell growth in lung tumor cell lines. Cai et al. (2011) demonstrated development of lung cancer in tobacco treated animal model by aberrant expression of NF-κB and oncogenic protein k-ras which leads to cell proliferation, inhibition of apoptosis, angiogenesis, inflammation, invasion and metastasis.

Tobacco smoke cause DNA damage in lung epithelial cells and develops oncogenic growth and cancer progression. Lung cancer cells implicated numerous mechanistic alterations to battle death signals and facilitate their growth. The evidence from the current discovery suggests that inophyllamine-1 isolated from *C. inophyllum* prevent the tumor development by regressing NF-κB and bcl-2 and trigging p53 and bax. The compound normalized the haematological and antioxidant variation effectively in lung cancer bearing animals. Further investigation will be addressed about exact mechanistic action of inophyllamine-1 involved in chemoprevention.

**ACKNOWLEDGMENTS**

The authors are grateful to authorities of Annamalai University for providing laboratory facilities and Dr. C. Ravinder Singh to UGC, Government of India for Dr. S. Kothari Post Doctoral Fellowship (No: BL/11-12/0037).

**REFERENCES**


Submit your manuscript at http://www.academiapublishing.org/journals/mms