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ORIGINAL ARTICLE

Antineoplastic Potential of Eupatilin against Benzo[a] pyrene-Induced Lung Carcinogenesis

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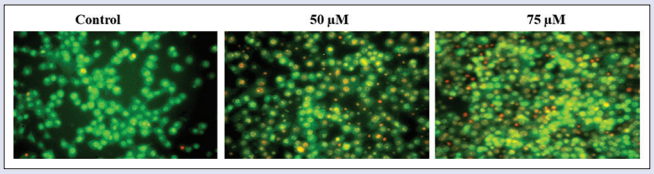
ABSTRACT

Background: Most of the conservative therapies used to treat lung cancer show serious side effects. In addition, the prevalence and death rates due to lung cancer have been increasing alarmingly across the globe. Eupatilin (EUP) is a naturally occurring flavone which is primarily the active ingredient of the traditional Chinese medicine *Artemisia asiatica*. **Materials and Methods:** In this study, we evaluated the antineoplastic effect of EUP against benzo(a) pyrene-induced lung cancer in Swiss albino mice. We analyzed the level of xenobiotics, liver dysfunction enzymes (LDEs), pro-inflammatory cytokines, and histology of the liver. Furthermore, we conducted *in vitro* experiments (A549 cells) to elucidate the amount of cell proliferation, apoptosis, and their markers (caspases 3 and 9). **Results:** The EUP (30 mg/kg bw) treatment of tumor-bearing mice with EUP revealed the normal levels of xenobiotic, LDEs, antioxidant enzymes, lipid peroxidation in the liver and further carcinoembryonic antigen, pro-inflammatory marker, and histology in lung tissues. EUP inhibited the proliferation of A549 cells and induced the formation of reactive oxygen species and apoptosis by upregulating the expression of caspases 3 and 9. **Conclusion:** Overall, these results substantiate the anti-neoplastic effects of EUP against carcinogen-induced lung cancer in *in vitro* and *in vivo* models.

Key words: Apoptosis, benzopyrene, eupatilin, lung carcinogenesis, MTT, reactive oxygen species

SUMMARY

- Eupatilin (EUP) stimulates apoptosis via mitochondrial apoptotic pathway in human lung cancer cells.
- EUP inhibits cancer cell proliferation via caspase-induced signaling.
- EUP exhibits good antitumor effect in tumor mice model of lung cancer therapy.



Abbreviations used: LPO: Lipid peroxidation; EUP: Eupatilin; CEA: Carcinoembryonic antigen; ROS: Reactive oxygen species.

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INTRODUCTION

Cancer is the leading cause of death and is a major health concern worldwide.[1] Globally, lung cancer (LC) is most prevalent and according to the World Health Organization, approximately 1.4 million patients are diagnosed with LC every year.[2] In the USA, approximately 228,190 people were diagnosed with LC in 2013, and approximately 159,480 people died due to LC.[3] Excessive smoking is an imperative cause of squamous cell carcinoma; continued use of cigarette increases the risk of LC.[4]

Benzo(a)pyrene (BaP) is a polycyclic aromatic hydrocarbon and a common procarcinogen observed in tobacco smoking. It contributes to the initiation and progression of LC.[5] During tumorigenesis, BaP is metabolized to BaP-7,8-dihydrodiol-9,10-epoxide (BPDE) by cytochrome P450, which is a highly reactive metabolite. BPDE is a highly reactive carcinogenic metabolite, which forms DNA adducts, leading to cancer formation. Even though the imbalance between metabolic regulation and detoxification may affect and exhibit the risks of cancer.[6]

Phytochemicals avert the oxidative damage caused due to the presence of toxic chemicals by altering various signaling pathways. Free radical scavenging and antioxidant mechanism may overcome degradation due to metabolism and reduce the side effects caused by the toxic chemicals.[7] Enzyme analysis in tissues is helpful in examining the chemopreventive potential of natural compounds.[8] Chemoprevention is a useful and novel approach in the development of therapeutics; it prevents the progression of the disease in patients with the use of natural products and synthetic agents. Triterpenes are structural components of plant. A number of terpenoids act as antineoplastic agents.[9,10]

Phytochemicals are safe and are widely distributed in the plant kingdom. Systematic studies on phytochemicals have lasted nearly half a century and show good antioxidant activity.[11] Eupatilin (EUP) (5,7-dihydrox y-3′,4′,6-trimethoxyflavone) is an *O*-methyl-flavonoid, and it is found in various parts of plants. It is responsible for the therapeutic activity of *Artemisia asiatica* (Compositae).[12] It shows broad-spectrum pharmacological and biological activity such as anti-inflammatory,

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anticancer, neuroprotective, cardioprotective, antioxidant, and anti-allergic.[13] EUP suppresses the cell proliferation in cancer cells of osteosarcome U-20S cells, which induces apoptotic mechanism via mitochondrial pathways.[14,15] It significantly suppresses the proliferation of gastric cancer cells by blocking STAT3-mediated vascular endothelial growth factor expression. It inhibits proliferation and invasion of cancer cells and decreases the activity of nuclear factor kappa B of MKN-1 cells.[16] Moreover, a study conducted on EUP in renal cancer and the mechanisms in renal cancer cells remains binuclear. Many articles have reported anticancer effect of EUP.[17-23]

In this study, we investigated the antineoplastic effect of EUP against A549 cells and the mice model. We analyzed the levels of antioxidant, lipid peroxides (LPO), xenobiotic and liver dysfunction enzymes, carcinoembryonic antigen, and pro-inflammatory cytokines. We also conducted histopathological analysis in *in vivo* model and studied the inhibition of cell proliferation, induction of reactive oxygen species (ROS), and apoptotic mitochondrial pathway by caspases 3 and 9 protein expression in A549 cells.

MATERIALS AND METHODS

Chemicals

BaP (≥95% purity) and EUP (≥95% purity, CAS NO: 22368-21-4) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of diagnostic range purchased from HiMedia (Mumbai, India).

Animals

Swiss albino mice weighing about 20–25 g (6–8 weeks old, male) were housed in polypropylene cages with pathogen-free air, 12:12 h light and dark cycles, temperature of 25°C ± °C, and humidity of 50% ± 10%. The animals were fed with standard animal pellet diet and filtered water. All investigations were conducted as per the regulation and guidelines provided by the Institutional Animal Ethics Committee.

Preparation of eupatilin

Each day, EUP was suspended in corn oil just before administration (30 mg/kg bw, for 18 weeks).

Experimental design

The investigational mice were divided into four groups with six mice in each group.

- Group I: (Vehicle control) corn oil was used as vehicle control
- Group II: (BaP) mice administered with BaP (50 mg/kg bw), orally (twice a week (1st and 4th day) for 4 weeks, from 2nd to 6th week)
- Group III: (BaP with EUP) mice were administered with EUP treated (30 mg/kg bw, suspended in corn oil) starting from 12th week of the experiment as in Group II up to the end of the experimental period (18th week)
- Group IV: (EUP) mice were orally administered with EUP alone (30 mg/kg bw, diluted in corn oil) for 18 weeks.

The effective dose of EUP (30 mg/kg bw) and BaP (50 mg/kg bw) was selected based on the literature data.[23,24] The post-intoxicated groups were utilized for the investigation of chemotherapeutic effect of EUP. All mice were weighed every week until the 18th week of investigational regimen. After 18 weeks, all animals were anesthetized and sacrificed via cervical dislocation.

Total protein from tumor-bearing and normal lung tissues was analyzed using Bradford method. A subsequent biochemical analysis was conducted using lung homogenate and serum.

Changes in bodyweight and lung weight of mice

Final bodyweight and lung weight of normal and tumor-bearing mice were measured throughout the experimental period. The mice were weighed at the initiation of the experiment and once in a week and finally before sacrifice. At the end of study, the lungs were cut out from the tumor-bearing mice, washed with normal saline, and weighed.

Biochemical analysis

Analysis of LPO (thiobarbituric acid reactive substances [TBARS]), enzymatic antioxidants (glutathione peroxidase [GPx], catalase [CAT], superoxide dismutase [SOD], glutathione-S-transferase [GST], and glutathione reductase [GR]), non-enzymatic (GSH) antioxidants, Vitamin E, Vitamin C, and total protein was done based on the previously described techniques.[25-32]

Biochemical analysis of lactate dehydrogenase, aryl hydrocarbon hydroxylase, γ-glutamyl transpeptidase, and *p*-nitroaniline, 5′nucleotidase

The activity of aryl hydrocarbon hydroxylase (AHH), γ-glutamyl transpeptidase (γ-GT), *p*-nitroaniline, 5′nucleotidase (5′-ND), and lactate dehydrogenase (LDH) was analyzed based on previous publications.[33-36] (Mildred *et al*.,[33] Rosalki and Rau,[34] Luly *et al*.,[35] and King,[36] respectively).

Estimation of carcinoembryonic antigen marker analysis in tumor tissue

Quantitative determination of carcinoembryonic antigen (CEA) was based on solid-phase ELISA (Pierce Biotechnology, Rockford, USA) kit.[37]

Estimation of pro-inflammatory marker analysis in lung cancer

The level of pro-inflammatory (tumor necrosis factor [TNF]-α, interleukin [IL]-16, and IL-1β) markers in the lung tissue samples was assessed. The lung tissue homogenate (10%) was prepared by using protease inhibitors in phosphate-buffered saline (PBS). The homogenate was centrifuged at 10,000 *g* for 20 min and the supernatant was collected. Next, the supernatant was used to analyze the level of TNF-α, IL-16, and IL-1β using ELISA kit (Pierce Biotechnology, Rockford, USA). The application of TNF-α, IL-16, and IL-1β in lung tumor was assessed and depicted as picogram per milligram protein.

Lung tumor tissue histology

Histopathological changes were analyzed to verify the incidence of LC and the status of EUP action on the BaP-treated mice. A tumor sample was fixed in formalin and then dried using series of ethanol. Then, the tissues were cleaned using xylene, fixed in paraffin wax, and a 4-mm-thick section was cut by using microtome. The section was stained with hematoxylin and eosin and observed under light microscope for histological changes.

Cell culture maintenance

Human LC (A549) cells were purchased from ATCC, USA. The cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (Himedia Pvt. Ltd) and incubated at 37°C in a humidified atmosphere with 5% CO_2 and the cells were used in the experiments after they reached confluency. The medium was changed every 2 days and the cells were maintained under controlled conditions.

Cytotoxicity assay

The effect of EUP on cell proliferation of A549 cells was investigated based on the method described by Mosmann.[38] A549 cells were seeded in 96-well plate. The cells were treated with various concentrations (5 to 100 μM/mL) of EUP and were incubated overnight in a CO_2 incubator. MTT was added to each well (1 mg/mL), and the cells were subsequently incubated for 4 h at 37°C. Then, the medium was replaced with DMSO to dissolve the formazan crystals. Finally, the absorbance of the color formed was read at 490 nm (Microplate reader, Bio-Rad, USA). The values of half-maximal inhibitory concentration (IC_{50}) were calculated and the optimum concentrations were calculated at different time period. The medium effective dose (IC_{50}) is the number of cells suppressed by the EUP at 50%, which was calculated graphically for each well growth curve.

Measurement of apoptotic induction using acridine orange–ethidium bromide dual staining method

To examine the cell proliferation of A549 cells after incubation with EUP (IC_{50}), we analyzed the level of apoptosis or necrosis by acridine orange–ethidium bromide (AO–EB) staining. Briefly, 5 μL of 100 μg/mL AO and EB staining solution was added to live cells at 37°C in the dark, followed by examining under the fluorescence microscope. The fluorescence microscopic observation of apoptotic cell was conducted based on Baskić *et al.*[39]

Measurement of reactive oxygen species

2′,7′-Dichlorofluorescein (DCF) is oxidized through radicals which were visualized at excitation 535 nm, emission 485 nm. DCF is not oxidized by H_2O_2 or superoxide radical. Briefly, the cells were plated in 6-well plate and treated with 50 and 75 μL/mL of EUP and then incubated for 24 h. After this, A549 cells were rinsed with PBS and dichloro-dihydro-fluorescein diacetate (DCFH-DA) (20 μM) in DMEM medium was added to it. The cells were incubated for 30 min at 37°C. Next, the cells were rinsed with PBS and fluorescence was recorded every 5 min up to 30 min using a spectrofluorimeter at 37°C.

Measurement of caspases 3 and 9

The level of caspases 3 and 9 was analyzed in A549 human LC cells using ELISA kit (Biovision Research Products, USA). The peroxidase activity of caspases 3 and 9 was tested colorimetrically by checking the development of oxidized *N*, *N*, *N*′, *N*′-tetra methyl-*p*-phenylenediamine at 590 nm. Caspases 3 and 9 of the chromophore *p*-nitroanilide after breakdown from labeled substrate DEVD-pNA and LEHD-pNA, respectively, at 405 nm was based on spectrophotometric detection in an ELISA reader.

Statistical study

Data were presented as arithmetic mean of three independent experiments in each group. The significance was calculated by one-way analysis of variance and Tukey's *post hoc* test by SPSS (16.0) tool (MO, USA). Differences in mean were regarded as statistically significant if their $P < 0.05$.

RESULTS

Effect of eupatilin on bodyweight, lung weight, and tumor incidence

Table 1 shows the effect of EUP on mean bodyweight, lung weight, and tumor incidences in investigational mice after 18 weeks of treatment. At the end of the experiment (18th week), BaP reduced weight gain, increased lung weight, and increased tumor incidence when compared with that of untreated mice. However, there was a significant ($P < 0.05$) delay in the development of tumor and tumor incidence reduced after supplementing EUP (30 mg/kg bw) to BaP-treated mice. However, EUP and control mice showed normal lung weight, bodyweight, and tumor incidence rate.

Effect of eupatilin on lipid peroxidation and on enzymatic and non-enzymatic antioxidants

Figures 1 and 2 show the status of LPO (TBARS) and enzymatic (GPx, CAT, SOD, GST, and GR) and non-enzymatic (GSH) antioxidants in the control and experimental mice. BaP noticeably increased the level of LPO and decreased the level of pulmonary enzymatic and non-enzymatic antioxidants in LC induced mice. EUP (30 mg/kg bw) offered significant ($P < 0.05$) defense against BaP-induced increase in LPO and returned the status of both enzymatic and non-enzymatic antioxidants to near-normal status when compared with that of BaP-treated mice. No significant differences were obtained in case of EUP alone treated and control mice.

Effect of eupatilin on xenobiotic and liver dysfunction enzymes

Figure 3 shows the levels of LDH, AHH, 5′-ND, and γ-GT in the tumor tissues obtained from the treated mice. These enzymes were notably ($P < 0.05$) increased in the BaP-induced mice than that of the control mice. The increase in the level of enzymes was significantly reduced ($P < 0.05$) in EUP-treated mice when compared to those in the BaP-treated control mice. There were no considerable differences between the mice treated with EUP alone and the control mice.

Effect of eupatilin on carcinoembryonic antigen and interleukin-6, interleukin-1β, and tumor necrosis factor-α

Figure 4a and b shows the effect of EUP treatment on the levels of CEA and IL-6, TNF-α, and IL-1β in experimental animals. BaP-induced LC bearing mice displayed increased in the levels of CEA and IL-6, TNF-α, and IL-1β significantly ($P < 0.05$) when compared with that of control and EUP alone treated mice, respectively. This effect drastically ($P < 0.05$) decreased after treatment of BaP-induced mice with EUP.

Histology of lung tissues

Figure 5 shows the histopathological analysis of lung tissue samples obtained from the experimental mice. We observed uniform nuclei and normal architecture in samples obtained from control mice. Cell abrasion with central alveolar and bronchiolar epithelial hyperplasia and thrashing of architecture with deformed alveoli was seen from augmented hyper chromatic nuclei in a tumor tissue revealed in BaP-treated mice. After the administration of EUP, there was a little condensed lung destruction with similar normal structural appearance. EUP decreased the level of alveolar damage and restored normal architecture of the lung tissue.

Effect of eupatilin on cell proliferation of A549 cells

A549 LC cells were incubated with EUP at different concentrations (5–100 μM/mL) for 12 h. According to the results, the

Table 1: Effect of eupatilin on body weight, lung weight, and tumor incidence in control and experimental animals

Groups/treatments	Number of animals	Body weight (g)	Lung weight (mg)	Tumor incidence
Group I	6	29.55±1.77	247.33±18.84	0
Group II	6	17.22±0.79*	342.69±26.10*	6
Group III	6	25.61±1.95#	284.83±21.81#	3
Group IV	6	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 significantly at $P<0.05$ (DMRT). SD: Standard deviation; DMRT: Duncan's Multiple Range test

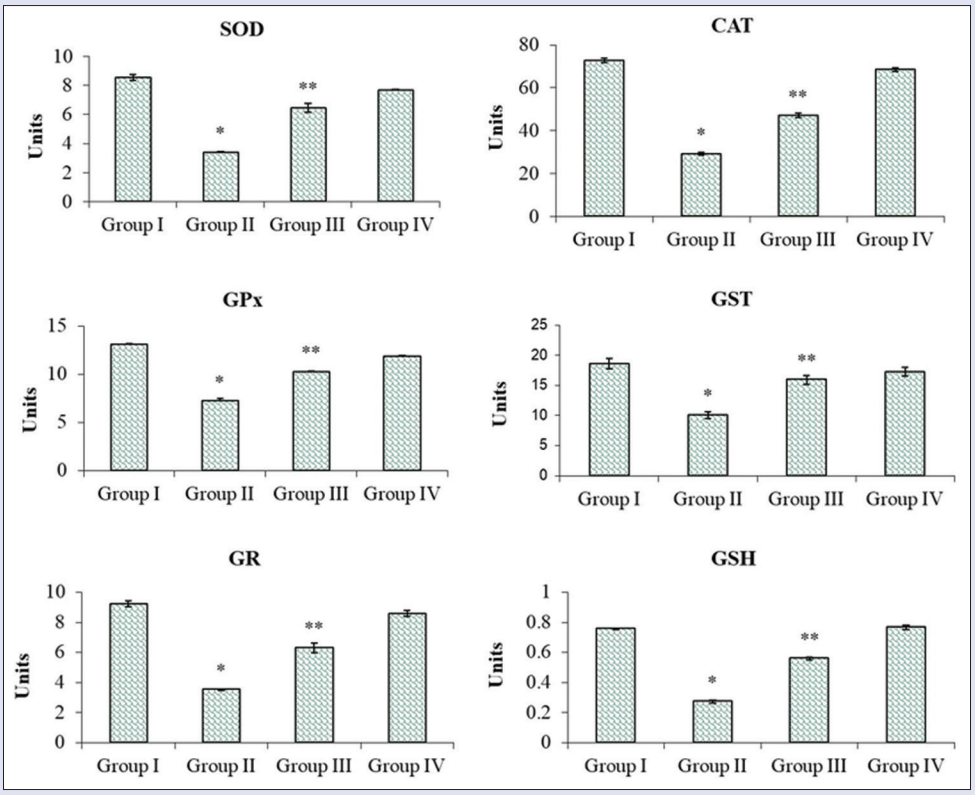

Figure 1: Effect of eupatilin on antioxidant activities in control and experimental animals. Results are expressed as mean ± standard deviation for six animals in each group. Data not sharing a common superscript letter (* - **) differ significantly at $P < 0.05$ Duncan's Multiple Range test (DMRT)

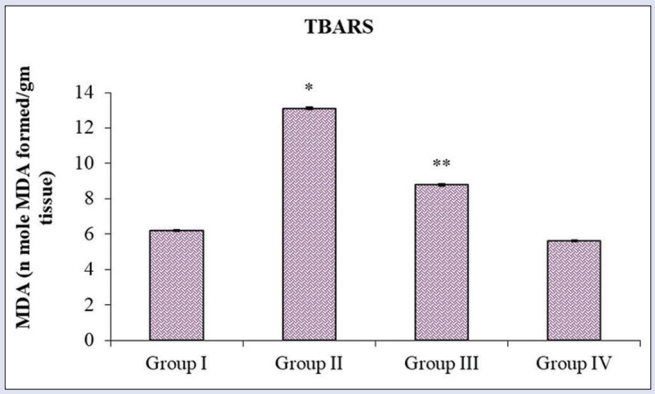

Figure 2: Effect of eupatilin on LPO in control and experimental animals. Results are expressed as mean ± standard deviation for six animals in each group. Data not sharing a common superscript letter (* - **) differ significantly at $P < 0.05$ Duncan's Multiple Range test (DMRT)

cell growth decreased significantly ($P < 0.05$) compared with the control cells. As shown in Figure 6a, increased level of EUP caused a decreased rate in cell proliferation. After 12 h, there was a decreased rate of cell proliferation in cells treated with 100 μM/mL of EUP. Furthermore, it is shown that more that 50% of the cells died after incubation with concentration of 50 μM/mL for 24 h.

Effect of eupatilin on A549 cells during apoptosis via acridine orange–ethidium bromide dual staining assay

A549 cells were treated with EUP (50 and 75 μM/mL) for 12 h and the morphological changes were detected via AO/EB staining. AO binds with DNA in live cells and emits green fluorescence. EB binds with DNA of dead cells and emits red fluorescence. Apoptotic morphological appearance of some of the chromatin condensation, alterations in the size, nuclear fragmentation, and the shape of cells, as examined through fluorescence microscopic, were measured predominantly after EUP treatment at (50 and 75 μM/mL) for 24 h. The maximum difference in apoptotic cells was recorded for EUP after 24 h (IC_{50}, 50 μM), which was higher than that of control cells. Figure 6b shows this clearly. The time interval exposure of the EUP treatment results in induced the necrotic-like cell death.

Reactive oxygen species measurement in A549 cells

EUP induced the production of ROS in A549 cells. This led to oxidative damage, thereby resulting in apoptosis of cancer cells. The level of ROS generated was analyzed via staining with DCFH-DA dye. In Figure 7a, the green florescence signal of DCF was revealed in A549 cells after incubation of cells EUP (12 h, 50 and 75 μM/mL) and in control cells. The fluorescence intensity increases intracellularly within 20 min after incubation of the cells with EUP at 12 h at CO_2 incubator. With increase in the concentration of EUP, the oxidized form of cells also increased.

Estimation of caspases 3 and 9 activities by ELISA

Figure 7b shows the pro-apoptotic protein expression of control and EUP-treated LC A549 cells. According to our results, the control cells showed reduced expression of caspases 3 and 9. EUP significantly increased the expression of pro-apoptotic markers when compared to control cells ($P < 0.05$). These findings show that EUP regulates pro-apoptotic proteins in A549 cells.

DISCUSSION

Worldwide, natural plant products are used as remedial therapeutic agents. Recently, there has been an increasing concern with respect to the efficacy of plant phytochemicals against tumor cell proliferation.[40,41]

This study shows that EUP regulated cell growth and cytotoxicity of A549 cells. It induced apoptosis and ROS via induction of caspases 3 and 9. MTT assay showed that EUP inhibited the viability of A549 cells in a dose-dependent manner. EUP induced cytotoxicity in A549 cells at minimal dose for a short time. In other words, EUP might be a safe and efficient alternative to treat lung cancer. Figure 5a shows apoptotic changes in A549 cells. When compared to the control cells, reduction in the number of cells with rounded morphology was noted in EUP-treated A549 cells. AO/EB staining assay revealed that EUP induced apoptosis in LC cells in a dose-dependent manner.

In this study, we found that after 24-h incubation, EUP induced apoptosis at 50 and 75 μM/mL concentrations. Furthermore, the measurement of mitochondrial membrane potential (MMP) revealed that EUP decreased MMP in A549 cells. Moreover, ROS plays a significant role in cancer cells by inducing apoptosis.[42] Taken together, this result shows that apoptotic cell death induced by EUP might be via mechanisms related to mitochondria.

In this study, the level of apoptotic proteins in A549 cells was analyzed by using ELISA kits. In apoptotic pathway, caspases 3 and 9 are the most important apoptotic markers; these apoptotic markers find their use in cancer. The level of caspases 3 and 9 was upregulated after incubation of the cells with EUP (50 and 75 μM/mL) for 24 h. These hallmarks of apoptosis may be mediated by the formation of PARP breakdown and occurrence of DNA fragmentation.[43] Taken together, this study shows that EUP potently suppresses cancer formation. ROS stimulated caspase-3 mediated intracellular pathway.

The critical rationales behind carcinogen induced cancer in mice, a diminished the antioxidant defense machinery were lowered in levels of anti-oxidative enzymes (SOD, CAT, and GSH).[44] SOD decreases superoxide radicals and guards the cells from superoxide. Numerous information have freshly cited diminished levels of SOD and CAT neoplasia.[45] CAT is extensively circulated in region of tissues and is recognized to stimulate the break of H_2O_2 generated by cancer cells. Conversion of cell viability rate is attended by revolutionized in their cytosolic GSH status. SOD, CAT, and GSH represent antioxidant defense system. The level of the aforementioned enzymes decreases in cancer-bearing mice. Deposition of ROS exists to be slightly higher in tumor-developed mice than in control mice because of the induction generated by BaP. These results suggests that EUP (30 mg/kg bw) decreases antioxidant levels and induces oxidative damage, leading to apoptosis cell death.

In this study, BaP-treated mice gained bodyweight and lung weight and showed increased tumor incidence. This drop/rise might have been due to LC formation. LC findings in progression failure of body weight due to destroy of the host body compartments. Typically, tumor-bearing mice show reduced bodyweight and tissue wasting.[46-48] Recent studies have shown that the reduction in bodyweight was due to abnormal diet.[49] However, EUP control mice did not show any significant changes in the lung weight, bodyweight, and tumor incidence compared to control mice.

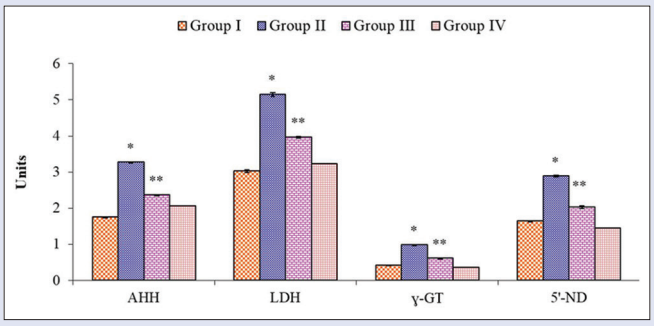

Figure 3: Effect of eupatilin on the activities of xenobiotic and liver dysfunction enzymes in the liver of the control and experimental animals. Results are expressed as mean ± standard deviation for six animals in each group. Data not sharing a common superscript letter (* - **) differ significantly at $P < 0.05$ (DMRT)

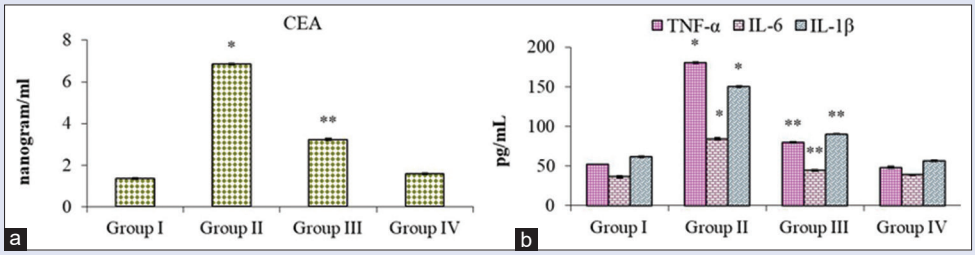

Figure 4: Effect of eupatilin on carcinoembryonic antigen and pro-inflammatory cytokines in lung tissue of control and experimental animals. Effects of eupatilin on serum carcinoembryonic antigen levels and pro-inflammation response in animals. (a) Activities of serum carcinoembryonic antigen and (b) ELISA was performed for tumor necrosis factor-α, interleukin-6 and interleukin-1β levels in mice induced by BaP. Each value is expressed as mean ± standard deviation for six mice in each group. Results are expressed as mean ± standard deviation for six animals in each group. Data not sharing a common superscript letter (* - **) differ significantly at $P < 0.05$ (DMRT)

In cancer, xenobiotic and hepatic marker enzymes are the best marker analysis. In this study, we analyzed AHH, γ-GT, 5′ND, and LDH as markers of liver and lung damage.[50] The activity of AHH, γ-GT, and 5′ND was greater in tumor-bearing mice.[51] This increase was found to be significantly reduced after the administration of EUP compared to control mice, which might be due to the anti-tumor effect on LC.[52] The increased level of LDH shows that glycolysis was increased in tumor-bearing mice, as glycolysis is the only energy-generating mechanism used by cancer cells. EUP decreased the level of LDH in cancer cells.

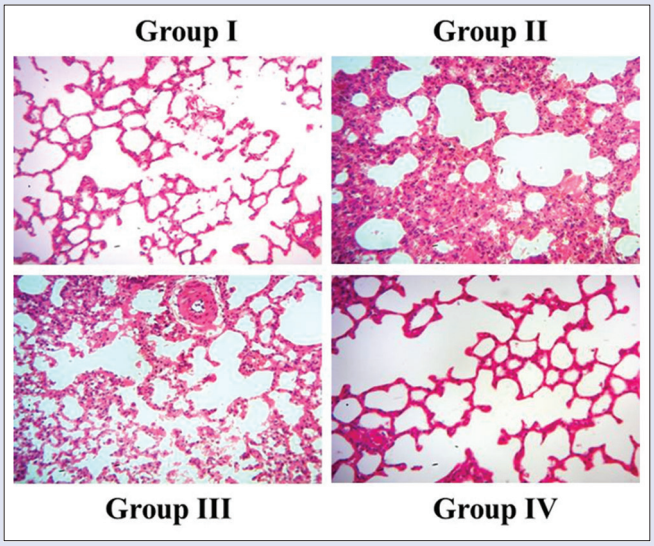

Figure 5: Histological examinations of the lung tissues of control and experimental animals. Group I revealed a normal architecture; Group II BaP alone showing alveolar damages with more number of pyknoic nuclei; Group III BaP + EUPATILIN (30 mg/kg bw) post-treated showing reduced alveolar damage and reduced irregular hyperchromatic cells and Group IV eupatilin alone showing no histological abnormalities

CEA is a one of the oncofetal antigens and tumor-associated glycoproteins that is usually upregulated in the malignant epithelial-type cancers including LC.[53] As a representative, TNF-α, IL-6, and IL-1β cytokines play a double role in cancer development. Several earlier therapeutic studies have suggested that TNF-α is a intracellular tumor promoter.[54,55] IL-6 alters the expression of proteins responsible for cell growth and suppression of apoptosis.[56] The increased expression of CEA, TNF-α, IL-6, and IL-1β in BaP induced LC mice, whereas decreased in the status of CEA and pro-inflammatory cytokines in EUP post-treated mice were found. These findings demonstrate the antiproliferative effect of EUP.

Histopathological analysis shows that EUP modifies the effect of BaP. In BaP-treated mice, we observed significantly increased hyperplastic nuclei with widespread multiplication of alveolar epithelium in lung tissue sections. EUP significantly recovered the BaP-induced histological modifications, which further suggests that EUP is a better antineoplastic agent in LC.

CONCLUSION

EUP inhibited the cell proliferation of A549 cells and induced apoptosis via increasing the formation of ROS in the mitochondria. This upregulated caspases 3 and 9. Taken together, our result shows that EUP protected cells against carcinogen-induced oxidative stress by ameliorating LPO and inducing antioxidant system. Furthermore, EUP decreased the level of CEA and pro-inflammatory cytokine markers. Taken together, this study shows that EUP can be used as a safe and useful chemotherapeutic agent to prevent human lung cancer.

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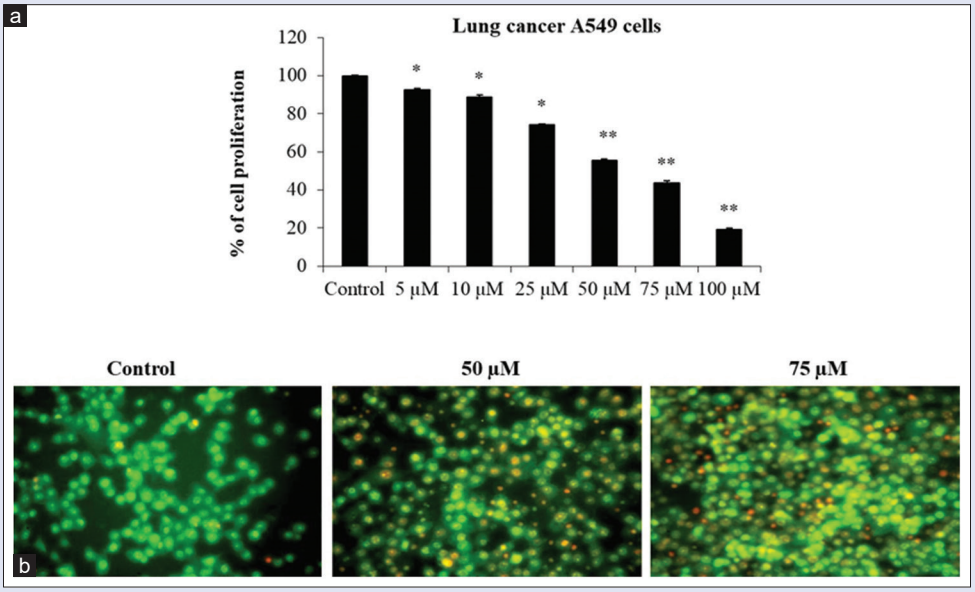

Figure 6: Effect of eupatilin on cell cytotoxicity and induced apoptosis incidence of A549 cells. (a) Results are expressed as lung cancer A549 cells treated with control and eupatilin (5–100 µM/ml) for 24 h. (b) A549 cells treated within control and eupatilin at different concentrations (50 and 75 µM/ml) at 24 h, stained with acridine orange–ethidium bromide staining and then analyzed by fluorescence microscopy. Values were presented as mean ± standard deviation of three independent experiments (analysis of variance) followed by DMRT. Data not sharing a common superscript letter (* - **) differ significantly at $P < 0.05$ (DMRT)

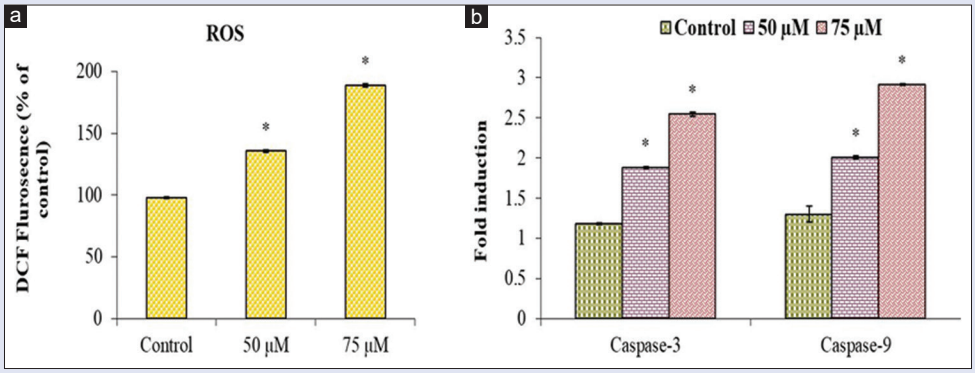

Figure 7: Effects of eupatilin on induces intracellular reactive oxygen species generation and caspase-3 and -9 in LC cells (A549). (a) A549 cells were treated with eupatilin at different concentrations (50–75 µM/ml) for 24 h, stained with dichloro-dihydro-fluorescein diacetate dye. (b) The A549 cells were treated with eupatilin for 24 h and then harvested. The protein status were examined by ELISA. Values were presented as mean ± standard deviation of three independent experiments (analysis of variance) followed by DMRT. Asterisks indicate statically different from control: *$P < 0.05$

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Nil.

Conflicts of interest

There are no conflicts of interest

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