Chapter V

Farnesol ameliorates massive inflammation, oxidative stress and lung injury induced by intratracheal instillation of cigarette smoke extract in rats: An initial step in lung chemoprevention
Introduction

Various plant constituents, other than polyphenols, possess preventive properties against a variety of toxicities (Imai et al., 2002; Salminen et al., 2008). Isoprenoids are plant compounds that have tumor suppressing activity in experimental animals (Burke et al., 1997; Mo and Elson, 2004). It is also reported that isoprenoids have been shown to modulate cell growth, induce cell cycle arrest, initiate apoptosis, and suppress cellular signaling activities (Elson and Yu, 1994; Mo and Elson, 1999). Farnesol is an isoprenoid obtained from the essential oils of *Vachellia farnesiana*, ambrette seeds, citronella, present in many aromatic plants (Zhao et al., 2004). Chemically farnesol is 3,7,11-Trimethyl-2,6,10-dodecatnene-1-ol.

![Chemical structure of Farnesol](image)

Previous studies from our laboratory have shown that it is a potent antioxidant and protects kidneys against oxidative damage (Jahangir et al., 2006). Farnesol is reported to induce apoptosis in lung carcinoma cells (Joo et al., 2007). Present study was designed to assess the role of farnesol against cigarette smoke toxicants induced lung damages in Wistar rats. Farnesol is reported to have protective effects even at lower doses (Jahangir et al.; 2006; Ong et al., 2006) moreover at higher doses it shows minimal toxic effects (Hern et al., 2005). In the present investigation, lower doses (50 and 100 mg/kg, b.wt.) of the farnesol were administered to avoid probable toxic effects of higher doses.
Treatment regimen

To study the effect of pretreatment of animals with farnesol on CSE induced acute lung toxicity, 25 male Wistar rats were randomly allocated to five groups of five rats in each. The animals of Group I & II served as control and toxicant (CSE) respectively, received corn oil orally (5 ml/kg b.wt., once daily, for 7 days). Group III received pretreatment with farnesol by gavages once daily for 7 days at a dose of 50 mg/kg b.wt. (FO I). Group IV and V received farnesol once daily for seven consecutive days at a dose level of 100 mg/kg b.wt. (FO II). On day 7, one hour after the last treatment with farnesol or corn oil the animals of Group II, III and IV were administered with a single i.t. dose of CSE (1.3 ml/kg b.wt.), Group I (control group) and Group V (only FO II group) was administered with DDW i.t. (1.3 ml/kg b.wt.). All the animals were sacrificed 24 h after last treatment.

Results

Inflammation and edema

CSE exposed animals (Group II) showed a significant increase in the total cell count of BALF (p < 0.01) when compared with control group (Group I) (Fig. 1). Pre-treatment of farnesol in Group III and IV animals (50 and 100 mg/kg b.wt. respectively) showed significant (p < 0.01) reduction in total cell count of BALF when compared with CSE treatment group (Group II). Intratracheal instillation of CSE led to discernible edema after 24 h in terms of protein content of BALF (Fig. 2). CSE administration caused significant edema formation in terms of total protein in BALF when compared with group I animals (p < 0.01). Group III and Group IV animals which were pre-treated with both the doses of farnesol (50 and 100 mg/kg b.wt.) show reduced lung edema response in CSE exposed rats and the changes in response was significant (p < 0.01) when compared with CSE-treated animals.
Administration of only farnesol (100 mg/kg b.wt.) (Group V) does not show any significant alterations and results remained close to normal.

**Cytotoxicity and membrane damage**

Group II animals (CSE-treated) showed significant enhancement in levels of cytotoxicity marker LDH (Fig. 3) and MDA formation (Fig. 4) in BALF when compared with Group I (control group). Group III and IV on farnesol pre-treatment showed a significant decrease in cytotoxicity and membrane lipid peroxidation indicating protective effect of farnesol against CSE lung toxicity.

**Alterations in lung surfactants**

Figure 5 shows the qualitative assessment of phospholipid content in BALF of all the animal groups. Group II animals (CSE-treated) showed significant reduction ($p < 0.01$) in levels of phospholipid in BALF when compared with Group I (control group) (Fig. 5). Farnesol pre-treatment in Group III and IV animals (50 and 100 mg/kg b.wt. respectively) showed a significant restoration ($p < 0.01$) of phospholipid content in BALF when compared with Group II. Only farnesol (100 mg/kg b.wt.) (Group V) administration does not show any significant alteration in phospholipid content when compared with Group I.

**Antioxidant status**

The intratracheal instillation of CSE (Group II) caused a significant decrease in the activities of glutathione metabolizing enzymes such as GR and GPx ($p < 0.01$), and catalase activity ($p < 0.05$) when compared with control (Group I) animals (Table 1). No significant change was observed in only farnesol treated animals (Group V) compared to control group data. Administration of FO I (farnesol 50 mg/kg b.wt.) + CSE in Group III animals showed a
significant increase in the activities of GR \( p < 0.01 \), GPx \( p < 0.01 \) and catalase \( p < 0.05 \) as compared to CSE-treated animals (Group II). Higher dose FO II (farnesol 100 mg/kg b.wt.) + CSE treatment (Group IV) also showed significant increase in the activities of GR \( p < 0.01 \), GPx \( p < 0.05 \) and catalase \( p < 0.01 \) as compared to CSE-treated animals (Group II). Significant \( p < 0.01 \) decrease in GSH was observed in the lungs from CSE-treated animals (Group II) when compared with Group I animals. FO I (farnesol 50 mg/kg b.wt.) + CSE (Group III) and dose FO II (farnesol 100 mg/kg b.wt.) + CSE (Group IV) treatments showed a significant increase in GSH when compared with Group II animals (Table 1). Only FO II (farnesol 100 mg/kg b.wt.) (Group V) significantly induces GSH levels \( p < 0.01 \) in lungs when compared with Group I.

\[ H_2O_2 \] content

CSE treatment in Group II animals showed significant enhancement in levels of \( H_2O_2 \) in lung tissue when compared with Group I (control group). Pre-treatment with farnesol (50 and 100 mg/kg b.wt. respectively) in Group III and IV showed a significant decrease in \( H_2O_2 \). Only Farnesol (Group V) also show a significant reduction \( p < 0.01 \) in \( H_2O_2 \) content when compared with Group I (Figure 6).

\[ Histopathological \] findings

Histological assessment at 400X enlargement reveals that CSE administration (group II) causes massive inflammatory cell infiltration, interstitial and alveolar edema, vascular congestion and alveolar collapse was also observed (Fig. 7B). CSE administration shows a uniform cellular infiltration and vascular congestion throughout the lung section, edema and alveolar collapse were seen as prominent patches under microscope and found to be uniform in distribution. In control (group I) a mild interstitial cellular infiltration was seen in complete
section of the lungs of different animals. Alveolar morphology changes were not observed in control group lung (Fig. 7A). Histology of Group III (FO I+CSE) and Group IV (FO II+CSE) exhibit a significant reduction in cellular infiltration and edema and show intact alveolar architecture (Fig. 7C & D). Group V (only FO II) shows histology similar to that of control (Fig. 7E).

Discussion

Lung injuries caused by inhalant toxicants present in cigarette smoke is a matter of major concern. It is reported that alteration in lung epithelium due to cigarette smoke exposure may result in permanent damage and lead to various respiratory disorders (Aoshiba and Nagai, 2003). During last years, knowledge of phytochemistry has been exploited to find out some plant based non-nutrient products having protective role against various organ toxicities (Jahangir et al., 2006; Lampc, 2003). Farnesol an isoprenoid is known to possess protective efficacy against such type of the disorders (Ong et al., 2006). In the present study farnesol reduced intense inflammatory responses and edema induced by CSE. Histological evaluations also support its anti-inflammatory activity in lung. Studies show that isoprenoids including farnesol reduces inflammation in mice. Farnesol is reported to down regulate the expression of Th1 cytokine IFN-γ and the Th1-inducing cytokine IL-12 in mice (Navarathna et al., 2007). In the present findings down regulation of IFN-γ and IL-12 may be a probable mechanism of farnesol against CSE induced inflammation in rat lung.

Cigarette smoke contains billions of free radicals (Church and Pryor, 1985) and chemicals that generate ROS in-vivo. This makes cigarette smoke highly oxidative and is responsible for most of the lung damages. The present data has demonstrated causal role of cigarette smoke oxidants in lung injury and farnesol is minimizing it significantly. According to present findings farnesol restored the levels of redox cycle components GR, GPx and GSH in
lungs of the rat experimental model. It also restored the levels of catalase, an enzymatic antioxidant responsible for the breakdown of hydrogen peroxide into water and oxygen. It can be said that farnesol potentiates endogenous defense enzymes in rat lungs against CSE-induced oxidative stress. Similar action of farnesol is reported from our lab, against Fe-NTA induced renal oxidative stress in rats (Jahangir et al., 2006). Some studies report isoprenoid mechanism of action at the molecular level and indicate that these mechanisms are in addition to antioxidant activities of natural compounds, like suppression of mevalonate pathway can be attributed to the chemopreventive activity of isoprenoids (Kline et al., 2001). Diverse isoprenoids modulate cell growth, induce cell cycle arrest, initiate apoptosis, and suppress cellular signaling activities (Mo and Elson, 2004). So it can be suggested that farnesol may involve some pathway other than induction of antioxidant armory against cigarette smoke-induced oxidative lung damage. Our data exhibit a marked reduction in lung injury markers in BALF. Decreased level of LDH with simultaneous reduction in MDA level of BALF point out the role of farnesol in protection of lung epithelial cells. Type II cells secrete lung surfactants which are essential for the maintenance of alveolar morphology (Fisher et al., 2005). Decreased phospholipids content plays a role in altering the biophysical characteristics of surfactant (Subramanian et al., 1995). Results show that farnesol administration restored the phospholipid level of BALF; this also indirectly demonstrates the role of farnesol in type II cell protection. Histological findings strongly support these biochemical observations. H$_2$O$_2$ content formed in the tissue is associated with oxidative DNA damage and it may lead to or play a role in cancer development (Stone et al., 1994). In the present finding, CSE-induced H$_2$O$_2$ content in lung tissue was significantly reduced to normal by both of the doses of farnesol. Oral administration of farnesol 100 mg/kg b.wt. alone (Group V) significantly increases GSH content (p<0.01) when compared with control group. These findings suggest
that farnesol play a major role against cigarette smoke toxicants induced lung injuries in Wistar rats.

**Conclusion**

On the basis of present findings it can be concluded that farnesol has protective effects on rat lungs against oxidative damage caused by cigarette smoke toxicants. It may have similar effects on cigarette smoke exposed human lungs. The present investigation gives a path to study the effects of farnesol more deeply on lung injuries caused by other air pollutants and pneumotoxicants present in the environment.
Figure 1: Effect of Farnesol against cigarette smoke extract (CSE) induced total cell count in BALF

Fig. 1. Effect of farnesol and CSE on total cell count in BALF of rats. Values are expressed as means ± S.D. (n = 5) measured as mn (10^6) cells/mL BALF. Significant differences are indicated by **p < 0.01 when compared with control animals (Group I) and ###p < 0.01 when compared with CSE-treated animals (Group II).
Fig. 2. Effect of farnesol and CSE on total protein in BALF of rats. Values are expressed as means±S.D. (n = 5) measured as mg protein/dL BALF. Significant differences are indicated by **p < 0.01 when compared with control animals (Group I) and ##p < 0.01 when compared with CSE-treated animals (Group II).
Figure 3: Effect of Farnesol against cigarette smoke extract (CSE) induced LDH activity in BALF

Fig. 3. Effect of farnesol and CSE on cytotoxicity marker LDH in BALF of rats. Values are expressed as means±S.D. (n = 5) measured as µmol NADH oxidized/mL BALF. Significant differences are indicated by **p < 0.01 when compared with control animals (Group I) and #p < 0.05 when compared with CSE-treated animals (Group II).
Fig. 4. Effect of farnesol and CSE on lipid peroxidation product MDA in BALF of rats. Values are expressed as means±S.D. (n = 5) measured as μmol MDA/mL BALF. Significant differences are indicated by **p < 0.01 when compared with control animals (Group I) and ##p < 0.01 when compared with CSE-treated animals (Group II).
Fig. 5. Effect of farnesol and CSE on phospholipid content in BALF of rats. The values are expressed as means±S.D. (n = 5) measured as Absorbance of thiocyanatoiron-phospholipid complex at 470 nm. Significant differences are indicated by **p < 0.01 when compared with control animals (Group I) and #p < 0.01 when compared with CSE-treated animals (Group II).
Figure 6: Effect of Farnesol against cigarette smoke extract (CSE) induced hydrogen peroxide level in lung tissue

Fig. 6. Effect of farnesol and CSE on H$_2$O$_2$ formation in lung tissue of rats. Values are expressed as means±S.D. (n = 5) measured as nmol H$_2$O$_2$ formed/h/g tissue. Significant differences are indicated by **p < 0.01 when compared with control animals (Group I) and ***p < 0.01 when compared with CSE-treated animals (Group II).
Figure 7: Lung histopathology of farnesol and cigarette smoke extract (CSE) treated animals.
Fig. 7. Histological slide (A) represents only vehicle (control) group, slide (B) represent CSE group. (C) and (D) are farnesol+CSE groups (50 and 100 mg/kg b.wt. respectively), slide (E) represents only farnesol group (100 mg/kg b.wt.). (A) There were no changes observed in alveolar architecture. Interstitial inflammatory cells (arrow) can be seen. (B) Massive infiltration of inflammatory cells (dotted arrow and arrow head) and alveolar epithelium thickening (arrow) is clear. Intra-alveolar protein (bold arrow) and edema is observed. Alveolar architecture is damaged by CSE administration. (C), (D) and (E), slides show that farnesol maintains the alveolar architecture and minimizes the recruitment of inflammatory cells (arrows). These observations show a relationship with cellular and biochemical estimations of the BALF of all of these groups.
Table 1: Effect of farnesol and cigarette smoke extract (CSE) on lung antioxidant status

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glutathione Reductase (nmol NADPH oxidized/min/mg protein)</th>
<th>Glutathione peroxidase (nmol NADPH oxidized/min/mg protein)</th>
<th>Catalase (as nmol H₂O₂ consumed/min/mg protein)</th>
<th>Reduced glutathione (GSH) (nmol GSH conjugates/gm tissue)</th>
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<tbody>
<tr>
<td>I [Only vehicle]</td>
<td>16.13 ± 0.47</td>
<td>239.62 ± 8.72</td>
<td>13.34 ± 0.31</td>
<td>305.14 ± 31.83</td>
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<tr>
<td>II [CSE]</td>
<td>6.07 ± 0.92**</td>
<td>205.54 ± 1.33**</td>
<td>9.87 ± 0.37*</td>
<td>213.23 ± 22.96**</td>
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<tr>
<td>III [FO I + CSE]</td>
<td>13.10 ± 0.89##</td>
<td>261.82 ± 13.68##</td>
<td>11.42 ± 0.29#</td>
<td>238.97 ± 6.36#</td>
</tr>
<tr>
<td>IV [FO II + CSE]</td>
<td>13.30 ± 0.77##</td>
<td>227.37 ± 7.37#</td>
<td>12.33 ± 0.37##</td>
<td>330.88 ± 19.1##</td>
</tr>
<tr>
<td>V [FO II]</td>
<td>16.34 ± 2.35</td>
<td>233.19 ± 5.91</td>
<td>13.46 ± 1.14</td>
<td>525.73 ± 27.75##</td>
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Values are means ± S.D. (n = 5). Significant differences are indicated by *p < 0.05 and **p < 0.01 when compared with control animals (Group I), and #p < 0.05, ##p < 0.01 when compared with CSE-treated animals (Group II). CSE = cigarette smoke extract; FO I = farnesol 50 mg/kg b.wt.; FO II = farnesol 100 mg/kg b.wt.